Sensors and Actuators B 259 (2018) 133–141

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Fabrication of a new electrochemical imprinted sensor for determination of ketamine based on modified polytyramine/sol-gel/f-MWCNTs@AuNPs nanocomposite/pencil graphite electrode

Behjat Deiminiat, Gholam Hossein Rounaghi *

Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

ARTICLE INFO

Article history:
Received 8 November 2017
Accepted 11 December 2017
Available online 12 December 2017

Keywords:
Ketamine
Molecularly imprinted polymer
Polytyramine
Sol-gel
f-MWCNTs @AuNPs nanocomposite

ABSTRACT

This paper proposes a new strategy for measurement of ketamine through one-step electropolymerization of the molecularly imprinted polymer (MIP) composed of polytyramine (pty), sol-gel, functionalized multiwall carbon nanotubes@gold nanoparticles (f-MWCNTs@AuNPs) nanocomposite and ketamine on a pencil graphite electrode (PGE) by cyclic voltammetric (CV) technique. F-MWCNTs@AuNPs nanocomposite was entrapped within the polymer network to enhance the electron transfer rate and sensitivity of the fabricated sensor. The f-MWCNTs@AuNPs nanocomposite was synthesized by the chemical reduction method and its structure characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDX). The electrochemical behavior of the modified electrode was investigated using cyclic voltammetry, electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV) techniques. Under the optimized experimental conditions, the calibration curve of the MIP electrode was plotted and two dynamic linear ranges from 1.0 to 50.0 nM and 50.0–1000.0 nM, with a limit of detection (LOD) of 0.7 nM were obtained for quantitative measurement of ketamine in solution. The obtained results show that the proposed sensor has an excellent stability, good reproducibility and repeatability, and high sensitivity and selectivity towards the ketamine molecules. Furthermore, the electrochemical imprinted sensor was successfully applied for determination of ketamine in biological samples with satisfactory results.

© 2017 Published by Elsevier B.V.

1. Introduction

Ketamine, (±)-2-(2-chlorophenyl)-2-(methylamino) cyclohexanone, which is a phencyclidine analogue is mainly used as an anesthetic drug that stimulate *N*-methyl-D- aspartate (NMDA) receptors on neuronal cells. It induces loss of consciousness, amnesia, immobility, pain relief and sedation while the cardiopulmonary functions and the protective airway reflexes remain unchanged [1]. Furthermore, ketamine is a popularly abused drug among teenagers at nightclubs and recreational parties due to its stimulant, dissociative and hallucinogenic effects [2]. The overdose of ketamine has been found to cause a variety of side effects such as dysfunctions of the nervous, urinary and cardiovascular systems [3]. Therefore, the identification and quantification of ketamine in biological fluids and pharmaceutical samples is of significant importance for clinical purposes, drug seizing and also controlling the industrial processes.

To date, various analytical techniques such as UV–vis spectrophotometry [4], gas chromatography [5–8], liquid chromatography [9–11] and micellar electrokinetic chromatography (MEC) [12,13] have been proposed in the literature for the determination of ketamine. However, most of these techniques are usually complicated, time consuming and they require the pretreatment step. Compared to these instrumental methods, the electrochemical measurement of ketamine is preferred due to its simplicity, high sensitivity, low cost instrumentation and fast response. However, until now, a few papers have reported the determination of ketamine based on electrochemical techniques [14–16].

Molecularly imprinted polymers (MIPs) are artificially synthesized receptors with specific molecular recognition capacity that have been extensively used in the construction of chemical sensors [17–20]. Although the use of MIPs as the recognition elements improves the selectivity, some limitations such as long response time, heterogeneous distribution of the binding sites, slow diffusion of the target molecule across the imprinted film and low sensitivity still exist in their applications to be surmounted [21]. Among the

* Corresponding author.
E-mail addresses: ghrounaghi@yahoo.com, ronaghi@um.ac.ir (G.H. Rounaghi).

https://doi.org/10.1016/j.snb.2017.12.062
0925-4005/© 2017 Published by Elsevier B.V.

various developed methods for the preparation of imprinting films, sol-gel imprinting has received increasing attention.

In sol–gel imprinting process a three dimensional silicate network is formed around a template through covalently/non-covalently interactions between a series of silane monomers and the template. The sol–gel MIP films have some specific advantages over conventional MIPs, such as easy preparation, mild reaction condition, physical rigidity, chemical inertness, thermal stability, excellent permeability and high porosity [22,23]. However, the low electrical conductivity of the sol–gel imprinted polymers is the main drawback of this technique. An excellent design for improvement the electrical properties of the sol–gel MIP based sensors, is the entrapment of conducting nanomaterials in the polymer network during the electrochemical deposition.

Among all kinds of the nanomaterials, carbon nanotubes (CNTs) and gold nanoparticles (AuNPs) with the unique properties such as high electrical conductivity, large specific surface area and good biocompatibility are very attractive materials for construction of electrochemical sensors and biosensors [24–27]. In order to enhance further the features of these nanomaterials, several methods have been developed to synthesize CNTs@AuNPs nanocomposite in which carbon nanotubes surface is decorated with AuNPs [28–30]. The incorporation of carbon nanotubes and gold nanoparticles not only prevent the aggregation of the Au nanoparticles, but also improves the electrochemical performance of the modified electrodes.

Tyramine (Ty), 4-(2-aminoethyl) phenol, one of the phenol derivatives, was chosen as an additional monomer of electropolymerization for the fabrication of a strongly adhering polymer film on the surface of the electrode. Recently, tyramine has been considered as an ideal monomer for development of biosensors [31–33] due to the presence of a free amine group which covalently attaches the bio-recognition molecules. The structure of the polytyramine (pty) film which deposited electrochemically on different substrates, has been investigated and it has been found that only the phenol moiety participates in polymerization process [34,35].

Recently, pencil graphite has been employed as a new electrode material in electrochemical sensing filed due to its availability, low cost, disposability, ease of surface modification, wide potential window and low background current. There are also different methods for pretreatment of pencil graphite electrode (PGE) which increase the electrode surface area and enhance the electron transfer rate [36,37].

The aim of the present work, is to develop a new sensitive and selective imprinted sensor for determination of ketamine by using the polytyramine and f-MWCNTs@AuNPs nanocomposite into the sol–gel network. To our knowledge, no report has been published to date on the application of a polytyramine, sol–gel and f-MWCNTs@AuNPs hybrid nanocomposite as an electrochemical imprinted sensor. The use of f-MWCNTs@AuNPs nanocomposite and polytyramine along with sol–gel MIP technique is expected to improve the stability, sensitivity and selectivity of the proposed sensor. The electrochemical characteristics of the imprinted sensor were investigated under optimized experimental conditions and it was used successfully for measurement of ketamine in real samples.

2. Experimental

2.1. Materials

Tetraethoxysilane (TEOS), phenyltriethoxysilane (PTEOS), trifluoroaceticacid (TFA), sodium dodecyl sulfate (SDS) and tetrakis (hydroxymethyl) phosphonium chloride (THPC) were purchased from Merck (Darmstadt, Germany) and tyramine was obtained from Acros (New Jersey, USA). MWCNTs (>95%, OD 5–15 nm) were purchased from US research nanomaterials, Houston, USA. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$) and ketamine were supplied from Sigma-Aldrich (USA). All other chemicals used in this study were obtained from Merck (Darmstadt, Germany).

2.2. Apparatus

An Autolab potentiostat/galvanostat model PGSTAT 101 (Netherlands) coupled with NOVA software was used for the voltammetric measurements, polymer deposition and pretreatment of the electrode surface. The electrochemical impedance spectroscopic measurements were performed using a Gill AC potentioastate (ACM instruments). All measurements were carried out in a three-electrode cell consisted of a pencil graphite working electrode (Rotring, 2B, 0.7 mm diameter, Germany), an Ag/AgCl reference electrode and a platinum wire counter electrode. The pH of the solutions was adjusted with Metrohm pH meter (model 827). The surface morphologies of the fabricated electrodes, were specified using a LEO 1450VP scanning electron microscope (SEM). The transmission electron microscope (TEM) image was taken using a LEO 912 AB transmission electron microscope. The surface elemental analysis was performed by the energy dispersive X–ray (EDX) technique using Oxford-7353 EDX microanalyzer. The X-ray diffraction (XRD) patterns were obtained by an X-ray diffractometer (Philips analytical X-ray).

2.3. Synthesis of f-MWCNTs@AuNPs nanocomposite

F-MWCNT@AuNPs nanocomposite was synthesized according to the procedure developed by our research group in a previous study [38]. In this way, a 100 mL flask was charged with 0.3 g of crude MWCNTs and 50 mL of HNO_3, and the resulting mixture was homogenized by ultrasonication. Then, it was refluxed at 120 °C under vigorous stirring for 24 h. After cooling to room temperature, the mixture was filtered and washed repeatedly with deionized water and finally dried under vacuum for 12 h. Subsequently, in order to prepare the colloidal gold nanoparticles, 1 mL of THPC solution and 0.5 mL of 1 M NaOH were added to 50 mL of HPLC grade water. After being stirred for a few minutes, the above solution was mixed with 1 mL of 1% $HAuCl_4$, and it was stirred for another 30 min. The resulting solution was kept in a refrigerator for at least 3 days. In the next step, the prepared colloidal gold nanoparticles were attached to the f-MWCNTs surface. For this purpose, 45 mL of gold nanoparticles solution was added to 6 mL of f-MWCNTs suspension. The mixture was gently stirred for 3 h and then centrifuged at 10,000 rpm. In order to grow the gold nanoparticles on the f-MWCNTs@AuNPs nanocomposite, 37.5 mg of potassium carbonate was dissolved in 150 mL of HPLC grade water. Then, 3 mL of 1% $HAuCl_4$ and 15 mL of f-MWCNTs@AuNPs suspension were added to the solution while it was stirred vigorously. To this solution, 225 μL of formaldehyde was injected slowly and it was stirred for 30 min while the temperature was kept at 65 °C. Then, the solution was left to cool to room temperature and it was centrifuged and redispersed in HPLC grade water to remove the residual reactants. The resulted black precipitate, was collected and dried under vacuum at 60 °C for 24 h to obtain f-MWCNTs@AuNPs nanocomposite.

2.4. Preparation of poltyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE

Prior to electrodeposition, the electrochemical treatment of the surface of PGE was performed in a 0.5 M acetate buffer solution (pH 4.8) containing 0.02 M sodium chloride under the potential of +1.4 V for a period of 300 s. The sol solution was prepared by mixing 75 μL of PTEOS, 75 μL of TEOS, 700 μL of water, 1100 μL

of ethanol and 10 μL of TFA with ketamine (2.0 mM) in a vial and stirring the mixture for 2 h at room temperature. Then, 8.0 mg of tyramine, 5.0 mg of SDS and 50 μL of 1.0 mg mL^{-1} suspension of f-MWCNTs@ AuNPs nanocomposite in dimethylformamide (DMF) were added to the homogeneous sol solution. The resulting solution was sonicated for 10 min. The imprinted film was then deposited on the pretreated electrode surface using cyclic voltammetry at the potential range between −0.8 and +1.2 V during 10 cycles with a scan rate of 50 mV s^{-1}. After the electropolymerization process, the prepared polytyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE was left to dry at room temperature (25 °C) for 2 h and then it was immersed in methanol-acetic acid (9:1, v/v) solution for 20 min to remove the template from polymeric network. The complete removal of the template from the electropolymerized film was verified electrochemically. For comparison, a non-imprinted polymer (NIP) electrode was prepared under the same condition without the presence of ketamine in the solution.

2.5. Electrochemical measurements

For electrochemical measurements, the imprinted sensor was first incubated into a phosphate buffer solution (PBS, pH 5.0) containing specific concentration of ketamine for 420 s to capture the analyte on the surface of the sensor. After that, the sensor was rinsed with double distilled water and electrochemical measurements were carried out in the presence of 0.1 M KCl and 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe solution. The cyclic voltammetry (CV) measurements were performed by cycling the electrode potential between −0.1 and 0.5 V at a scan rate of 50 mVs^{-1}. The electrochemical impedance spectroscopy experiments were carried out under the potential of 0.2 V, amplitude of 10 mV and in the frequency range of 0.1–30,000 Hz. The square wave voltammograms (SWVs) were recorded in the potential scan range from −0.10 to 0.50 V and with the amplitude, frequency and step potential of 20 mV, 15 Hz and 1 mV, respectively. All of the electrochemical measurements were conducted at room temperature.

2.6. Sample preparation

Human plasma sample was taken from a healthy volunteer. 2.0 mL of methanol was added to 2.0 mL of plasma sample to separate the proteins. After shaking, the mixture was centrifuged for 10 min at 3500 rpm, and then the supernatant was diluted with appropriate amount of PBS and spiked with standard ketamin solution.

Drug-free urine sample was collected in a sterile bottle and stored at 4 °C. The sample was centrifuged for 10 min at 3500 rpm and the supernatant was filtered and diluted with phosphate buffer solution. Then, different concentrations of ketamine were spiked into the sample and the standard addition method was used to obtain the electrochemical results.

3. Results and discussion

3.1. Characterization of the synthesized f-MWCNTs@AuNPs nanocomposite

Transmission electron microscopy (TEM), energy dispersive X-ray analysis (EDX) and X-ray diffraction analysis (XRD) were utilized for characterization of the f-MWCNTs@AuNPs nanocomposite. Fig. 1A, shows the TEM image of a single strand of f-MWCNTs@AuNPs nanocomposite. As is evident in this Figure, the AuNPs with the size about 12 nm have been successfully attached onto the f-MWCNTs surface and show no tendency for aggregation at the surface of the f-MWCNTs. The presence of Au and C at

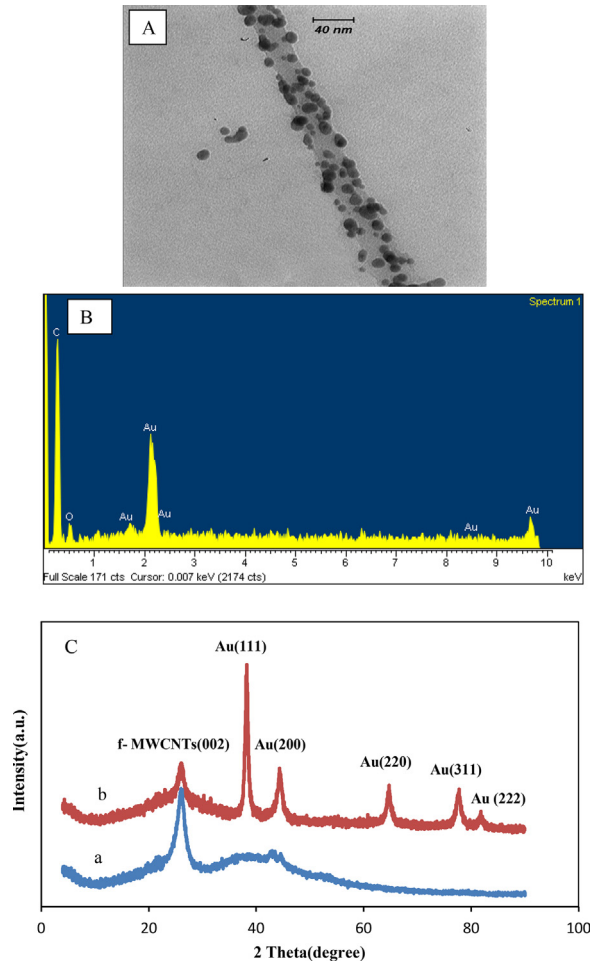

Fig. 1. A) Transmission electron microscope (TEM) image of f-MWCNTs@AuNPs nanocomposite, B) energy dispersive X-ray analysis (EDX) spectrum of f-MWCNTs@AuNPs nanocomposite and C) XRD patterns of (a) f-MWCNTs and (b) f-MWCNTs@AuNPs nanocomposite.

the chemical composition of the nanocomposite was investigated using the energy dispersion X-ray analysis (Fig. 1B). Fig. 1C, shows the XRD patterns of f-MWCNTs and f-MWCNTs@AuNPs nanocomposite. The presence of the (111), (200), (220), (311) and (222) crystalline plane diffraction peaks of gold in the XRD pattern of nanocomposite, confirms the formation of gold nanoparticles on the surface of the f-MWCNTs.

3.2. Preparation and characterization of imprinted sensor

The most important requirement in molecularly imprinting process is the existence of different type of interactions between the target molecule and functional monomers. Therefore, selection of suitable functional monomers for successful imprinting is necessary. In this paper, TEOS, PTEOS and tyramine were chosen as the monomers for preparation of so-gel MIP film for the first time. TEOS is a silane monomer that accelerates the condensation process. It was utilized as cross-linker to create a polymeric network around the target molecule via hydrogen bonds and ionic interactions. PTEOS was used as functional monomer because of its phenyl group, to interact with the aromatic ring of the template through π–π interaction. In order to increase the stability of the polymer, tyramine functional monomer was also incorporated into the sol solution. The free amine group of the tyramine can form hydrogen bonds with the C=O and —N—H groups of ketamine molecules. The combination of tyramine and silane monomers results in the

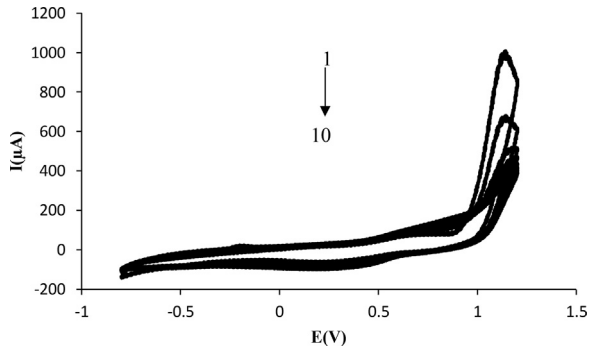

Fig. 2. Cyclic voltammograms for electropolymerization of the imprinted sol–gel on the PGE surface. Conditions: Scan rate 50 mV s^{-1}, number of scans10, potential range −0.8 to 1.2 V, ketamine concentration 2.0 mM.

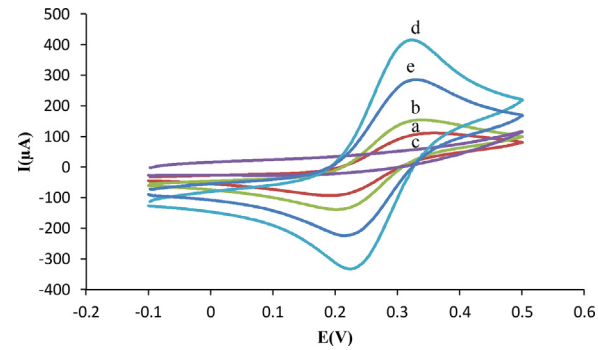

Fig. 4. Cyclic voltammograms (CVs) of (a) bare PGE, (b) treated PGE, (c) MIP/PGE, (d) MIP/PGE after removal of ketamine and (e) MIP/PGE after 420 s incubating in 20.0 nM ketamine solution. Conditions: Potential scan range −0.1 V to +0.5 V, Scan rate 50 mVs^{-1}.

preparation of a new MIP that possesses the properties of both these compounds.

The electrochemical entrapment of the ketamine and f-MWCNTs@AuNPs nanocomposite into the polymer network was performed by cyclic voltammetric technique and the corresponding voltammograms, are shown in Fig. 2. During the electrochemical polymerization, the ketamine molecules with several functional groups, interact with the functional monomers and entrap in the polymer matrix. The f-MWCNTs@AuNPs nanocomposite increases the electron transfer rate from sol-gel MIP film to the electrode surface which may be attributed to the high conductivity and large specific surface area of the f-MWCNTs@AuNPs nanocomposite. As can be seen in Fig. 2, an irreversible anodic peak appears at potential of 1.1 V which is attributed to the oxidation of the tyramine. During the next cycles, the peak current decreases significantly with increasing the number of scan cycles from the 1 st to the 10th, which indicates the covering of the electrode surface by a non-conductive polymer film. Also, the formation of the sol-gel MIP film on the surface of the electrode was confirmed by CV and EIS in the presence of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox pair. The surface morphologies of the bare PGE, treated PGE and polytyramine/sol-gel/f-MWCNTs @AuNPs MIP/PGE were characterized by scanning electron microscopy (SEM), and the results are shown in Fig. 3. A comparison reveals that an imprinted layer has been attached to the surface of the electrode which is suitable for effective diffusion of ketamine molecules to the electrode surface.

3.3. Electrochemical characterization of polytyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE

Cyclic voltammetry (CV) was used to characterize the stepwise construction process of the modified electrode. Fig. 4, shows the cyclic voltammograms of the bare PGE, treated PGE and polytyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE in $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ probe solution. Curve a, shows the electrochemical signal of the probe at the surface of the bare PGE. After electrochemical treatment of PGE in acetate buffer solu-

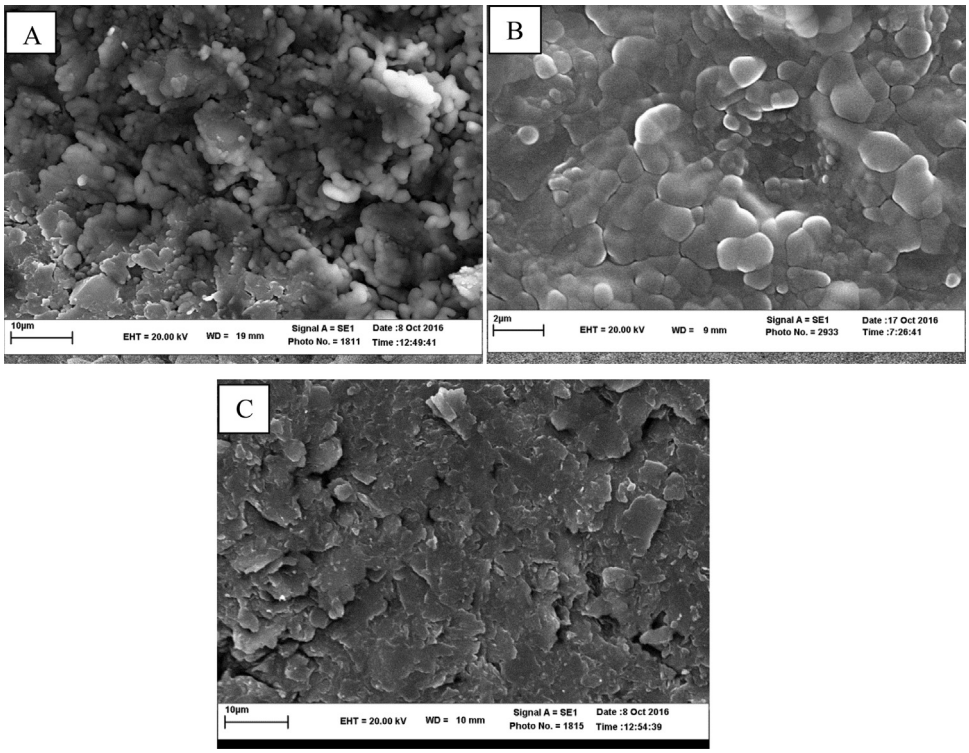

Fig. 3. Scanning electron microscope (SEM) images of (A) bare PGE, (B) treated PGE and (C) MIP/GCE.

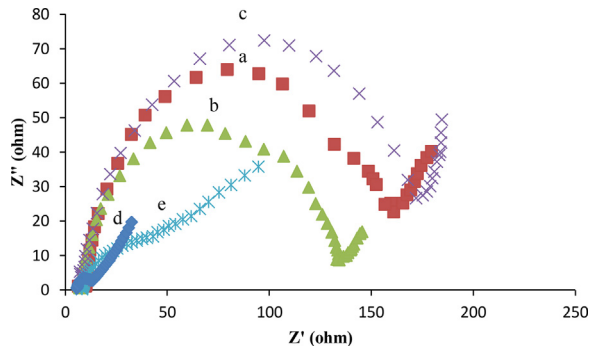

Fig. 5. Nyquist plots of (a) bare PGE, (b) treated PGE, (c) MIP/PGE, (d) MIP/PGE after removal of ketamine and (e) MIP/PGE after 420 s incubating in 20.0 nM ketamine solution. Conditions: Potential 0.20 V, frequency range of 0.1–30,000 Hz and amplitude of 10 mV.

tion, the probe peak current increases and the peak separation (ΔEp) decreases. When the polymer film is electrodeposited on to the treated electrode surface, no peak is observed at the MIP electrode, indicating that the prepared MIP film is very compact and $[Fe(CN)_6]^{3-/4-}$ anions cannot reach to the electrode surface for electrochemical reaction and the electron transfer of the probe is blocked. However, after removing the template from the polymer matrix, the current response increases because the $[Fe(CN)_6]^{3-/4-}$ anions can reach easily to the electrode surface through the vacant recognition sites which are produced at the surface of the electrode. Finally a decrease in the current response of the electrode was observed after incubation the polytyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE in 20.0 nM ketamine solution for 420 s. This result shows the ability of the imprinted electrode to rebind with ketamine molecules which limit the electron transfer of the probe on the electrode surface.

In order to characterize further the electrochemical behavior of the prepared sensor, the electrochemical impedance spectroscopy (EIS) measurements were carried out in the probe solution. The Nyquist plot consists of a semicircular part at higher frequencies and a linear part at lower frequencies. The semicircle part of the Nyquist plot represents the electron-transfer limited process, and the linear part represents the diffusion process. In the impedance spectrum, the semicircle diameter is equal to the electron transfer resistance (R_{et}) and it has an inverse relation with the electrochemical activity of the species in solution. The Nyquist plots of the electrode at various modification steps are presented in Fig. 5. Inspection of this Figure, shows that after PGE treatment, the electron transfer resistance (R_{et}) value decreases to R_{et} = 122.7 Ω due to the faster electron transfer of the redox probe on the electrode surface. The electropolymerization of polytyramine/sol-gel/f-MWCNTs@AuNPs MIP on the surface of the treated electrode displays an increase in semicircle diameter (curve c), which is an evidence for formation of a compact polymer film on the electrode surface that would hinder the access of $[Fe(CN)_6]^{3-/4-}$ probe anions to the electrode surface. When the template molecules are removed from the surface of the electrode, the R_{et} value decreases, because the specific binding cavities which are formed on the polymer layer can facilitate the diffusion of the $[Fe(CN)_6]^{3-/4}$ probe through the sol-gel MIP film and enhance the electron transfer rate. Furthermore, after the imprinted sensor is incubated in a 20.0 nM solution of ketamine for 420 s, the electron transfer resistance increases to R_{et} = 41.85 Ω (curve e), due to the occupation of vacant recognition sites by the ketamine template molecules which prevents the arrival of the redox probe to the electrode surface.

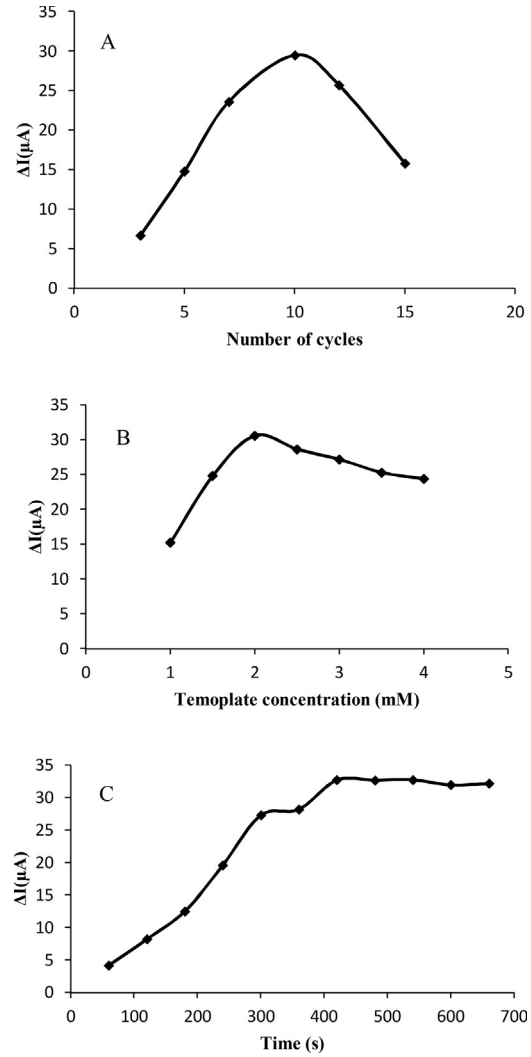

Fig. 6. Effect of (A) number of scan cycles, (B) template concentration and (C) incubation time on the response current of the MIP sensor.

3.4. Optimization of experimental conditions

In order to achieve the best sensor performance, it is necessary to optimize several key parameters including potential interval, the number of scan cycles, amount of the monomers, template concentration, amount of the nanocomposite, pH of the ketamine solution and the incubation time. The experimental parameters were optimized based on the change of the probe peak current (Δi_p) by subtracting the recorded currents in the presence and absence of ketamine molecules in solutions.

3.4.1. Electropolymerization conditions of MIP film

The thickness of the polymeric film is an important factor that affects the final sensor performance and it can be easily controlled by changing the number of scan cycles during the electropolymerization process. In order to obtain the optimum film thickness, the number of electropolymerization cycles was varied from 3 to 15. As is evident in Fig. 6A, the peak current value increases with the cycle number and reaches a maximum at 10 cycles. However, the peak current value decreases when the cycle number is more than 10, because at higher cycles, the imprinted polymer becomes very thick and the less binding sites are accessible for the template molecules. [39]. According to these results, the 10 cycles were selected as optimum scan cycles. The electrochemical polymerization of the sol-gel

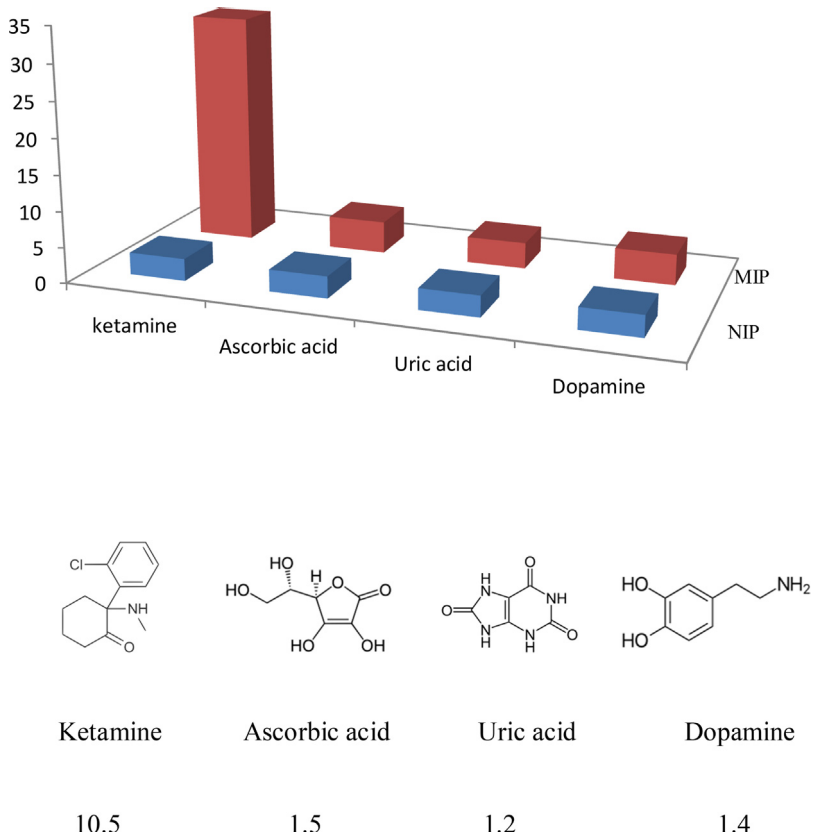

Fig. 7. Selectivity of the imprinted and non-imprinted sensors for ketamine molecules and some interferences.

MIP film on the surface of PGE was carried out in various potential intervals and the best result was obtained in the range of −0.8 to +1.2 V.

3.4.2. Conditions of the sol solution

The electrochemical response of the proposed sensor can be affected by the composition of the imprinted polymer. Therefore, the amount of monomers and f-MWCNTs@AuNPs nanocomposite was investigated. In order to optimize the composition of the sol solution, a series of electrodes was constructed by varying the amount of silane monomers from 100 to 300 μL and the tyramine concentration from 5 to 100 mM. The best response was obtained with 150 μL of silane monomers (75 μL of TEOS and 75 μL of PTEOS) and 30 mM tyramine monomer. Therefore, these amounts were used as the optimum concentrations for the fabrication of the polytyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE. Lower amounts of functional monomers lead to the formation of a restricted number of imprinted cavities. On the other hand, at higher concentration, the thickness of the imprinted polymer film increases and the ketamine molecules are embedded at the central area of the polymer and they cannot be completely removed from the polymer film [40].

The influence of nanocomposite amount on the peak current was also investigated and it was found that 50 μL of 1.0 mg mL^{-1} suspension of f-MWCNTs@AuNPs is the optimum amount for fabrication of the proposed sensor.

The number of recognition sites in the polymer film for selective rebinding of the template molecules has a direct relationship with the concentration of template. As is evident in Fig. 6B, the number of binding sites increases as the concentration of the template molecules increases up to 2.0 mM. It seems that at higher concentrations, the template molecules are agglomerated at the surface

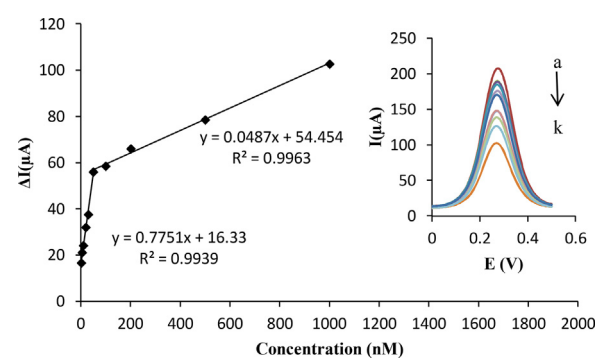

Fig. 8. Calibration curve of the imprinted sensor in different concentrations of keamine solutions. Inset is the square wave voltammograms of polytyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE before (a) and after incubation in 1.0 (b), 5.0 (c), 10.0 (d), 20.0 (e), 30.0 (f), 50.0 (g), 100.0 (h), 200.0 (i), 500.0 (j) and 1000.0 (k) nM of ketamine solutions. Conditions: Potential scan range −0.1 V to +0.50 V, Step potential 1 mV, Frequency15 Hz, Amplitude, 20 mV in phosphate buffer pH 5.0 containing 0.1 M KCl and 1.0 mM $[Fe(CN)_6]^{3-/4-}$.

of the electrode and hence, the peak current decreases. As a result, the optimum concentration of template was found to be 2.0 mM.

3.4.3. Rebinding conditions

The effect of the incubation time on the ketamine rebinding at the surface of the MIP electrode was also evaluated. After removing the template from the polymer matrix, the prepared electrode was placed in 20.0 nM ketamine solution for different accumulation times and the response currents were recorded (Fig. 6C). As is shown in this Figure, the response current increases with increment of the incubation time up to 420 s and then it is almost unchanged indicating that the adsorption of ketamine molecules on the surface of the electrode reaches to equilibrium. Therefore, an accumula-

Table 1
Comparison of analytical performance of the different electrodes for detection of ketamine.

Method	Linear range (μmol L^{-1})	Detection limit (μmol L^{-1})	Reference
ISE	10–10,000	5	[42]
Genosensor	0.001–0.6	1.98×10^{-3}	[43]
Au/Pt	7.3–3600	1.8	[44]
NPtCPE	0.1–2.0	0.1	[16]
ISE	1.5–10,000	0.12	[45]
Polytyramine/sol-gel/f-MWCNT@AuNPs MIP/PGE	1×10^{-3}–1	7×10^{-4}	This work

ISE: Ion-selective membrane electrode.
Au/Pt: Pulse plating gold film modified platinum electrode.
NPtCPE: Platinum nanoparticle modified carbon paste electrode.

tion time of 420 s was chosen for determination of ketamine in the subsequent studies.

The effect of the pH of ketamine solution on the electrochemical response of the imprinted sensor was investigated in the pH range from 4.0 to 9.0. It was observed that the best response is obtained at pH 5.0, suggesting that this pH value can facilitate the penetration of the ketamine molecules in the binding cavities. Therefore, the pH of ketamine solution was adjusted at 5.0 in the incubation process.

3.5. Interferences effect

In order to study the selectivity of the imprinted sensor, the influence of some of the interfering species existing in biological fluids such as ascorbic acid, uric acid and dopamine on determination of ketamine was investigated in 20.0 nM ketamine solution and the corresponding results are shown in Fig. 7. As is evident in this Figure, the current response of the MIP electrode is higher than that of the NIP electrode, and the presence of the interferences in solution has no obvious effect on the electrochemical response of the MIP electrode to ketamine molecules. The imprinting factor (IF) which is defined as the ratio of the Δi_{MIP} to the Δi_{NIP}, was used for further evaluation of the selectivity of the constructed sensor. As is evident in Fig. 7, the imprinting factor value for ketamine is 10.5 while for the other species it is in the range of 1.2–1.5. All of the obtained results show that the proposed electrochemical sensor has a good selectivity towards the ketamine molecules. This good selectivity can be attributed to the shape and size of the binding cavities that match the ketamine template molecules in the imprinted polymer film.

3.6. Analytical performance of the imprinted sensor

The square wave voltammetry technique was used for determination of ketamine in standard solutions using polytyramine/sol-gel/f-MWCNTs@AuNPs MIP/PGE under the optimized experimental conditions. After template removal, the imprinted sensor was immersed in different concentrations of ketamine solution and the probe peak currents were recorded. The inset of Fig. 8, shows the square wave voltammograms of the modified electrode in the concentration range of 1.0–1000.0 nM. When the imprinted sensor is placed in the solution containing ketakmine, the ketamine molecules occupy the binding cavities in the sol-gel MIP film, which leads to the decrease of the probe response current. Upon increasing the concentration of ketamine, more imprinted cavities are occupied by ketamine molecules and the response current further decreases.

The calibration curve of the MIP electrode shows two linear regions from 1.0 to 50.0 and 50.0–1000.0 nM for concentration of ketamine with the regression equations of $\Delta i = 0.7751C + 16.33$ ($R^2 = 0.9939$) and $\Delta i = 0.0487C + 54.454$ ($R^2 = 0.9963$), respectively. The detection limit (LOD) was estimated to be 0.7 nM according to $3s_b/m$, where S_b is the standard deviation for 5 replicate measurements of the blank and m is the slope of the calibration curve.

Table 2
Determination of ketamine in human plasma and urine samples (n = 3).

Sample	Detected (nM)	Added (nM)	Recovery (%)	RSD (%)
Plasma	Not detected	–	–	–
	5.4	5.0	108	3.5
	9.5	10.0	95	4.2
	19.6	20.0	98	3.0
Urine	Not detected	–	–	–
	5.5	5.0	110	3.8
	10.3	10.0	103	4.1
	19.4	20.0	97	3.6

The performance of the present sensor and some of the previously reported electrochemical sensors for determination of ketamine is compared in Table 1. The data in this Table, show that the value of LOD obtained in this work is lower than those obtained in the other methods for measurement the concentration of ketamine in solutions.

In order to investigate the repeatability of one of the prepared sensors, the electrochemical response of a 20.0 nM ketamine solution was measured successively for 14 times and the relative standard deviation (RSD) was obtained 3.4% (n = 14). The sensor to sensor reproducibility was also evaluated by measurement of 20.0 nM ketamine solution using five similarly MIP electrodes. The RSD value was found to be 4.4% for 5 measurements. These values, indicate that the proposed electrochemical sensor has an acceptable repeatability and reproducibility. To investigate the stability, the prepared MIP electrode was stored at 4 °C in the refrigerator for a period of two weeks. This electrode was used to detect the same concentration of ketamine every day. The response current of the electrode retained 92.8% of its initial value after two weeks, which confirms the good stability of the developed imprinted sensor. This good stability can be attributed to the formation of the sol-gel MIP stiff film on the electrode surface [41].

In order to evaluate the analytical performance of the proposed sensor in real samples, the electrochemical measurements of ketamine in urine and plasma samples were performed using the standard addition method. The real samples were prepared according to the methods which are described in the sample preparation section. The certain amounts of ketamine were added to the prepared samples and the ketamine concentration was measured by the proposed sensor. Three measurements were performed at each concentration. The experimental results are summarized in Table 2. As is seen in Table 2, the obtained recoveries are in the range of 95%–110% with RSDs less than 4.2%, which demonstrate the capability of the proposed MIP sensor for determination of ketamine in biological samples.

4. Conclusion

The present study, reports a new approach for the sensitive and selective detection of ketamine molecules in solutions based on one-step electropolymerization of the sol-gel MIP film at the

surface of a pencil graphite electrode for the first time. The presence of f-MWCNTs@AuNPs nanocomposite in the polymer matrix, effectively facilitates the electron transmission from the molecularly imprinted polymer film to the surface of the electrode. The combination of f-MWCNTs@AuNPs nanocomposite, polytyramine and the sol–gel MIP technology, led to the fabrication of a new electrochemical imprinted sensor with a low detection limit, high selectivity as well as good stability, reproducibility and repeatability for measurement of ketamine in solutions. In addition, the acceptable recoveries which obtained from the real sample analysis, reveals that the sensor performance is not affected significantly by the matrix of the real samples and the proposed electrochemical sensor, can be used for determination of ketamine in urine and plasma samples.

Acknowledgments

The financial support from the Ferdowsi University of Mashhad, Mashhad, Iran (Grant No. 3.32159) is gratefully acknowledged.

References

[1] A. Pai, M. Heining, Ketamine, continuing education in anaesthesia, Crit. Care Pain 7 (2007) 59–63.
[2] N. Porpiglia, G. Musile, F. Bortolotti, E.F.D. Palo, F. Tagliaro, Chiral separation and determination of ketamine and norketamine in hair by capillary electrophoresis, Forensic Sci. Int. 266 (2016) 304–310.
[3] M.F. Chen, S.Y. Lai, P.C. Kung, Y.C. Lin, H.I. Yang, P.Y. Chen, I.Y. Liu, A.C. Lua, T.J.F. Lee, Inhibition by ketamine and amphetamine analogs of the neurogenic nitrergic vasodilations in porcine basilar arteries, Toxicol. Appl. Pharm. 305 (2016) 75–82.
[4] R. El-Sheikh, M. Zaky, F.Z. Mohamed, A.S. Amin, A.A.E.F. Gouda, Spectrophotometric determination of dextromethorphan hydrobromide and ketamine hydrochloride in pure and dosage forms, J. Chin. Chem. Soc. 53 (2006) 831–838.
[5] J. Xiong, J. Chen, M. He, B. Hu, Simultaneous quantification of amphetamines, caffeine and ketamine in urine by hollow fiber liquid phase microextraction combined with gas chromatography-flame ionization detector, Talanta 82 (2010) 969–975.
[6] K. Lian, P. Zhang, L. Niu, S. Bi, S. Liu, L. Jiang, W. Kang, A novel derivatization approach for determination of ketamine in urine and plasma by gas chromatography–mass spectrometry, J. Chromatogr. A 1264 (2012) 104–109.
[7] I. Moreno, M. Barroso, A. Martinho, A. Cruz, E. Gallardo, Determination of ketamine and its major metabolite norketamine, in urine and plasma samples using microextraction by packed sorbent and gas chromatography-tandem mass spectrometry, J. Chromatogr. B 1004 (2015) 67–78.
[8] S. Gentili, C. Mortali, L. Mastrobattista, P. Berretta, S. Zaami, Determination of different recreational drugs in sweat by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME GC/MS): application to drugged drivers, J. Pharm. Biomed. Anal. 129 (2016) 282–287.
[9] H.R. Lin, K.I. Choi, T.C. Lin, A. Hu, Simultaneous quantification of amphetamine opiates, ketamine and relative metabolites in urine for confirmatory analysis by liquid chromatography tandem mass spectrometry, J. Chromatogr. B 929 (2013) 133–141.
[10] N.S. Nosseir, G. Michels, P. Binder, M.H.J. Wiesen, C. Müller, Simultaneous detection of ketamine, lorazepam, midazolam and sufentanil in human serum with liquid chromatography-tandem mass spectrometry for monitoring of analgosedation in critically ill patients, J. Chromatogr. B 973 (2014) 133–141.
[11] M.C. Parkin, A.M. Longmoore, S.C. Turfus, R.A. Braithwaite, D.A. Cowan, S. Elliott, A.T. Kicman, Detection of ketamine and its metabolites in human hair using an integrated nanoflow liquid chromatography column and electrospray emitter fritted with a single porous 10 μm bead, J. Chromatogr. A 1277 (2013) 1–6.
[12] H.P. Jen, Y.C. Tsai, H.L. Su, Y.Z. Hsieh, On-line preconcentration and determination of ketamine and norketamine by micellar electrokinetic chromatography: complementary method to gas chromatography/mass spectrometry, J. Chromatogr. A 1111 (2006) 159–165.
[13] Y.H. Lin, M.R. Lee, R.J. Lee, W. Kung Ko, S.M. Wu, Hair analysis for methamphetamine, ketamine, morphine and codeine by cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography, J. Chromatogr. A 1145 (2007) 234–240.
[14] Y. Chen, Y. Yang, Y. Tu, An electrochemical impedimetric immunosensor for ultrasensitive determination of ketamine hydrochloride, Sens. Actuators B 183 (2013) 150–156.
[15] H.M. Abu Shawish, S.M. Saadeh, H. Tamos, K.I. Abed-Almonem, O. Al khalili, A novel coated silver ketamine(I) electrode for potentiometric determination of ketamine hydrochloride in ampoules and urine samples, Anal. Chem. Res. 2 (2014) 30–36.
[16] Z. Bagheryan, J.B. Raoof, R. Ojani, E. Hamidi-Asl, Introduction of ketamine as a G-quadruplex-binding ligand using platinum nanoparticle modified carbon paste electrode, Electroanal. 25 (2013) 2659–2667.
[17] K. Kor, K. Zarei, Development and characterization of an electrochemical sensor for furosemide detection based on electropolymerized molecularly imprinted polymer, Talanta 146 (2016) 181–187.
[18] M. Cui, J. Huang, Y. Wang, Y. Wu, X. Luo, Molecularly imprinted electrochemical sensor for propyl gallate based on PtAu bimetallic nanoparticles modified graphene-carbon nanotube composites, Biosens. Bioelectron. 68 (2015) 563–569.
[19] H. Silva, J. Pacheco, J. Silva, S. Viswanathan, C. Delerue-Matos, Molecularly imprinted sensor for voltammetric detection of norfloxacin, Sens. Actuators B 219 (2015) 301–307.
[20] B.V.M. Silva, B.A.G. Rodríguez, G.F. Sales, M.D.P.T. Sotomayor, R.F. Dutra, An ultrasensitive human cardiac troponin T graphene screen-printed electrode based on electropolymerized-molecularly imprinted conducting polymer, Biosens. Bioelectron. 77 (2016) 978–985.
[21] B. Rezaei, H. Lotfi-Forushani, A.A. Ensafi, Modified Au nanoparticles-imprinted sol–gel, multiwall carbon nanotubes pencil graphite electrode used as a sensor for ranitidine determination, Mater. Sci. Eng. C 37 (2014) 113–119.
[22] Y. Yang, G. Fang, G. Liu, M. Pan, X. Wang, L. Kong, X. He, S. Wang, Electrochemical sensor based on molecularly imprinted polymer film via sol–gel technology and multi-walled carbon nanotubes-chitosan functional layer for sensitive determination of quinoxaline-2-carboxylic acid, Biosens. Bioelectron. 47 (2013) 475–481.
[23] J. Zhang, Y. Niu, S. Li, R. Luo, C. Wang, A molecularly imprinted electrochemical sensor based on sol–gel technology and multiwalled carbon nanotubes–nafion functional layer for determination of 2-nonylphenol in environmental samples, Sens. Actuators B 193 (2014) 844–850.
[24] S. Cheemalapati, S. Palanisamy, V. Mani, S.M. Chen, Simultaneous electrochemical determination of dopamine and paracetamol on multiwalled carbon nanotubes/graphene oxide nanocomposite-modified glassy carbon electrode, Talanta 117 (2013) 297–304.
[25] J. Bai, X. Zhang, Y. Peng, X. Hong, Y. Liu, S. Jiang, B. Ning, Z. Gao, Ultrasensitive sensing of diethylstilbestrol based on AuNPs/MWCNTs-CS composites coupling with sol-gel molecularly imprinted polymer as a recognition element of an electrochemical sensor, Sens. Actuators B 238 (2017) 420–426.
[26] D. Yu, Y. Zeng, Y. Qi, T. Zhou, G. Shi, A novel electrochemical sensor for determination of dopamine based on AuNPs@SiO2 core-shell imprinted composite, Biosens. Bioelectron. 38 (2012) 270–277.
[27] X. Niu, W. Yang, G. Wang, J. Ren, H. Guo, J. Gao, A novel electrochemical sensor of bisphenol A based on stacked graphene nanofibers/gold nanoparticles composite modified glassy carbon electrode, Electrochim. Acta 98 (2013) 167–175.
[28] X. Hou, L. Wang, X. Wang, Z. Li, Coating multiwalled carbon nanotubes with gold nanoparticles derived from gold salt precursors, Diamond Relat. Mater. 20 (2011) 1329–1332.
[29] H. Ismaili, F. Lagugne'-Labarthet, M.S. Workentin, Covalently assembled gold nanoparticle-carbon nanotube hybrids via a photoinitiated carbene addition reaction, Chem. Mater. 23 (2011) 1519–1525.
[30] D. Gingery, P. Buhlmann, Formation of gold nanoparticles on multiwalled carbon nanotubes by thermal evaporation, Carbon 46 (2008) 1966–1972.
[31] S.A. Miscoria, G.D. Barrera, G.A. Rivas, Glucose biosensors based on the immobilization of glucose oxidase and polytyramine on rodhinized glassy carbon and screen printed electrodes, Sens. Actuators B 115 (2006) 205–211.
[32] M.Q. Liu, J.H. Jiang, Y.L. Feng, G.L. Shen, R.Q. Yu, Glucose biosensor based on immobilization of glucose oxidase in electrochemically polymerized polytyramine film and overoxidised polypyrrole film on platinized carbon paste electrode, Chin. J. Anal. Chem. 35 (2007) 1435–1438.
[33] Z.S. Wu, J.S. Li, T. Deng, M.H. Luo, G.L. Shen, R.Q. Yu, A sensitive immunoassay based on electropolymerized films by capacitance measurements for direct detection of immunespecies, Anal. Biochem. 337 (2005) 308–315.
[34] A.M. Tenreiro, C. Nabais, J.P. Correia, F.M.S.S. Fernandes, J.R. Romero, L.M. Abrantes, Progress in the understanding of tyramine electropolymerisation mechanism, J. Solid State Electrochem. 11 (2007) 1059–1069.
[35] C.M. de Castro, S.N. Vieira, R.A. Goncalves, A.G. Brito-Madurro, J.M. Madurro, Electrochemical, morphologic studies of nickel incorporation on graphite electrodes modified with polytyramine, J. Mater. Sci. 43 (2008) 475–482.
[36] B. Rezaei, M. Khalili Boroujeni, A.A. Ensafi, A novel electrochemical nanocomposite imprinted sensor for the determination of lorazepam based on modified polypyrrole@sol-gel@gold nanoparticles/pencil graphite electrode, Electrochim. Acta 123 (2014) 332–339.
[37] A. Özcan, Y. Şahin, Preparation of selective and sensitive electrochemically treated pencil graphite electrodes for the determination of uric acid in urine and blood serum, Biosens. Bioelectron. 25 (2010) 2497–2502.
[38] B. Deiminiat, G.H. Rounaghi, M.H. Arbab-Zavar, I. Razavipanah, A novel electrochemical aptasensor based on f-MWCNTs/AuNPsnanocomposite for label-free detection of bisphenol A, Sens. Actuators B 242 (2017) 158–166.
[39] W. Lian, S. Liu, J. Yu, J. Li, M. Cui, W. Xu, J. Huang, Electrochemical sensor using neomycin-imprinted film as recognition element based on chitosan-silver nanoparticles/graphene-multiwalled carbon nanotubes composites modified electrode, Biosens. Bioelectron. 44 (2013) 70–76.
[40] B.L. Li, J.H. Luo, H.Q. Luo, N.B. Li, A novel strategy for selective determination of d-penicillamine based on molecularly imprinted polypyrrole electrode via the electrochemical oxidation with ferrocyanide, Sens. Actuators B 186 (2013) 96–102.

[41] A. Taheri, M. Noroozifar, M. Khorasani-Motlagh, Investigation of a new electrochemical cyanide sensor based on Ag nanoparticles embedded in a three-dimensional sol–gel, J. Electroanal. Chem. 628 (2009) 48–54.
[42] N. Alizadeh, R. Mehdipour, Drug-selective electrode for ketamine determination in pharmaceutical preparations and electrochemical study of drug with BSA, J. Pharm. Biomed. Anal. 30 (2002) 725–731.
[43] M. Asghary, J.B. Raoof, R. Ojani, E. Hamidi-Asl, A genosensor based on CPE for study the interaction between ketamine as an anesthesia drug with DNA, Int. J. Biol. Macromol. 80 (2015) 512–519.
[44] J. Zhou, Y. Xu, L. Wang, J. Liu, Y. Li, B. Ye, Electrochemical behavior and voltammetric determination of ketamine at pulse plating gold film modified platinum electrode, J. Chin. Chem. Soc. 59 (2012) 879–883.
[45] H.M. Abu Shawish, H. Tamous, S.M. Saadeh, K.I. Abed-Almonem, O. Al khalili, A new approach for decreasing the detection limit for a ketamine(I) ion-selective electrode, Mater. Sci. Eng. C 49 (2015) 445–451.