Analytical Methods

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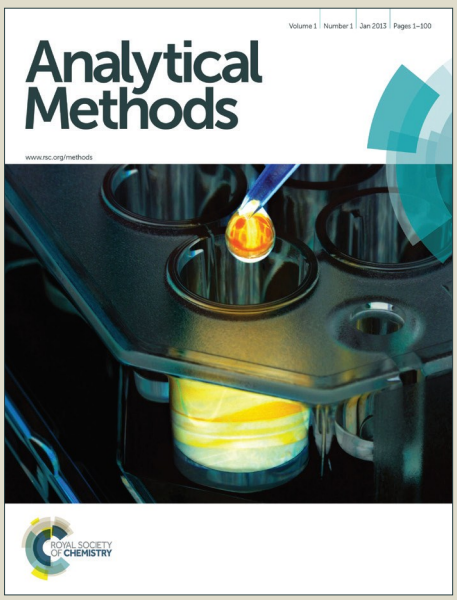

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ARTICLE

Electrochemical sensor based on carbon paste electrode modified by graphene nanosheets and molecularly imprinted polymer nanoparticles for determination of chlordiazepoxide drug

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A selective and sensitive voltammetric sensor based on graphene – carbon paste electrode (GCPE) modified with imprinted polymer nanoparticles (Nano–MIP) for determination of chlordiazepoxide (CDP) drug has been developed. The chlordiazepoxide binding experiments indicated that the sensor modified by Nano–MIP have much higher adsorption ability to CDP molecules than non–imprinted polymer nanoparticles (Nano–NIP) based sensor. Also, using of graphene in preparation of CPE leads to a significant improvement in response of electrode to CDP drug. The effect of crosslinker type and amount of template molecule on the MIP nanoparticles properties and also other parameters affecting sensor response were studied. Under optimized extraction and analysis conditions, the peak current obtained at Nano–MIP modified graphene–carbon paste electrode (Nano–MIP–GCPE) was proportional to chlordiazepoxide concentration within the range of 6.0×10^{-10} to 7.5×10^{-8} M ($R^2 = 0.9982$) with a detection limit of 2.61×10^{-10} M. The repeatability of developed sensor in terms of relative standard deviation was 3.2%. This sensor was successfully applied for determination of chlordiazepoxide in pharmaceutical formulation and biological fluids samples.

Introduction

Developments of sensitive and selective analytical methods for determination of pharmaceutical compounds in different real complicated matrices in order to various clinical studies and to minimize adverse effects(toxicity symptoms) are highly required.[1]

Chlordiazepoxide (CDP) (7-chloro-N-methyl-5-phenyl-3H-1,4-benzodiazepine-2-amino-4-oxide) is one of the benzodiazepines drugs with medicinal properties such as antianxiety, sedative, anticonvulsant, and muscle relaxant. This compound has high half-life time (>24 h) and therefore, it has a great effect on the actions of other benzodiazepines. Chlordiazepoxide inhibits monosynaptic and polysynaptic reflexes by inactivity neuronal transmitters or by blocking excitatory synaptic transmission.[2,3]

Several analytical methods including spectrophotometry[4-7], different chromatographic techniques (HPLC, LC/MS and GC/MS)[2, 8-13], capillary electrophoresis[14, 15] and electrochemical techniques (potentiometric, polarography and voltammetry)[1, 16-24] were reported for determination of chlordiazepoxide in pharmaceutical formulations and plasma samples.

Among these methods, electrochemical techniques usually provide greater sensitivity, in addition to its other outstanding features such as low cost, easy operation, fast response time and excellent potential for miniaturization and construction for portable equipment applications.[25]

Among the working electrodes in electrochemistry carbon paste electrode (CPE) due to its inherent advantages, such as: ease of electrode preparation and regeneration, stability, good electrical conductivity, broad potential window, low cost and chemical inertness, are widely used in electrochemical measurements mainly for preparation of electrochemical sensors.[26]

CPE usually is prepared by mixing graphite powder with a hydrophobic binder to form a homogeneous paste, followed by filling a tube holder with the resulting paste. However, more binders used in CPE preparation, are usually less or no conducting, which leading to relatively slow electron transfer kinetics and thus decreased detection sensitivity.[27]

The graphene with supreme physicochemical properties such as extremely high surface area, high thermal and electrical conductivity and robust mechanical strength is an excellent candidate to improve the electrochemical performance of CPE[28]. In addition to providing a great domain for analyte binding, this material accelerates electron transfer between electrode surface and probe molecules. Both of these effects result in signal amplification and so more sensitivity in graphene-based electrochemical detection platforms.[29]

However, in addition to sensitivity, the selectivity is one of the most important characteristics in an analytical procedure and is strong incentive to find new materials for modification of working electrodes to develop in electrochemical sensors.[30]

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Molecular imprinting is an attractive approach to create the mimic natural molecular recognition systems via the preparation of synthetic recognition sites in a polymer matrix with predetermined selectivity for various target analytes.[31]
It has also been shown that the preparation of imprinted polymers at nanoscale, leading to a significant increase in efficiency of MIP materials in sensing and separation events through increasing the total surface area per material weight that provide more recognition sites and better accessibility to them.[32] In recent years, numerous articles on the use of MIP nanoparticles in preparation of chemical sensors has been published.[33]
To the best of our knowledge, there are no previous reports on the preparation of electrochemical sensor based on MIP for chlordiazepoxide drug. Actually, so far, chlordiazepoxide not used as a template in molecular imprinting process.
In our previous research[30], we developed an electrochemical sensor based on nanocomposite of MWCNTs–MIP for determination of diazepam (a medication of the benzodiazepine family) drug and in present study we've tried to preparation of new electrochemical sensors based on MIP for chlordiazepoxide (another of benzodiazepine drugs) with improved properties through the preparation of nano sized MIP particles by suspension polymerization method for simple and reliable voltammetric determination of chlordiazepoxide drug.

Experimental

Apparatus

Electrochemical studies were carried out with an Autolab potentiostat/ galvanostat-model of PGSTAT 100 (Echo Chemie, B.V., Netherlands) through a three-electrode system includes modified CPE as working, platinum rod as auxiliary and Ag/AgCl as reference electrodes. Corning - PH meter (Model 140) with a combined glass electrode was used to adjust the solutions pH. Surface morphological images were recorded with a Field Emission Scanning Electron Microscope (FE–SEM), Hitachi, model S–4160.

Materials and solutions

Chlordiazepoxide hydrochloride (Analytical grade) was obtained from Pharmaceutical Research Centre, Mashhad University of Medical Sciences (Mashhad, Iran). Methacrylic acid (MAA), Ethylene glycol dimethacrylate (EDMA), Graphite fine powder (spectroscopic grade, particle size < 50 µm) obtained from Merck (Darmstadt, Germany). 2, 2–azobisisobutyronitrile (AIBN), trimethylolpropane tri-acrylate (TMPTA) and silicone oil were supplied by Sigma–Aldrich (Germany). Other chemicals were analytical grade and purchased from Merck.
Graphene oxide (GO) was prepared from purified natural graphite powder by the Hummers method[34] and then reduced according to method reported by Stankovich et al.[35]
The standard stock solution (1×10^{-3} M) of chlordiazepoxide was prepared by dissolving the appropriate amount of CDP in distilled deionized water and used to preparation of other standard concentrations of CDP by dilution with Britton–Robinson (B.R.) buffer solution to the mark.

Preparation micro and nanoparticles of MIP

CDP imprinted polymer nanoparticles were prepared by suspension polymerization method according to the work reported by T. Alizadeh.[36] Briefly, 0.5 mmol CDP (template), 2 mmol MAA (functional monomer), 10 mmol EGDMA (cross–linker) and 0.05 g AIBN (initiator) were dissolved in 5 mL of acetonitrile. The pre-polymerization mixture was added to the 60 ml treated silicon oil (purged with N_2 for 15 min) and dispersed by stirring at 800 rpm for 5 min. In order to prepare smaller polymerizable droplets, the solution was further mixed by ultrasonic mixer. Next, the suspension was purged with nitrogen for 10 min and heated at 65 ºC for 12 h to complete polymerization. The synthesized polymer particles were filtered and washed with petroleum ether and toluene solvents several times. CDP and unpolymerized components were removed from the polymer matrix by washing with methanol : acetic acid (9 : 1, v/v) and then ethanol : water (9 : 1, v/v)solutions. Finally, the MIP nanoparticles were dried in vacuum at 60°C overnight.
The MIP microparticles, were prepared with similar manner but without adding the silicon oil to polymerization media. The corresponding non-imprinted polymers (NIP) were also formed by following the same procedures, but in absence of template molecule.

Preparation of the sensors

In order to preparation of modified carbon paste electrodes, 0.045 g of graphite powder, 0.01 g graphene and 0.015 g modifier (Nano–MIP, Nano–NIP, MIP, and NIP) were mixed and homogenized in a mortar. The mixture was added to 0.030 g melted n-eicosane (at 45–50 °C) as a binder and was thoroughly mixed. The final paste was tightly packed into the end of a glass holder (2.5 mm, *i.d.*) equipped with a copper wire through the paste to make an electrical connection. The excess of solidified material on the electrode surface was removed by polishing it onto a weighting paper until the surface is shiny appearance and then it was rinsed with distilled water. The electrode can be reused after each experiment through the cut a thin layer of paste and then polish the new surface. The graphene–carbon paste electrode (GCPE) was prepared in a similar manner but with the paste contains 0.060 g of graphite, 0.01 g of graphene, and 0.030 g of n-eicosane. The bare carbon paste electrode (CPE) was also includes 0.070 g graphite and 0.03 g n-eicosane.

General procedure for chlordiazepoxide determination using the modified electrodes

The modified electrode was incubated in CDP solution (pH=3.5) for 10 min under stirring at 500 rpm. After that, the electrode was washed and placed in the electrochemical cell containing 10 mL 0.1 M H_2SO_4 solution (as supporting electrolyte). The square wave voltammogram (SWV) was obtained under the frequency of 30 Hz, pulse amplitude of 40

mV and step potential of 5 mV over the potential range of −0.20 to −0.50 V versus Ag/AgCl. The results are reported based on triplicate analysis and the average of reduction peaks height was used for construction of calibration curve. All measurements were conducted at room temperature.

Determination of chlordiazepoxide in real samples

Tablet

For determination of CDP in pharmaceutical formulations, ten tablets containing 5 mg of chlordiazepoxide (per tablet) were weighed accurately and crushed into a fine powder. A sufficient amount of powder for preparing a stock solution of 1.0×10^{-4} mol L^{-1} CDP was weighed and transferred into a 25 mL volumetric flask contained 20 mL ethanol. The content of the flask was sonicated for 10 min and then diluted to mark with the same solvent. The solution was next filtrated and desired concentrations of drug were obtained by accurate dilutions with B.R. buffer at pH= 3.5. Finally, these samples were analyzed according to the proposed method.

Biological fluids

For CDP assay in human blood plasma, an aliquot of CDP standard stock solutions were fortified with chlordiazepoxide free human serum samples (obtained from a local pathology laboratory). One milliliter of each of these solutions was diluted to 1.5 ml volume, with ethanol (as serum protein precipitating agent) in a 2.0 ml volume centrifuge tube. After vortexing for 30 s, the precipitated proteins were collected by centrifugation for 5 min at 14,000 rpm. An aliquot of the clear supernatant was transferred to 10 mL volumetric flasks and diluted with B.R. buffer solution (pH = 3.5) to operate in linear range of the proposed method.

Finally, in order to measurement of CDP in urine samples that collected from healthy volunteers (Informed consents were obtained prior to the urine sampling), in four centrifuge tubes, 1.0 ml of urine sample was spiked with an appropriate volume of CDP standard solution and then centrifuged at 14,000 rpm for 5 min. Then, 0.5mL of each sample was transferred to 10 mL volumetric flask and diluted with B.R. buffer solution (pH = 3.5). The chlordiazepoxide content of both biological real samples was determined by recommended procedure.

All experiments were performed in compliance with the relevant laws and institutional guidelines and were approved by local research ethics committee.

Results and discussion

Surface characterization of graphene nanosheets, microparticles and nanoparticles of MIP

Scanning electron microscopy images of graphene nanosheets and also obtained MIP particles, by both precipitation and suspension polymerization techniques are shown in Fig.1.

SEM images revealed that the synthesized graphene consists of thin and crumpled sheets that randomly associated with each other (Fig. 1I). The average thickness of the graphene sheets was obtained about 18 nm (Fig. 1II).

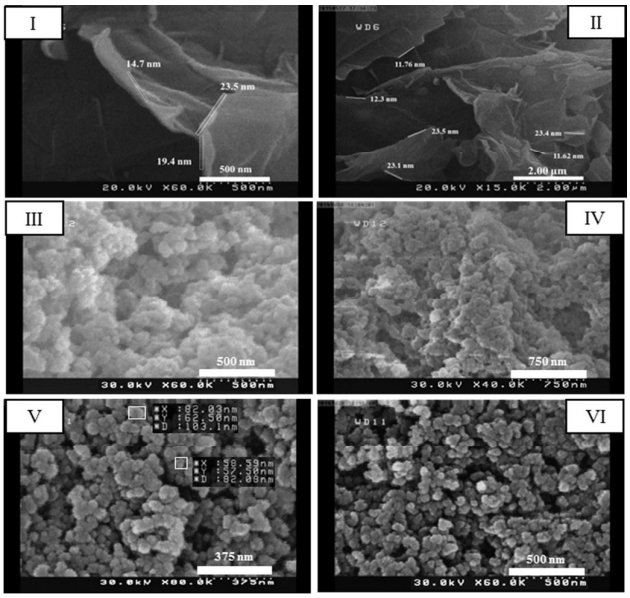

Fig. 1 Scanning electron microscopy images of graphene nanosheets (I & II), microparticles(III & IV) and nanoparticles (V – VI) of MIP.

Also, as can be seen in Fig.1, MIP obtained from the precipitation method, have a micro−sized dimension (Fig.1, III, and IV) whereas, suspension polymerization in silicon oil leading to production of MIP nanoparticles with particles size in the range of 50 – 100 nm (Fig. 1, V and VI).

Electrochemical behavior of chlordiazepoxide

The preliminary cyclic voltammetry experiment was carried out to study voltammetric behavior of 5.0×10^{-5} M CDP in H_2SO_4 0.1 M at the CP and GCP electrodes in potential range of 0 to -1.0 V (vs. Ag/AgCl). The voltammograms obtained for CDP(Fig. 2 A) exhibited two irreversible reduction waves in –0.49 V and – 0.68 V at CPE and in –0.42 V and – 0.60 V at GCPE surfaces which the first peak has been attributed to $2e^-$, $2H^+$ reduction of N-oxide group in position 4 and the second one was related to $2e^-$, $2H^+$ reduction of 4,5- azomethine group[1, 17, 18, 23].

As can be seen in figure 2A, using of graphene in preparation of CPE, in addition to peak shift toward more positive values, leading to a significant increase in intensity of voltammetric signals that this is due to unique properties of graphene that previously mentioned. Thus, graphene was used for preparation of CPE.

order to verify CDP recognition ability of MIP-based sensors and also, evaluate the effect of particle size on improving the sensors response, bare GCP and GCP electrodes modified with nano and micro particles of MIP (NIP) were prepared and then each of them, incubated in 1.0×10^{-6} mol L^{-1} CDP solution at pH 4.5 for 10 min under stirring. Next, the electrodes were washed with B.R. buffer (pH = 4.5) to remove any weakly adsorption of analyte and then, were transferred into an electrochemical cell containing H_2SO_4 0.1M.

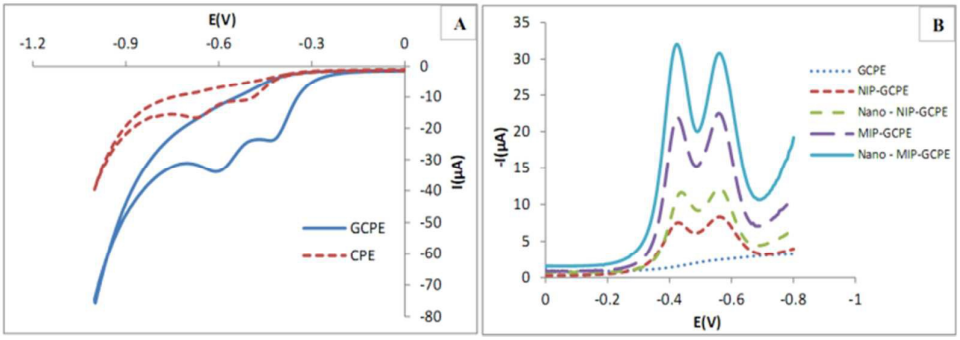

Fig. 2 (A) Cyclic voltammetry behaviour of 5.0×10^{-5} M CDP solution on the CPE and GCPE. **(B)** Square wave voltammetric signals of GCP and modified GCP electrodes after 10 min extraction from 1.0×10^{-6} M CDP solutions (pH= 4.5). (The SW conditions: ΔE= 20 mV, Vstep= 5 mV, tstep = 0.3 s, Frequency = 20 HZ)

The amount of adsorbed CDP was evaluated by SWV method. As can be seen in Fig. 2B, there was no significant signal for bare GCP electrode while the current obtained with MIPs–GCP is noticeably higher than that obtained for NIPs–GCP indicating non-selective rebinding of CDP with improper sites in NIPs that could be removed from the surface of sensor during washing process before determination, whilst in MIPs based sensors, most of adsorbed CDP molecules were trapped in imprinted sites and strongly bonded to these recognition sites through the hydrogen bonding and not be removed easily during washing process[31].

Also, the signal of Nano–MIP–GCPE is higher than that of MIP–GCP electrode. These observations show that the adsorption capability of MIP nanoparticles for CDP is considerably higher than that of micro–sized MIP.

In MIP nanoparticles, the majority of imprinted cavities are located at surface or approximately near the surface of polymer particles that leads to higher binding capacity for MIP nanoparticles and also enhances affinity of imprinted sites to target molecules. Thus, proposed sensor was fabricated by using of MIP nanoparticles as selective recognition elements.

Optimization parameters affecting the response of sensor

After securing the initial response of sensor to CDP, factors affecting the performance of sensor such as components of synthesized Nano–MIP and variables involved in extraction and analysis of CDP were optimized. A 5.0×10^{-7} mol L^{-1} CDP solution was used for extraction step in optimization process. Also, the first CDP reduction signal was selected for further studies.

Effect of crosslinker type and amount of template molecule on MIP nanoparticles properties

Cross-linker is primarily responsible for improvement of mechanical and thermal stability of polymer, yield of polymer matrix, stabilizes selective recognition sites, and providing adequate porosity to ensure accessibility of analyte to cavities.[37]

Also, a suitable ratio between components of polymerization mixture plays a key role on mentioned characteristics of imprinted polymer materials.

In order to obtain the good recognition characteristic, four different MIP nanoparticles were synthesized according to methods mentioned in experimental section using methacrylic acid (MAA) as a functional monomer, a cross-linking agent (ethylene glycol dimethacrylate (EGDMA) for MIP_1 and trimethylolpropane tri-acrylate (TMPTA) for MIP_2) with two different amount of CDP. The obtained polymers were used for fabrication of modified GCP electrodes and after extraction and washing steps; the response of sensors was evaluated by SWV method. The results (table 1) showed that the MIP nanoparticles prepared with TMPTA as cross-linker, have greater rebinding ability to CDP. Also, optimum molar ratio between template molecules, functional monomers, and cross-linkers were 1:4:20. The SWV signals decreased at higher molar ratio, apparently due to highly agglomeration of Nano–MIP particles that lead to a poor accessibility of target molecules to recognition sites. Therefore, Nano–MIP_{2a} was chosen to preparation of sensor in this work.

Table 1 Effect of crosslinker type and amount of template molecule used for preparation of MIP nanoparticle, on SWV signal of modified CPE.(n=3)

Polymers	Template (CDP) (mmol)	Monomer (MAA) (mmol)	Cross-linker (EGDMA) (mmol)	Cross-linker (TRIM) (mmol)	Initiator (g)	I(μA)
Nano-MIP_{1a}	0.5	2	10	-	0.05	16.34±0.81
Nano-MIP_{1b}	0.4	2	10	-	0.05	14.32±0.60
Nano-MIP_{2a}	0.5	2	-	10	0.05	21.71±0.65
Nano-MIP_{2b}	0.4	2	-	10	0.05	18.67±0.87

Optimization of Nano–MIP–GCPE composition

To achieve the best composition for Nano–MIP–GCP electrodes, the amounts of electrode components include Nano–MIP, graphite, graphene and n–eicosane were optimized. For this purpose, at first, amounts of graphite and Nano–MIP are fixed (0.045 and 0.015 g respectively) and the mass ratio of n-eicosane to graphene is changed. The prepared electrodes were used for CDP extraction and analysis. According to obtained results, best sensor response was obtained in a mass ratio of 2.64 (the sensor with 0.029 g n-eicosane and 0.011g graphene). The higher amounts of n-eicosane lead to a decrease in reduction current response due to increasing of electrode resistance. In addition, although the electrode signal is increased with increasing of graphene, too much amount of graphene lead to undesirable mechanical properties of CPE which correspondingly decreased the rate of electron transfer. Accordingly, the mass ratio of 2.64 (for n-eicosane to graphene) was selected as optimized value for preparation of modified CPE. Thereafter, the Nano–MIP based sensors were prepared in different mass ratios of Nano–MIP to graphite powder at optimized amounts of n-eicosane and graphene. The obtained results showed that with increasing Nano–MIP content, the reduction peak current is gradually increased up to mass ratio of 0.36, because of an increase in the number of imprinted sites on the electrode surface. However, response of sensor is decreased at higher mass ratios might be due to decrease in graphite content and consequently decrease in conductivity of sensor. Thus, sensor with mass percentages of 44.0, 11.0, 16.0 and 29.0 % respectively for graphite, graphene, Nano–MIP, and n–eicosane, has the best answer to CDP and used for other studies.

Effects of extraction and analysis pH

The dependence of sensor response to extraction solution pH was evaluated by incubating the Nano–MIP–GCPE with optimized composition, into 5.0×10^{-7} M CDP solutions with different pH values (2.0 to 7.0). The sensor was then washed and SWV signal was recorded. As shown in Fig. 3A, reduction current increased with increasing pH up to 3.5 and then decreased gradually. By consideration pKa values of MAA (pKa = 4.7), and CDP (pKa = 4.8), the carboxylic groups situated on the polymer are ionized at pH values higher than pKa of MAA and do not interact with CDP. Also at very low pH (< 3.5), CDP exists in cationic form which is not a favorable species for interaction with imprinted cavities in polymer, and the extraction amount of CDP is reduced.[31] Thus, the pH of 3.5, fixed by B.R. buffer, was selected as optimum for CDP extraction step in developed method.

In addition to extraction pH, effect of analysis solution pH on the Nano–MIP based sensor response at pH range from 2.0 to 7.0 was investigated and the results are illustrated in Fig 3B. As can be seen, the cathodic peak current is dependent to pH and decreased with increasing pH.

Also, the plot of peak potential against pH (Fig. 3C) shown that the reduction peak potential shifted toward more negative values upon increasing of pH that indicated the participation of proton in reduction process and higher pH values are unfavorable for CDP reduction.

Furthermore, this plot shows two linear portions, with only one break at 4.3 which is a little lower than pK_a of CDP molecule determined by potentiometry (4.79) and spectrophotometry (4.82) methods.[38] With respect to dependence of CDP reduction mechanism to pH and this fact that the acidic pH is more appropriate to CDP reduction, in order to obtain high electrochemical responses of sensor,

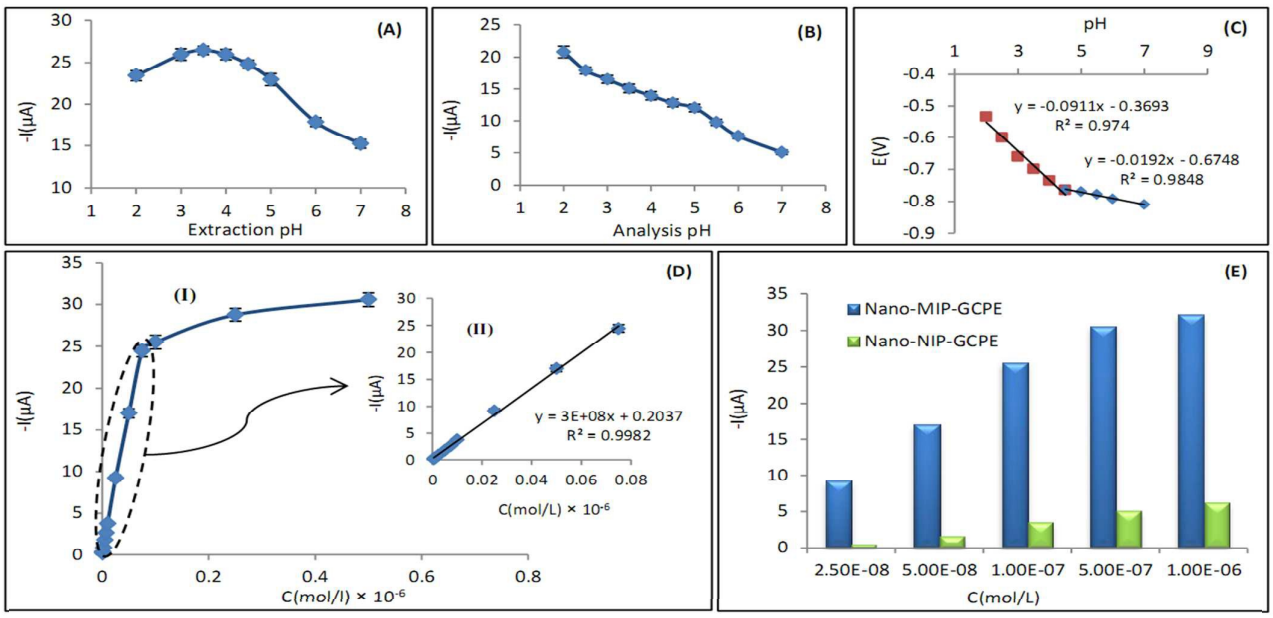

Fig. 3 Effect of Extraction pH**(A)**, and Analysis pH**(B & C)**, on the SWV response of modified CPE after extraction from 5.0×10^{-7} M CDP solution. **(D)** The calibration curve (I) and linear range related to it (II), obtained for developed method. **(E)** Comparison of SWV responses obtained at nano–MIP and nano–NIP based sensors under optimum conditions.

H_2SO_4 0.1 M was used as supporting electrolyte.

Optimization of extraction time and stirring rate of sample solution

The results of evaluation the effect of extraction time and stirring rate on the CDP extraction showed that with an increase of extraction time and stirring rate of extraction solution until about 9 min and 500 rpm respectively, the CDP SWV signal increased noticeably and longer extraction times and higher stirring speed did not significant affect on the CDP extraction. Therefore, these values were selected as the optimal.

Electrochemical condition optimization

Finally, dependent of CDP reduction signal on the two important square wave voltammetry parameters including pulse amplitude (in the range of 30 – 100 mv) and square wave frequency (in the range of 10 – 80 Hz), was studied. The obtained results showed that the best response with highest ratio of CDP reduction peak height to peak width was obtained for pulse amplitude of 40 mV and frequency of 30 Hz. Therefore, these values were selected for other studies.

Analytical characteristics

The developed sensor at optimized conditions was used for determination of CDP at various concentrations for plotting of calibration curve. The calibration graph (Fig. 3D) showed a linear relationship with CDP concentration in the range of 6.0×10^{-10} to 7.5×10^{-8} M ($R^2 = 0.9982$).

The calculated limits of detection (LOD) and quantification (LOQ) for Nano–MIP based sensor were 2.61×10^{-10} and 8.69×10^{-10} M respectively, according to relation of kS_b /m where k = 3 for LOD and 10 for LOQ. The S_b represented the standard deviation of peak current for blank solution (n = 7) and m is slope of calibration curve.

The repeatability (intra-day) of proposed sensor was examined for five measuring of 7.5×10^{-9} M CDP solution and relative standard deviation (%RSD) was obtained 3.2%. For each measurement electrode surface was renewed.

The reproducibility (intra-day) of the proposed method was evaluated through measurement of 7.5×10^{-9} M CDP solution by five different electrodes and the value of %RSD was obtained 4.1%.

Also, determination of 7.5×10^{-9} M CDP solution after 6 weeks with same sensor, showed that the response of sensor remained up to 95.2% (RSD=4.07%, n=5) of its initial value which indicates that the Nano–MIP based sensor has excellent stability.

Another study that is conducted, comparison of Nano–NIP and Nano–MIP based sensors under optimal conditions in various CDP concentrations. As can be seen in Fig. 3E, Nano–MIP based sensor shows a much higher voltammetric response to CDP compared to Nano–NIP based sensor.

To assess the selectivity of proposed sensor, interference of some ions and molecules that exist in biological fluids was evaluated. The interference level was considered as the error of 5% in determination of 7.5×10^{-9} M CDP solution by aimed interfering compounds. Also, the interference influence of compounds with a similar structure to CDP (i.e. alprazolam, oxazepam and diazepam) was investigated. The results are listed in Table 2 and show that except for compounds with similar structures, the performance of developed sensor did not significantly affected by presence of various species studied. This should be ascribed to the effect of rigid imprinted cavities formed in Nano–MIP matrix which enhances the selectivity of sensor to CDP.

Table 2 Tolerance limit with respect to CDP for some interfering substances and ions using Nano–MIP based sensor.

Interferents	Tolerance limit (mol ratio)
Li^+, Na^+, K^+	2000
Mg^{2+},Ca^{2+}	1500
CO_3^{2-}, HCO_3	1000
Cl^-	2000
SO_4^{2-}	800
Urea	200
Dopamine	200
Glucose	180
Uric acid	50
Ascorbic acid	70
Alprazolam	10
Diazepam	7
Oxazepam	6

Analysis of real samples

The usefulness of proposed Nano–MIP sensor was evaluated by determination of CDP in three real samples includes: CDP tablets, human serum, and urine.

The real samples were spiked with CDP standard solutions at certain concentration and were accomplished according to procedures described in experimental section. The results are summarized in Table 3. The obtained recovery values showed that the Nano–MIP based sensor have a great ability to assay of CDP in complex matrices and the real samples matrices did not considerable interference in determination of CDP.

Therapeutic and toxic concentrations range of CDP in serum are 0.7 – 2.0 mg/L (2.3 – 6.7 μM) and 3.5 – 10.0 mg/L (11.7 – 33.4 μM) respectively which is much higher than detectable concentrations by proposed method and indicates that the proposed method is sensitive enough for determination of CDP in biological samples.[39]

Comparison of developed sensor with other electrodes

In Table 4, performance of Nano–MIP based sensor is compared with other electrodes reported for CDP determination. As it is obvious, the performance of proposed sensor is superior to other reported electrodes in terms of linear range, detection limit and biomimetic material used in construction of sensor.

Table 3 Results of CDP determination in real samples (n=3)

Sample	Added (nmol L^{-1})	Detected (nmol L^{-1})	Recovery (%)	RSD %
Tablet	0	9.95	99.5	4.0
	20.0	30.1	100.3	4.5
	40.0	48.97	97.9	3.8
	60.0	68.61	98.0	2.9
Serum	–	–	–	–
	1.0	0.94	93.8	3.4
	5.0	4.79	95.7	4.2
	25.0	23.51	94.0	4.6
Urine	–	–	–	–
	2.0	1.95	97.3	4.4
	10.0	9.82	98.2	3.5

Conclusions

In this article, a selective and sensitive modified CPE with graphene nanosheets and MIP nanoparticles was fabricated for voltammetric determination of chlordiazepoxide at very low concentrations. The use of graphene in preparation of CPE leads to a dramatically improvement in performance of CPE due to excellent electrical conductivity and large surface area of graphene. Incorporating of MIP nanoparticles as recognition element in proposed sensor leads to high selectivity and sensitivity towards chlordiazepoxide. Also, the results indicate that the use of TMPTA as crosslinker leading to more effective rebinding ability and so higher yield of MIP nanoparticles than EGDMA. The proposed sensor was used successfully for chlordiazepoxide determination in pharmaceutical formulation and biological fluids.

Acknowledgements

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Table 4 Comparison of some characteristics of developed sensor and other electrodes reported for CDP determination.

Electrode	Technique	Linear range (mol L^{-1})	Detection limit (mol L^{-1})	Real samples	References
HMDE[a]	DPAdSV	$8.0\times10^{-9}-11.0\times10^{-8}$	0.9×10^{-9}	Human serum	40
Sonogel–Carbon	SWAdSV	$1.13\times10^{-7}-1.01\times10^{-6}$	1.67×10^{-8}	Tablet Urine	1
HMDE	SWAdSV	$5\times10^{-9}-2\times10^{-7}$ M	4.4×10^{-10}	Tablet Human serum	17
DME[b]	LSP[d]	$3.20\times10^{-8}-1.60\times10^{-7}$ $1.60\times10^{-7}-1.44\times10^{-6}$ $1.44\times10^{-6}-1.44\times10^{-5}$	9.0×10^{-9}	Tablet	20
GCE[c]	CAdSV[e]	$2\times10^{-7}-5\times10^{-6}$	5.0×10^{-8}	Tablet	18
Nano-MIP-GCPE	SWV	$6.0\times10^{-10}-7.5\times10^{-8}$	2.61×10^{-10}	Tablet Human serum Urine	This work

[a] Hanging mercury drop electrode
[b] Dropping mercury electrode
[c] Glassy carbon electrode
[d] Linear-sweep polarography
[e] Linear-sweep Cathodic adsorptive stripping voltammetry

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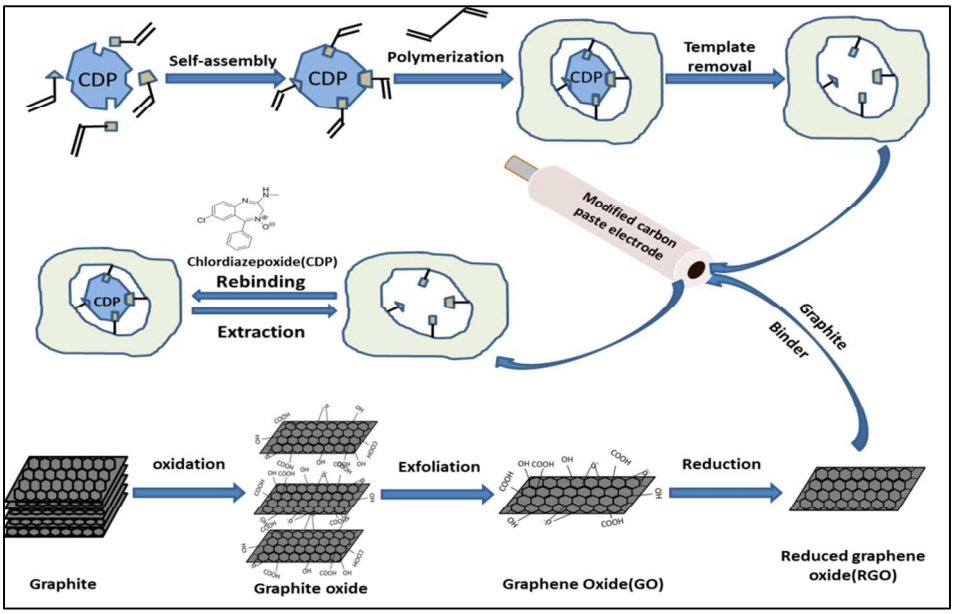
Graphical abstract



In this study, we introduce a Molecularly imprinted polymer for chlordiazepoxide(CDP) drug with improved properties through the preparation of MIP particles at nano-dimension and its use for modification of Graphene-carbon paste electrode to preparation of new electrochemical sensors for selective and sensitive determination of CDP.