Anal. Bioanal. Electrochem., Vol. 12, No. 1, 2020, 128-140

Analytical &
Bioanalytical
Electrochemistry

2020 by CEE
www.abechem.com

Full Paper

Molecularly Imprinted Polymer (MIP) Electrochemical Sensor based on Graphene Modified Platinum Electrode for Sertraline Determination

Mohammad-Reza Milani-Hosseini*, Sanaz Karamdoust, Mina Bahman, Ali Motaharian, and Saeideh Mohammadsadegh

Research Laboratory of Real Samples Analysis, Department of Analytical Chemistry, Faculty of Chemistry, Iran University of Science and Technology, Tehran, Iran

*Corresponding Author, Tel.: +982177491208; Fax: +982177491204

E-Mail: drmilani@iust.ac.ir

Received: 7 December 2019 / Received in revised form: 13 January 2020

Accepted: 16 January 2020 / Published online: 31 January 2020

Abstract- In this report, a new electrochemical sensor was developed for sertraline (STR) analysis. The sensor was prepared based on modified Pt electrode with graphene nanoparticles and molecular imprinting polymer (MIP) by coating a very thin layer of polymer onto a support. Through this approach, all of the binding sites of the polymer immobilized on the electrode surface are available for target molecules. The STR binding experiments indicated that the sensor modified by MIP have much higher adsorption ability than non−imprinted polymer (NIP) based sensor. Also, using of graphene in preparation of Pt electrode leads to a significant improvement in response of electrode because of their high electrical conductivity and large surface areas as the platform of the polymer that makes the graphene-MIP an excellent electrical transducer for direct electrical sensing. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) tests were used to evaluate the performance of the electrochemical sensor. Some parameters affecting the sensor performance such as MIP suspension, extraction pH and preconcentration time were optimized and under optimal conditions, the modified sensor with MIP-graphene showed linear responses with STR concentration in the range of 1.0×10^{-8} to 1.0×10^{-6} mol L^{-1} ($R^2 = 0.985$) with a detection limit of 7.0×10^{-9} mol L^{-1}. This developed sensor with the MIP was consequently capable of selective sensing of STR in human serum sample with recovery values in the range of 98.2-103.5%.

Keywords- Sensor, Molecularly Imprinted Polymer, Graphene, Sertraline

---

1. INTRODUCTION

One of the big problems for people worldwide which affect both economic and social life is depression. Sertraline (STR), Fig. 1, as one of the active drugs is mostly recommended for psychological diseases. This chemical has been used for the treatment of many diseases such as, obsessive-compulsive, social anxiety and panic disorders due to its minimal known side effects. For pharmaceutical use, STR generally is available as salt. As it is shown in Fig.1the structure includes a carbon side-chain containing an amino group and it is a secondary amine that exhibits two asymmetric centers, but has only a single enantiomer [1-5].

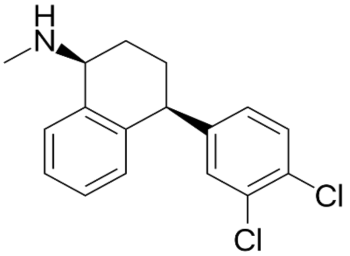

Fig. 1. Chemical structure of Sertraline (STR)

During the previous years variety of electroanalytical and spectrophotometric methods have been reported in the literature for the detection of STR as a kind of antidepressant substance and its metabolites in pharmaceutical formulations. For instance, HPLC, GC with the combination of different detectors such as LC tandem MS (LC–MS/MS), and GC–MS have been most commonly reported in the literature [6-10].Moreover, other techniques as chromatography, spectroscopy and electrochemical have also been reported [11-14]. As compared by the mentioned methods, electrochemical techniques usually provide considerable benefits such as easy operation as well as comfort preparation of electrode, sensitivity, low expense, good electrical conductivity, fast response time, plus broad potential window for miniaturization and construction for portable equipment applications[15-17].

In order to advance the electrochemical methods, graphene has been under high attention and selected as an excellent candidate with ultimate physicochemical properties, such as, extremely high surface area, high thermal and electrical conductivity and robust mechanical strength. Further on, to provide a great area 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 [18-20].

In an analytical procedure sensitivity and selectivity are the vital characteristics which are strong incentives to search novel materials for working electrodes alteration to develop in electrochemical sensors [21].The increasing pharmaceutical interest in molecularly imprinted polymers (MIP) is possibly a direct consequence of its major benefits related to other analytical techniques, mostly, improved selectivity and sensitivity of the method. Moreover, other significant characteristics of MIPs to be considered are facile preparation, specific identification, and wide workability. The preparation of MIP involves covalence, non-covalence, or semi-covalence binding interactions between the templates and monomers. Due to the type of binding interactions that occur during pre-assembly, two main approaches can be distinguished. The subsequent step of template removal leaves complementary cavities which should, in theory, be capable of rebinding [22, 23].

During the recent years, numerous articles on the use of MIP nanoparticles in preparation of chemical sensors have been published. Yet, there are no earlier reports on the preparation of electrochemical sensor based on MIP for STR and this medication has not been used as a template in Molecular Imprinting process.

2. EXPERIMENTAL

2.1. Apparatus

Electrochemical research contained a three-electrode system consisting of a modified Pt-electrode as working, platinum rod as auxiliary and Ag/AgCl as reference electrodes, plus a pH meter with a combined glass electrode to adjust the pH of solutions. All studies were carried out with an Autolabpotentiostat/ galvanostat-model of PGSTAT 100.

2.2. Materials and solutions

STR was purchased from Razak pharmaceutical Company, Cellulose acetate ($[C_6H_7O_2(OH)_3m(OOCH_3)]$), Azobisisobutylonitrile (AIBN), Methacrylic acid (MAA), Potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$), Ethylene glycol dimethyl acrylate (EGDMA) and Graphite fine powder. All products were acquired from Merck Company as well as other chemicals in analytical grades. With reference to Hummers method, Graphene oxide (GO) was prepared from purified natural graphite powder and then reduced according to method reported by Stankovich et al. [23].The standard stock solution of STR was prepared by dissolving the appropriate amount of STR in distilled deionized water and used to prepare other standard concentrations of STR by dilution with Britton−Robinson (B.R.) buffer solution.

2.3. Preparation of MIP and NIP

MIPs were prepared as the following procedure which the amount of each component is presented accordingly. 0.2 mmol of STR (template) was dissolved in 20 mL chloroform in a 100 mL glass flask and 2.0 mmol MAA (monomer) was added to the above solution. The mixture was stirred for 90 min to form a pre-complex of monomer-template molecules. Next, 1.8 mmol of EGDMA (cross-linker) and 0.03 g AIBN (initiator) were added into the above solution and stirred for 30 min, then, suspension was purged with nitrogen for 10 min and heated at 60 ºC for 24 h to complete polymerization. Unpolymerized components and STR 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 resulting MIP were dried in vacuum at 60 °C overnight. The non-imprinted polymers (NIPs) were also synthesized by following the same procedure but in the absence of STR molecules.

2.4. Preparation of the modified electrodes (so called Sensor)

In order to prepare modified Pt electrodes with graphene, 1 g of graphite powder, 0.004 g graphene were mixed to obtain a homogenized blend. The above mixture was added to 0.0001 g melted cellulose acetate as a binder and was thoroughly mixed. A thin layer of the final paste was placed on a graphene modified pt-electrode. The prepared electrodes were polished and finally, rinsed with distilled water. For making the sensor, an appropriate amount of MIP was then placed on the surface of the modified electrode.

2.5. General procedure for STR determination using the modified electrodes

The modified electrode was incubated in STR solution (pH=10) for 20 min under stirring condition. Then, the electrode was inserted in the washing solution (B.R. buffer) for 5 min to remove any weakly adsorbed analyte. After that, it was placed in the electrochemical cell containing 0.5 mM Potassium hexacyanoferrate ($K_3Fe(CN)_6$) in 0.1 M KCl solution.

2.6. Determination of STR in real samples

For determination of STR in pharmaceutical formulations, three different STR concentrations as 2×10^{-7}, 5×10^{-7}, and 1×10^{-6} mol L^{-1} were injected to human blood plasma. For this purpose, initially, 0.6 L human plasma and 0.5 mL acetonitrile was purred in a small cell; afterwards, for sedimentation of protein deposits, the content was centrifuged at 13500 rpm for 15 min. Further on, 0.3 mL STR standard solution was added to human blood plasma and finally, 0.25 mL of the solution in cell was transferred to a 25 mL-flask and diluted to volume with B.R. buffer (pH = 10).

3. RESULTS AND DISCUSSION

3.1. Characterizations of the synthesized Graphene and MIP

Size and morphology of the graphene sheets as well as synthesized MIP particles are shown in SEM images Fig.2A and 2B. As can be seen, the thickness of graphene sheets Fig. 3 (A) is below 30 nm. Moreover, as shown in Fig. 2B, the MIP particles have also nano-dimension with particles size in the range of 50 – 100 nm.

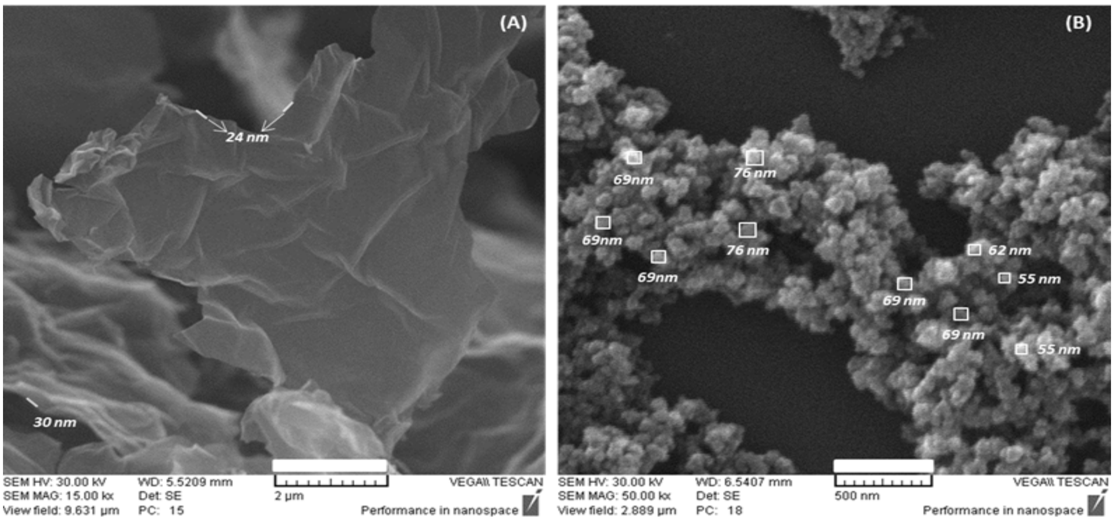

Fig. 2. Scanning electron microscopy images of grapheme (A) and synthesized MIP particles (B)

3.2. Effect of graphene as modifier

In order to study the voltammetric behavior of electro-active compounds on the surface of the electrode, cyclic voltammetry (CV) was obtained under the potential range of −0.50 V to 1 V. In addition, to achieve high sensitivity and appropriate limit of detection (LOD) in quantitative electrochemical analysis, differential pulse voltammetry (DPV) under the range 0 V to 0.4 V was also determined for electroanalysis of STR.

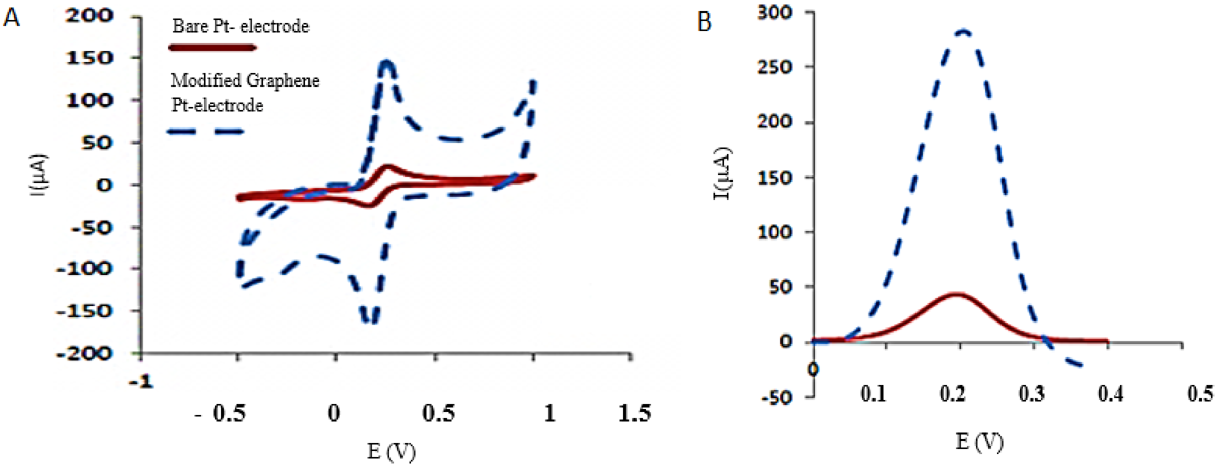

Fig. 3. (A) Cyclic voltrammetry of 5mM potasium hexacyanoferrate in KCl 0.1 M at potential range of −0.50 V to 1 V *vs.* Ag-AgCl on a bare Pt-electrode and graphene modified electrode. (B) Differential Pulse Voltammetry of 5mM potassium hexacyanoferrate in KCl 0.1 M, in the range of 0 V to 0.4 V on a bare Pt-electrode and graphene modified electrode

Fig. 2 shows the voltammetric behavior of 5mM potassium hexacyanoferrate ions on a bare Pt-electrode and graphene modified electrode in KCl 0.1 M at potential range of −0.50 V to 1 V. As can be demonstrated from Fig. 3A and 3B, after the paste of graphene, a noticeable increase in the height of cyclic voltammogram is observed. Also, as it is clear, in the differential pulse voltammogram height variation is illustrated, which this is related to the increase of electro-conductivity and high electron transfer rate for graphene modified electrode.

3.3. Study of the sensor response to SRT

In order to asses DPV for MIP/NIP graphene modified electrodes, the electrode signals were recorded in 0.5 mM of potassium hexacyanoferrate solutions. As it is shown in Fig. 4, DPV signal for graphene modified Pt-electrode is strongly higher that the bare Pt electrode. However, by pasting MIP on the graphene modified surface, the current intensity of the voltammogram decreased. This is probably due to the placing of a nonconductive polymeric layer on the electrode surface, which reduces electron transfer on the surface compared to modified graphene electrode.

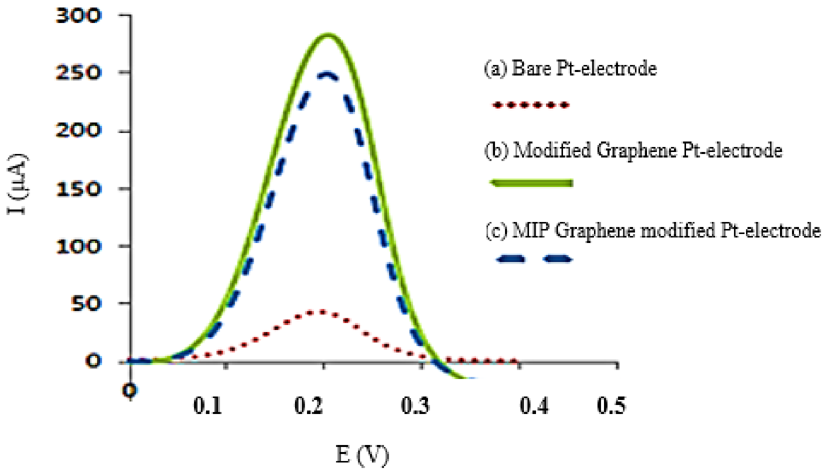

Fig. 4. Differential pulse voltammetry in potassium hexacyanoferrate for a) Bare Pt-electrode b) Graphene modified Pt-electrode c) MIP graphene modified Pt-electrode

In the same situation, NIP is pasted on the modified graphene and the DPV signal for 0.5 mM potassium hexacyanoferrate solutions is recorded. As it is shown in Fig. 5, by the comparison of different states, the current intensity voltammetry signal for grapheme modified Pt electrode in potassium hexacyanoferrate solutions increased compared to bare Pt electrode and by pasting NIP, the current intensity has dropped. This signal decrease is also seen in Fig. 5 for MIP, whilst in NIP based sensors this reduction is more.

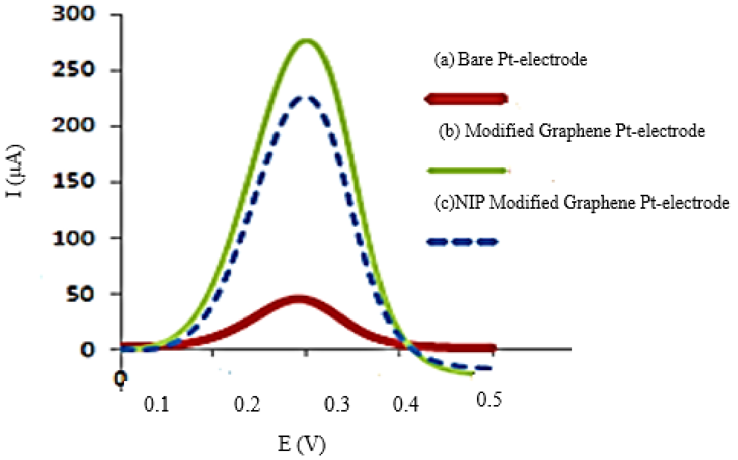

Fig. 5. Differential pulse voltammetry in potassium hexacyanoferrate for a) Bare Pt-electrode b) Graphene modified Pt-electrode c) NIP graphene modified Pt-electrode

In order to verify STR recognition ability of MIP-based sensors, the sensor was incubated in 5.0×10^{-6} M STR solution at pH =10 for 20 min under stirring condition. Next, the electrodes were washed with B.R. buffer to remove any weakly adsorption of analyte, and then DPV in potassium hexacyanoferrate solution was recorded. The result is indicated in Fig. 6.

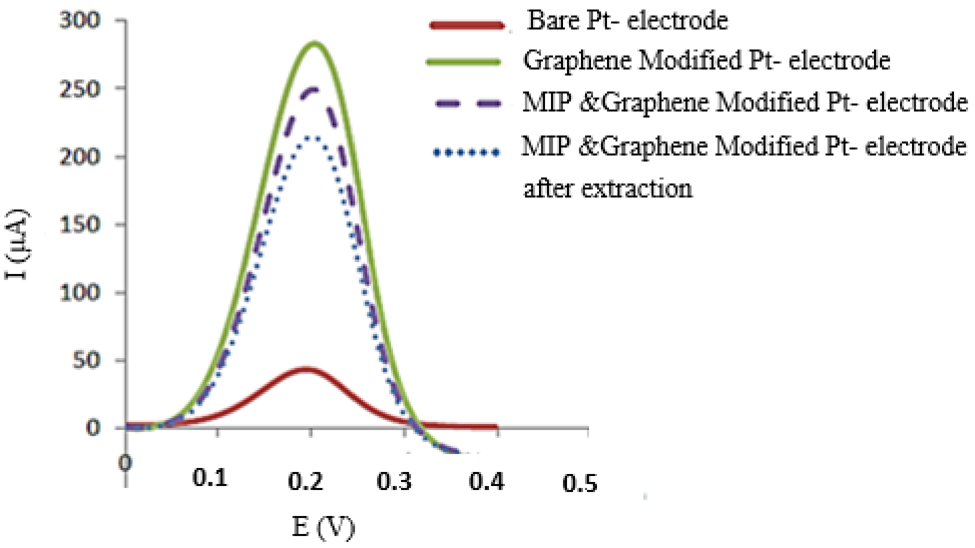

Fig. 6. Differential pulse voltammetry signals in potassium hexacyanoferrate in KCl 0.1 M, for Bare Pt and MIP &Graphene modified Pt electrodes after 20 min in B.R. buffer (pH= 10) including 5×10^{-6} M STR.

As it can be illustrated from Fig. 6 reduction of peak height and current differentiation ($\Delta I=33.86$) related to re-adsorption of STR, is very higher than the same parameters in Fig. 7, (ΔI=7.11). This is because the re-adsorption on MIP surface is more than NIP surface. Therefore, due to this we can see more decrease in potassium hexacyanoferrate signal in MIP modified electrode, which demonstrates suitable performance of the modified sensor in re-adsorption of STR.

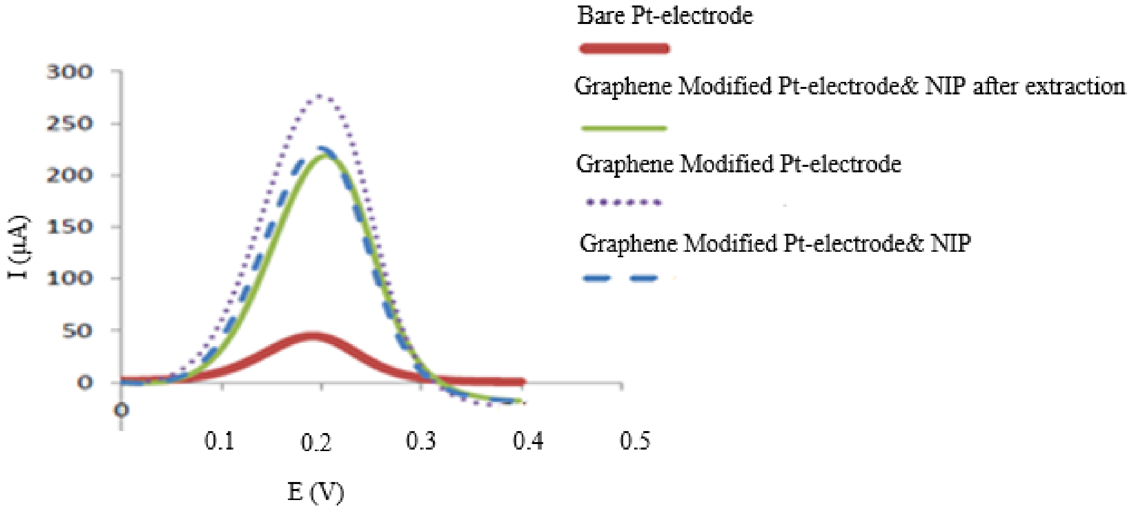

Fig. 7. Differential pulse voltammetry signals in potassium hexacyanoferrate in KCl 0.1 M, for Bare Pt-electrode and Graphene Modified Pt electrodes & NIP after 20 min in B.R. buffer (pH= 10) including 5×10^{-6} M STR.

3.4. Optimization of MIP−Graphene Pt electrode composition

To attain the best composition for MIP−Graphene Pt electrode, the amounts of electrode components contain graphite, graphene; MIP and cellulose acetate were optimized. For this purpose, amounts of graphite and graphene are used (1g and 0.004 g respectively) and the mass ratio of graphene to cellulose acetate and MIP to cellulose acetate are altered. The prepared sensor was used for STR extraction and analysis. The effect of graphene to cellulose acetate ratios in the fabrication of a suitable suspension solution for graphene depositing on the surface of Pt electrode was obtained. Also, current difference in differential pulse voltammetry peaks was investigated, which the results are listed in Table 1. As can be seen in Table 1, measured ratios of cellulose acetate and graphene (1 to 25, 30, 35, 40 and 45) illustrate that difference of DPV peak height between Pt electrode and graphene modified Pt electrode improved by increasing 1:40 the ratio graphene to cellulose acetate (No. 4), after that by the ratio of 1:45 (No. 5), it remains constant approximately. In addition, resistance of graphene on the electrode surface is significant, so the ratio of 1:40 graphene to cellulose acetate was selected as the optimum value for modified Pt electrode preparation. Thereafter, the ratio of MIP to cellulose acetate was optimized. Here, two objectives are considered: first, a rise of STR extraction and thus an increase of difference in the hexacyanoferrate current before and after extraction and second, stability of the coated mechanical layer. The amount of current difference moved to higher values by increasing the MIP to cellulose acetate ratio, but it is noticeable that the obtained results displayed that the coated polymeric layer in the ratio of 1:5 MIP to cellulose acetate and higher wasn't mechanically stable and was extracted from the electrode surface at the extraction stage. Consequently, the ratio of 1:3 MIP to cellulose acetate was selected as optimal.

Table 1. Optimization of the ratio of graphene to cellulose acetate in solvent constant volume (DMF) and intensity of DPV current difference between Pt electrode and graphene modified Pt electrode

No	Amount of graphene (%)	Amount of cellulose acetate (%)	DMF (mL)	Current difference between Pt electrode and graphene modified Pt electrode (μA)
1	96.16	3.84	0.5	57.06
2	96.78	3.22	0.5	75.41
3	97.22	2.78	0.5	103.05
4	97.56	2.44	0.5	201.17
5	97.82	2.18	0.5	214.31

3.5. Optimization of the of extraction pH

The effect of pH sample solution on the sensor response was investigated by incubating the MIP−Graphene Pt electrode with optimized composition, into 1.0×10^{-6} M STR solutions in the pH range of 2-12. The sensor was then washed and DPV signal was recorded. As can be seen in Fig. 8, reduction current increased by the rise of pH up to 10 and then dropped gradually. Thus, pH=10 by B.R. buffer, was chosen as optimum pH for STR extraction in the developed method.

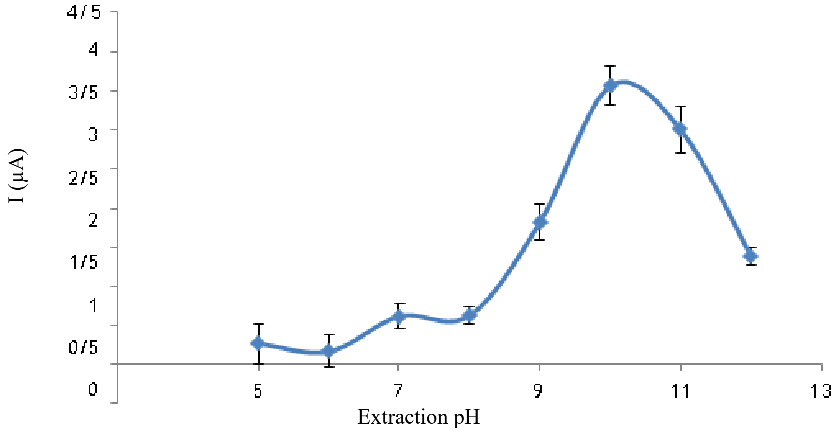

Fig. 8. Effect of extraction pH

3.6. Optimization of extraction time of sample solution

In this work the effect of sample solution extraction time as an important factor on the sensor response was also studied. For this purpose, 1.0×10^{-6} M STR solution (pH=10) extraction was carried out at different extraction times from 5 to 35 min with 5 min as the interval. The best time extraction is when the maximum decrease observed in hexacyanoferrate differential pulse voltammetric signal after the target molecule re-adsorption.

3.7. Optimization of electrochemical condition

The cyclic voltammetry was recorded between -0.5 to 1 V with scan rate of 0.05 V/s and the differential pulse voltammetry was studied in the range of 0 -0.4 V in 0.5 mM ($K_3Fe(CN)_6$) vs 0.1 M KCl solution with pulse time of 0.05 s and plus height of 2mV. In both cases reduction signal was determined. Yet the signal intensity reduction for NIP based sensors ΔI= 53.08 (μA) compared to MIP based sensors ΔI= 33.08 (μA) is higher.

3.8. Analytical performance and real sample analysis

Considering the optimal conditions, the prepared sensor was used for determination of STR at different concentrations for plotting calibration curve. The calibration curve exhibited a linear relationship with STR concentration in the range of 1.0×10^{-8} to 1.0×10^{-6} mol L^{-1} (R^2= 0.985).

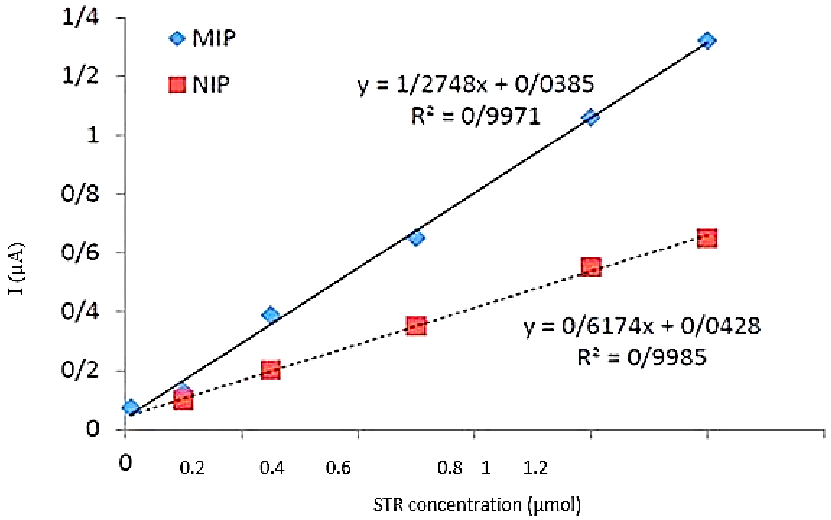

Fig. 9. Comparison of DPV responses obtained at MIP and NIP based sensors under optimum conditions

The detection limit of the suggested sensor was 7.0×10^{-9} mol L^{-1}, according to the relation of $3S_b$/ where the S_b indicates the standard deviation of the current peak for blank solution (n=5) and m is slope of calibration curve. Furthermore, the repeatability of this sensor was calculated for five measurements of 5.0×10^{-7} mol L^{-1} STR solution and relative standard deviation (RSD%) was achieved 4.9%.

Also, the comparison of MIP and NIP based sensors under optimal conditions in various STR concentrations was carried out as Fig. 9. As indicated MIP based sensor demonstrates a much higher response to STR compared to NIP based sensor.

Table 2. Tolerance limit of some interfering molecules and ions in determination of 1.0×10^{-7} M STR solution using MIP based sensor.

Interferes	Tolerance limit
Glucose	18
Na^+	120
K^+	130
Cl^-	120
CO_3^{2-}	30
Ca^{2+}	100

Selectivity is one of the fundamental indicators to estimate the determination ability of electrochemical sensors. In order to assess the selectivity of the suggested sensor, several ions and molecules that are present in blood serum were chosen. The tolerance limit was considered as the error of 5%in determination of 1.0×10^{-7} mol L^{-1} STR solution which leads to change the value of the initial signal. Tolerance limits of some interfering molecules and ions are listed in Table 2 which indicates that interfered by different species has negligible effect on the performance of constructed sensor for detecting STR. This is owing to being of molecular identification sites in MIP matrix which increase the selectivity of sensor to STR.

To prove the performance of the suggested sensor in practical applications, STR determination in human serum samples was tested. The real sample was spiked with STR standard solutions at certain concentration and the samples preparation was described in experimental section. The detection results were listed in Table 3.The result determines that this sensor was applicable and reliable. Thus, as a result it can be suitable to be used for detection in real sample matrices.

Table 3. Results of STR determination in human serum sample (n = 3)

Sample	Added (mol L^{-1})	Amount recovery	Recovery (%)	Average recovery	RSD%
1	-	-	-	99.16	-
2	2×10^{-7}	2.07×10^{-7}	103.56		3.7
3	5×10^{-7}	4.783×10^{-6}	95.66		4.1
4	10^{-6}	0.982×10^{-6}	98.2		3.5

4. CONCLUSION

In this work, we presented a selective and sensitive voltammetric sensor based on modified Pt electrode with graphene nanoparticles and MIP for recognition of STR in human serum sample. Graphene in preparation of the sensor were used because of high electrical conductivity and large surface areas as the platform of the polymer. The MIP was synthesized by precipitation polymerization, using STR hydrochloride as a template molecule, MAA as a functional monomer and EGDMA as a cross-linking agent that cause to high selectivity and sensitivity towards STR. The designed sensor showed a good linear relationship over the range from1.0×10^{-8} to 1.0×10^{-6} mol L-1 (R^2=0.985) with a detection limit of 7×10^{-9} mol L^{-1}. This sensor was successfully applied to the detection of STR in human serum sample.

REFERENCES

[1] T. Gornik, A. Vozic, E. Heath, J. Trontelj, R. Roskar, D. Zigon, and et al. Pollution 256 (2020) 113431.

[2] A. K. Attia, N. S. Rashed, and O. A. Mohamed, American J. Chem. Appl. 5 (2018) 35.

[3] N. Alizadeh and E. Barati, Anal. Methods (2020) DOI: 10.1039/C9AY02001B.

[4] E. S. Koçoğlu, S. Bakırdere, and S. Keyf, J. AOAC Int. 99 (2016) 1527.

[5] S. Tajik, M. Safaei, and H. Beitollahi, Measurement 143 (2019) 51.

[6] T. Rosado, A. Gonçalves, A. Martinho, G. Alves, A. P. Duarte, and F. Domingues, Chromatographia 80 (2017) 301.

[7] A. Babaei, M. Afrasiabi, and A. Yousefi, Anal. Bioanal. Electrochem. 11 (2019) 1.

[8] T. Ramon-Marquez, A. L. Medina-Castillo, A. Fernandez-Gutierrez, and J. F. Fernandez-Sanchez, Biosens. Bioelectron. 82 (2016) 217.

[9] M. Khater, Y. Issa, H. Hassib, and S. Mohammed, J. Adv. Res. 6 (2015) 459.

[10] M. Shahlaei, B. Rahimi, A. Nowroozi, M. R. Ashrafi-Kooshk, K. Sadrjavadi, and R. Khodarahmi, Chemico-biological Interacins 242 (2015) 235.

[11] S. Dermiş and H. Cay, Die Pharmazie-An Int. J. Pharm. Sci. 65 (2010) 182.

[12] H. P. Nouws, C. Delerue-Matos, A. A. Barros, and J. A. Rodrigues, J. Pharm. Biomed. Anal. 39 (2005) 290.

[13] A. Khraiwesh, I. Papoutsis, P. Nikolaou, C. Pistos, C. Spiliopoulou, and S. Athanaselis, J. Chromatogr. B 879 (2011) 2576.

[14] E. Weisskopf, A. Panchaud, K. A. Nguyen, D. Grosjean, J.M. Hascoët, C. Csajka, and et al., J. Chromatogr. B 1057 (2017) 101.

[15] H. Cheng, J. Liang, Q. Zhang, and Y. Tu, J. Electroanal. Chem. 674 (2012) 7.

[16] S. A. Zaidi and J. H. Shin, Int. J. Electrochem. Sci. 9 (2014) 4598.

[17] T. Alizadeh and S. Azizi, Biosens. Bioelectron. 81 (2016) 198.

[18] T. C. Pereira and N. R. Stradiotto, Microchim. Acta 186 (2019) 764.

[19] Y. Panahi, A. Motaharian, M. R. M. Hosseini, and O. Mehrpour, Sens. Actuators B: Chemical 273 (2018) 1579.

[20] A. Motaharian, M. R. M. Hosseini, and K. Naseri, Sens. Actuators B: Chemical 288 (2019) 356.

[21] M. Akhoundian, T. Alizadeh, M. R. Ganjali, and F. Rafiei, Biosens. Bioelectron. 111 (2018) 27.

[22] S. Nayeri and T. Alizadeh, Anal. Bianal. Electrochem. 10 (2018) 840.

[23] S. Stankovich, D. A. Dikin, R. D. Piner, K. A. Kohlhaas, A. Kleinhammes, and Y. Jia, Carbon 45 (2007) 1558.