Asian Journal of Nanoscience and Materials, 2018, 1(2), 63-73.

FULL PAPER

Ultra-sensitive electrochemical on-line determination of Clarithromycin based on Poly(L-Aspartic Acid)/Graphite Oxide/Pristine Graphene/Glassy Carbon Electrode

Navid Rabiee[a], Moein Safarkhani[a], Mohammad Rabiee[b,c]*

[a]Department of Chemistry, Shahid Beheshti University, Tehran, Iran

[b]Biomaterials Group, Faculty of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran

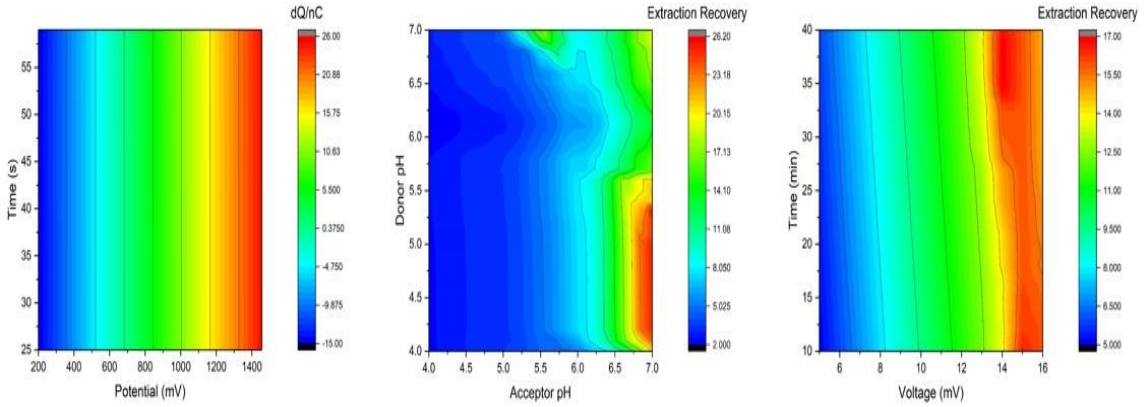
[c]Engineering Institute of Medical Diagnosis Systems, AmirKabir University of Technology, Tehran, Iran

Received: 20 January 2018, Revised: 09 March 2018 and Accepted: 11 March 2018

ABSTRACT: In this work, a novel and extra sensitive method for on-line monitoring of Clarithromycin in the whole blood sample was introduced based on coupling of electro-membrane extraction (EME) and stripping fast Fourier transform continuous cyclic voltammetry (SFFTCCV). In this method, the potential waveform was continuously applied on a Poly(L-Aspartic Acid)/Graphite Oxide/Pristine Graphene/Glassy Carbon Electrode and the electrode response was obtained by detracting the background current and also in the following integration with the current one in the specific potential range of oxidation of the analyte. This method was performed by applying a DC potential and migration of Clarithromycin from the sample solution into a layer of 4-methyl-2-pentanol that is immobilized in the pores of the sheet membrane and then migrated into the acceptor solution. A low and valuable detection limit of 1.0 ng ml-1 and quantification limit of 6.0 ng ml-1are considered as a part of the sensible results of this experiment. Furthermore, an efficient linearity in the range of 6.0-1000 ng ml-1 was found.

KEYWORDS: Clarithromycin, electrochemical, on-line determination, pristine graphene, Poly(L-Aspartic Acid).

GRAPPHICAL ABSTACT:



Introduction

Clarithromycin as an antibiotic to treat bacterial infections such as *streptococcus pyogenes*, *Helicobacter pylori*, and *Moraxelacatarhallis*etc was developed at

*Corresponding author: Mohammad Rabiee, Email: mrabiee@aut.ac.ir, Tel: 00218926622741

Asian Journal of Nanoscience and Materials

the late twentieth century. There is an indispensable requirement to find a simple and economical rout or to make an appropriate technique for its determination in various biological systems. Up to now, different methods in various biological matrices have been developed but according to our knowledge, the electrochemical methods are cost-effective and simpler.

Due to cases such as sensitivity of blood and microorganisms, finding the optimum technique to utilize an effective sample preparation to detect is a challengeable field. Among various techniques, hollow fiber liquid phase micro-extraction (HF-LPME) is known as a very effective and simple method which enhances significant pre-concentration factors and also the sensitivity. In other words, there are two phases in which the donor phase belongs to aqueous phase and the acceptor one belongs to organic solvent. These two phases in HP-LPME have a close physical contact via the pores of the membrane. During the last decade, researchers focused on a new HF-LPME technique as a novel concept for rapid sample preparation of biological fluids which is based on Elektrokinetic migration across artificial liquid membranes due to the fact that kinetic should be considered as a critical factor in all possible path of mass transport. This method can be described as HF-LPME except some changes such as platinum electrodes which take place inside the lumen of the hollow fiber[1, 2]. Combination of this method with electrochemical methods have been introduced as a promising technique to determine some compounds.

Recently, the novel technique based on the combination of stripping fast Fourier transforming continuous cyclic voltammetry (SFFTCCV) with Electro-membrane extraction (EME) for the extraction and electrochemical determination in a blood sample was introduced[3]. In the present work, the simple approach to boost the speed of analysis was used to determine the Clarithromycin in a blood sample. To be more precise, GO/PG was prepared by an economical and quick method and successfully modified on GCE, then P(L-Asp) was successfully modified on the surface and applied in fast Fourier transform continuous cyclic voltammetry-based system to determine Clarithromycin in the whole blood sample. In fact few researches on modifying the glassy carbon electrode with a novel composite based on GO/PG for ultra-sensitive determination techniques have been reported. In addition, this combination leads to take an efficient sample preparation technique and ultrasensitive detection, which in turn results to increase the lifetime of the electrode. As a result, a central composite design (CCD) was pragmatic for optimization of the critical parameters successfully leading to determine Clarithromycin in a blood sample[4, 5].

1. Experimental

1.1. Chemicals and materials

Clarithromycin was obtained from Hakim Pharmaceutical Company, Iran. L-Aspartic acid was obtained from Pharmacos Ltd, England.4-methyl-2-pentanol (98%), Graphite powder (particle size $\leq$ 30µm), sodium hydroxide (98%), ethanol (96%), orthophosphoric acid (85%, w/w) and sodium citrate ($Na_3C_6H_5O_72H_2O$) were purchased from Sigma-Aldrich Chemie GmbH, Germany. Polytetrafluoroethylene (PTFE) sheets with diameter of 20 mm were purchased from Darmstadt, Germany. Ultra-pure water was obtained from Milli-Q water purification system (Millipore, Spain).

1.2. Apparatus and software

XPS (PHI-5000 Versaprobe X-ray photoelectron spectrometer) was applied to characterize pristine graphene (PG). A Potentiostat-Galvanostat Model PG581 and Autolabpotentialstat PGSTAT 30 (Eco Chemie, Netherlands) were used for

Asian Journal of Nanoscience and Materials

voltammetry measurements. PG modified glassy carbon electrode (GCE) was utilized for electrochemical detection. DigiElch electrochemical simulation software and electrochemical software based on MATLAB were coupled to ORIGIN 2017 and were also applied to process the attained data.

1.3.The preparation of pristine graphene (PG)

1.5 g of graphite powder and 3 g of $Na_3C_6H_5O_72H_2O$ were added in 100 ml DMSO and the mixture was placed in ultrasonic bath with a frequency of 40 KHz for 4 hours at room temperature and ambient pressure. The resulted dispersion was centrifuged (7500 rpm for 20 minutes). Moreover, the aggregation washed with ultrapure water and centrifuged (7500 rpm for 5 minutes) and, then, the sediment was collected. Consequently, 1.52 mg $mL^{-1}$was obtained. As XPS analysis results shown (Fig. 1), The Pristine Graphene was prepared properly.

1.4.The preparation of Graphite oxide (GO)

Graphite oxide was synthesized by sonication of the natural graphite powder (a particle size of ≤ 30 μm) in water for 1.5 hour.

1.5. The preparation of binary GO/PG composite

First of all, the resultants from PG preparation and GO preparation were solved in their solvents. Then, 2 mg ml^{-1} of GO/H_2O and 1 mg ml^{-1} of PG/DMSO were directly mixed to obtain GO/PG composite with 3:2 mass ratio and highly controlled volume ratio of 3:4, afterwards, another sonication was applied for 45 minute. Subsequently, the resulted dispersion was centrifuged (7500 rpm for 15 minutes).

For comparison, poly(l-aspartic acid)/electrochemically which reduced graphene oxide composite were prepared similarly.

1.6.Procedure for electro-membrane extraction

The extraction cell that includes two glass compartments with two PTFE O-rings part clamped together and this membrane is sandwiched between them. To inoculate the solvent in the pores of the flat sheet membrane, first the surface was purified carefully and, then, dipped in 4-methyl-2-pentanol (MIBC) for 15 minute and in the next step 14 ml of the sample solution which consists of 0.1 M phosphate buffer with pH 4.0 and 80 ng ml^{-1} of Clarithromycin and the acceptor solution consisting of 6.0 ml of 0.1 M phosphate buffer with pH 7.0 were conveyed into the cells. Also, gold wires were used in association with DC power supply to obtain different voltages within the specific range across the supported liquid membrane (SLM). During the on-line SFFTCCV measurement of Clarithromycin, both of the solutions were stirred at 800 rpm for 35 minute.

1.7.The preparation of the solutions

A stock solution that contains 80 mg ml^{-1} of the analyte was prepared and maintained at -4^o C. this solution should be preserved from the light carefully. The blood sample was provided from a volunteer (24-year-old man) with respect to human ethical guidelines and stored at 4^o C. The blood sample was diluted with a ratio of 1:3 using triple distilled water. Also the pH value was carefully controlled and directly used in EME-SFFTCCV system.

1.8. The preparation of PG modified glassy carbon electrode

The glassy carbon electrode (GCE) was polished with 0.8 μm alumina powder and sonicated in 50 vol% ethanol/water for 1 minute. After that, the electrode was sonicated with ultrapure water for 2 minutes. The resultant PG was coated on GCE surface by drop casting and dried at room temperature. In addition, Electrochemical Impedance Spectroscopy (EIS) has been

applied to prove the PG/GCE preparation (Fig. 2)

1.9. The preparation of GO/PG modified glassy carbon electrode

The glassy carbon electrode (GCE) was polished with 0.8 µm alumina powder and sonicated in 50 vol% ethanol/water for 1 minute. After that, the electrode was sonicated with ultrapure water for 2 minutes. The resultant GO/PG was coated on GCE surface by drop casting and dried at room temperature.

1.10. The preparation of the modified GO/PG glassy carbon electrode with poly(l-aspartic acid)

The GO/PG/GCE was placed in 2 mM l-aspartic acid monomer solution and cyclic scanning in the range of -1.0-1.8 V at a scan rate of 100 mV s^{-1} for 30 cycle in pH 6.0 PBS was applied to growing poly(l-aspartic acid). The resultant electrode was washed with ultrapure water and then dried at room temperature.

2. Calculation of pre-concentration factor, extraction recovery, relative recovery and error

The pre-concentration factor (PF) was described as the ratio between the final analyte concentration in the acceptor phase ($C_{f,a}$) and the initial concentration of analyte ($C_{i,s}$) in the sample solution:

$$PF = \frac{C_{f,a}}{C_{i,s}} \quad (1)$$

Wherein, $C_{f,a}$was calculated from a calibration graph that was obtained from direct electrochemical determination of Clarithromycin standard solutions (5.0-500 ng ml^{-1}). Relative recovery (*RR%*), accuracy (*Error%*) and extraction recovery (*ER%*) were calculated by the equations below:

$$RR(\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (2)$$

$$\text{Error}(\%) = RR(\%) - 100 \quad (3)$$

$$ER(\%) = \frac{n_{f,a}}{n_{i,s}} \times 100 = \frac{C_{f,a} \times V_{f,a}}{C_{i,s} \times V_{i,s}} \times 100 \quad (4)$$

Where C_{found} is the concentration of analyte after addition of the known amount of the standard into the real sample, C_{real} is the concentration of the analyte in the real sample and C_{added}isthe concentration of the known amount of the standard that is spiked into the real sample. Also, the extraction recovery (*ER%*) is described as the ratio of the extracted moles of analyte in the acceptor phase ($n_{f,a}$) to those originally present in the sample solution ($n_{i,s}$). $V_{f,a}$ and $V_{i,s}$ are the volumes of the acceptor phase and the sample solution respectively.

3. Results and discussion

3.1. Characterizations of PG, GO, PG/GCE and P(L-Asp)/GO/PG/GCE

The elemental substance of graphite and PG were analyzed by XPS (Fig. 1). The analysis results represented that carbon contents are 95.28 and 96.83 atom% for PG and graphite respectively. In addition, oxygen contents are 4.72 and 3.17 atom% respectively. It should be noted that the resultant PG has high carbon content and low oxygen content.

Asian Journal of Nanoscience and Materials

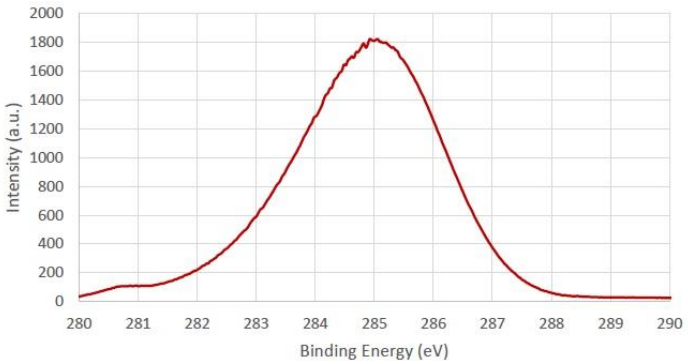


Fig. 1. The C 1s peak (XPS) of PG.

Electrochemical impedance spectroscopy (EIS) results of GCE and PG/GCE in 0.1 M KCl containing 10 mManalyte represented that the high frequency is applied the electron transfer resistance at the surface of the electrode (Fig. 2). Also, there is no charge transfer resistance on PG/GCE surface coming from the fast electron transfer on this modified electrode surface. In addition, we have estimated that the surface area of the electrode was potentially activated. The estimated data has shown that about 0.164 cm^2 of the activated surface area of PG/GCE and GCE with a diameter of 0.3 cm are used.

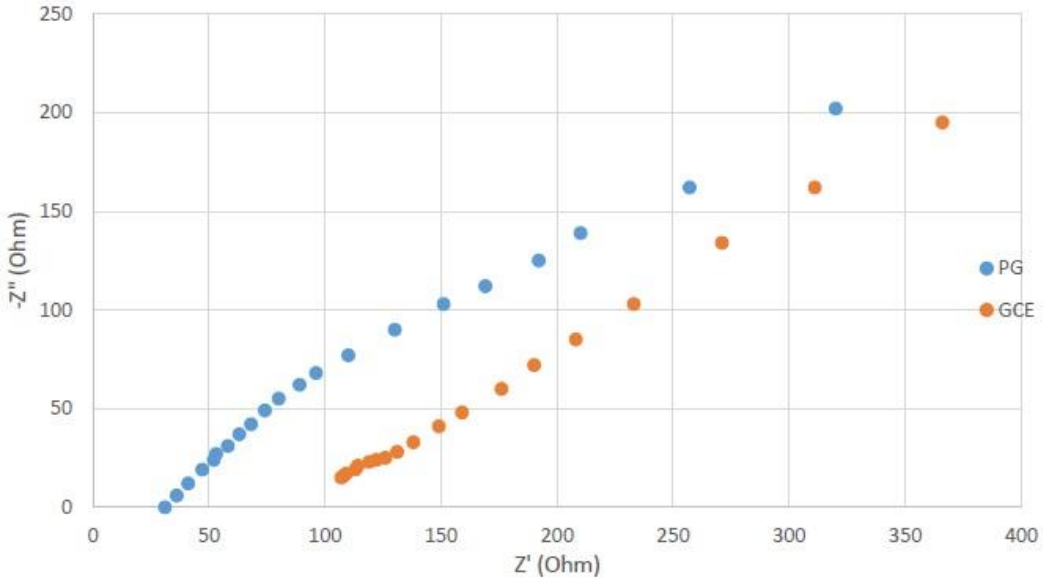


Fig. 2. EIS of GCE and PG/GCE in 0.1 M KCl containing 10 mManalyte.

By applying UV-Vis spectroscopy, the spectral band of l-aspartic acid is observed at 202 nm. Also, the spectral band of GO is observed at 226 nm which is assigned to π-π* transition of the aromatic C-C bonds and the shoulder assigned as n-π* transition of the C=O bond. For the P(L-Asp)/ERGO composite in comparison to GO, slightly red shift is observed which confirmed the electrochemical reduction of GO and formation of the composite (Fig. 4).

Asian Journal of Nanoscience and Materials

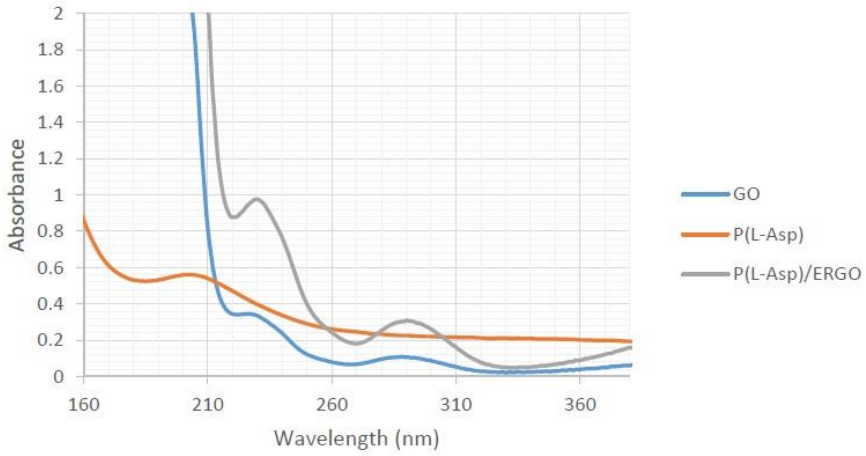


Fig. 3. The UV-Visible spectra of GO, P(L-Asp) and P(L-asp)/ERGO

3.2.Electrochemical optimization procedure of determination of Clarithromycin

Initially, the consistent factors were optimized and investigated electrochemically in order to obtain the signals and survey the sensitivity. Then, with the results, the factors which has significant impact on the EME-SFFTCCV method were investigated. In the next stage, the results were extended to blood sample.

3.2.1. Voltammetric behavior of Clarithromycin at the surface of Electrode

In this step, Cyclic voltammetry was used for investigation of the electrochemical behavior of 80 ng ml^{-1} of Clarithromycin at the surface of P(L-Asp)/GO/PG/GCE in 0.1 M of phosphate buffer solution with pH 7.0 (Fig. 3). It is crystal clear that there is a peak current in the presence of the Clarithromycin which appears at 1455. To be exact, the voltammogram of Clarithromycin at the surface of P(L-Asp)/GO/PG/GCE showed two anodic peaks which come from the occurrence of oxidation process to the two exposed OH- groups. By increasing the pH above 4.0, just one anodic peak was observed which came from the solubility of Clarithromycin affected by the pH.

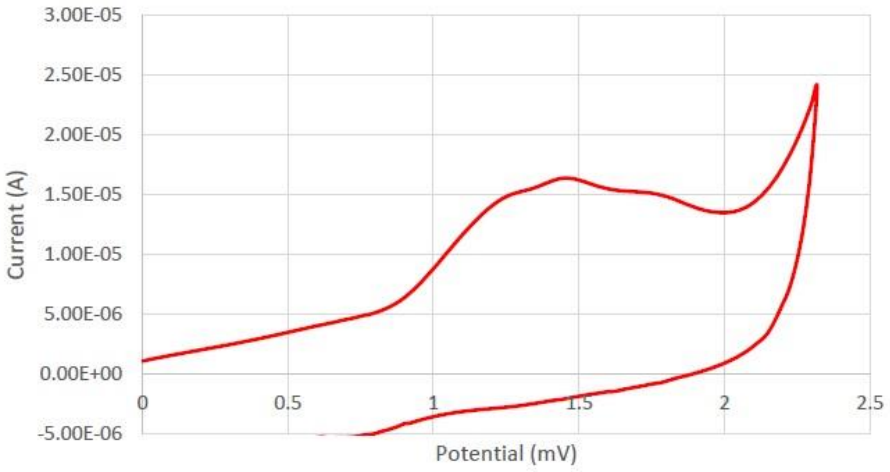


Fig. 4.Voltammogram of 80 ng ml-1 Clarithromycin on the P(L-Asp)/GO/PG/GCE at pH 7.0.

In addition, the resulted changes in the voltammetric responses of the P(L-Asp)/GO/PG/GCE were investigated while adding Clarithromycin solution into the

Asian Journal of Nanoscience and Materials

phosphate buffer with pH 7.0. There is a time axis that represents the time of a run. To obtain these data, a potential waveform was continuously applied several times. These data are in good agreement with the oxidation peak of Clarithromycin obtained from cyclic voltammetry. In this technique, the electrode response can be defined as the charge changes (ΔQ) during the correct potential scans and limited potential range, E_1 to E_2 (200-1500) as follows:

$$\Delta Q_n = Q_n - Q_{ave2} \quad (5)$$

$$\Delta Q(st) = \Delta t\left[\sum_{E=E_1}^{E=E_2} \left|i(s,E) - i(s_r,E)\right|\right] \quad (6)$$

Where Q_{ave} and Q_n are the calculated average charges at the specific potential range, s is the sweep number, t is the time period between the further potential scan, Δt is the time difference, $i(s,E)$ showed the current of the cyclic voltammetry during the s-th scan and at last $i(s_t,E)$ showed the reference current of the cyclic voltammetry. The reference cyclic voltammetry was obtained by averaging four cyclic voltammetry's before addition of the analyte.

3.3. Optimization of electromembrane composition

3.3.1. Effect of liquid membrane composition

Based on recent research, the chemical nature of the SLM was remotely related to the success of this technique, on the other side, long-chain alcohols with higher number of Hydrogen donor groups are suitable solvents for the extraction of these compounds[6]. In this work, 4-methyl-2-pentanol have been chosen due to their water-immiscible feature and enough compatibility with the wall of the hollow fiber for determination of Clarithromycin by EME[7].

3.3.2. The effect of time and voltage in extraction recovery

In EME system, time and voltage investigations have a critical role for providing an efficient extraction. Enhancing both of these factors would increase the extraction efficiency[8]. Besides, application of high voltages could restrict applying long extraction period and vice versa. In addition, to iron out this issue, these factors were simultaneously optimized using a central composite design (CCD) and Design-Expert software version 8.0 (Stat-Ease Inc, MN, USA) was applied to generate the matrix and investigate the results. One considerable type of CCD is face-centered design (FCCD) which include 11 experiments with three central points that α, star point, defined as unity[9].

The obtained results are represented using response surface methodology (RSM). Three dimensional recovery percentages of 80 ng ml^{-1} Clarithromycin show that the recovery percentage was increased by increasing the extraction time. On the one hand, voltage reached to the maximum value after 35 minute with an applied voltage of 14.0 V. On the other, the possible electrolysis reactions in the both acceptor and donor phases leads to decreasing the recovery percentage (Fig. 5-7)[10, 11].

Asian Journal of Nanoscience and Materials

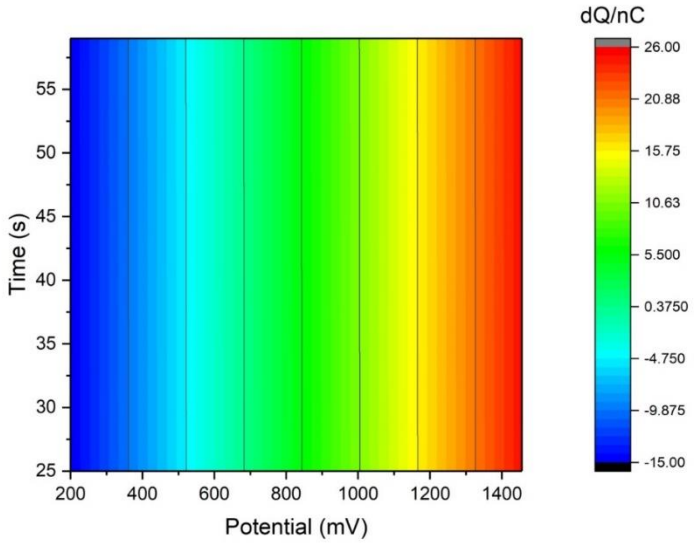


Fig. 5. The contour plots of redox behavior of 80 ng ml-1 Clarithromycin in SFFTCCV on the electrode in 0.1 M phosphate buffer pH 7.0

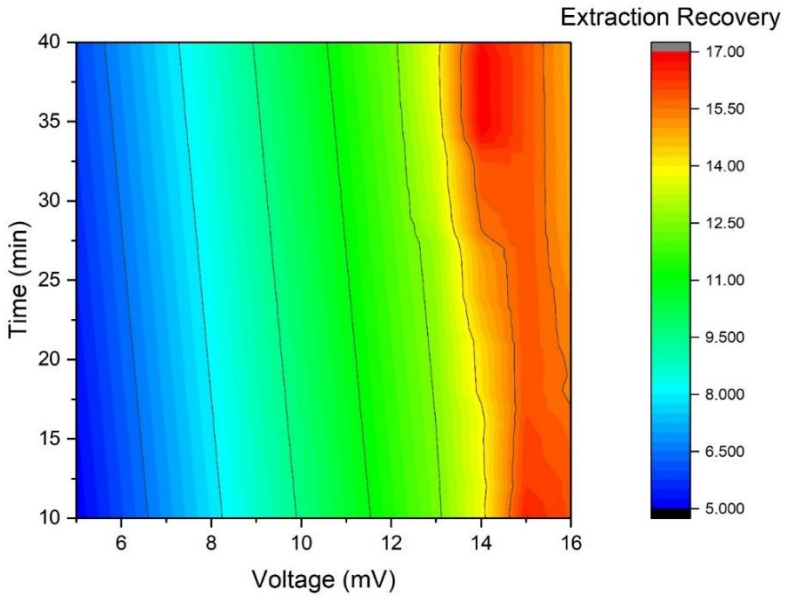


Fig. 6. The contour plots of recovery percentage against time and voltage for extraction of 80 ng ml-1 Clarithromycin

Asian Journal of Nanoscience and Materials

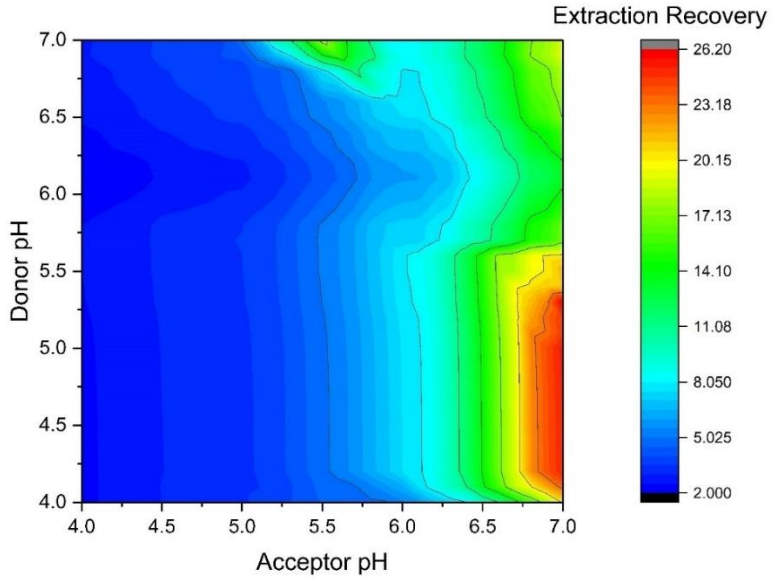


Fig. 7. The contour plots of recovery percentage against pH of acceptor and donor phase for extraction of Clarithromycin, applied voltage: 14.0 V, extraction time: 35 minute.

3.3.3. pH of acceptor and donor phases

For this intent, the electrode response to Clarithromycin was measured in the presence of phosphate buffer solutions within different pHs in the range of 4.0-7.0 as shown in Fig. 7. It is vividly depicted that the maximum recovery was obtained at the pH range of donor and acceptor phases, 4.0 and 7.0 respectively. In this sense, Clarithromycin ions remain in their anionic form and the detectable contest among the analyte and the hydroxide ions become manifested during the procedure. There is some recombination which leads to enhancing the electrolysis reactions in both the acceptor and donor phases coming from high concentration of OH^- ions[12, 13].

3.4. Analytical performance

After precise evaluation on effective factors, applied voltage, extraction time, SLM and pH, the optimized condition were set up to investigate for determination of Clarithromycin in the biological matrices. Limit of detection (LOD) and Limit of quantification (LOQ) were calculated precisely and found to be 1.0 ng ml^{-1} and 6.0 ng ml^{-1}, respectively. According to the results that obtained from the calibration curve, which was plotted in a concentration range of 6-1600 ng ml^{-1}, a plausible linearity in the range of 6.0-1000 ng $ml^{-1}$was observed as shown in Fig. 8.

Asian Journal of Nanoscience and Materials

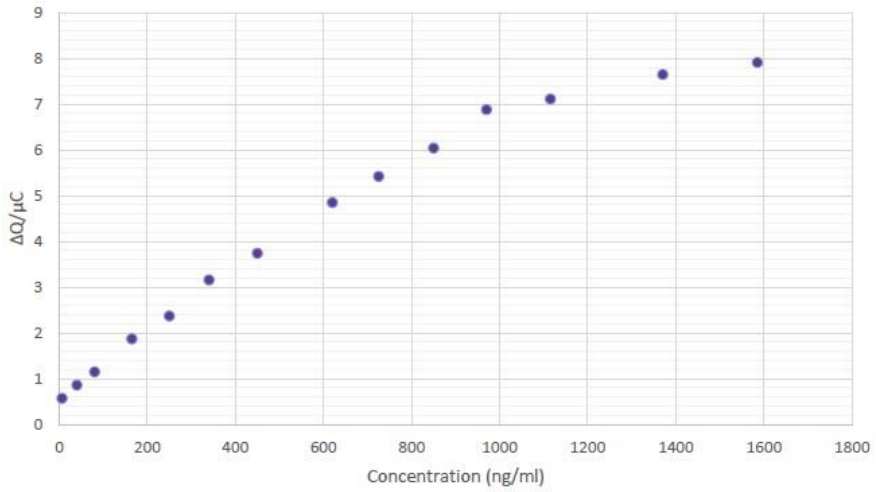


Fig. 8. Calibration curve applied for electrochemical determination of Clarithromycin under the optimum extraction conditions

3.5.Analysis of real sample

The optimized condition was obtained in term of critical effective factors to evaluate the applicability of the EME-SFFTCCV technique in order to determin Clarithromycin in whole blood sample (Table 1).

Table 1. Determination of Clarithromycin in whole blood sample		
Sample		
The Sample	C_{real}(ng ml^{-1})	Not Detected
	C_{add}(ng ml^{-1})	15
	C_{found}(ng ml^{-1})	14.2
	RR%	94.6
	Error%	0.53
	RSD%	8.81

Conclusions

In the present work, an ultra-sensitive method for online continuous monitoring of Clarithromycin in whole blood sample was introduced based on a novel electrode, P(L-Asp)/GO/PG/GCE. This method is based on combination of SFFTCCV and EME to optimiz an efficient sample preparation and enhancing the accuracy and sensitivity of this electrochemical detection technique.Also, GO/PG was prepared by an economical and quick method and was successfully modified on GCE, then P(L-Asp) was successfully modified on the surface and applied in fast Fourier transform continuous cyclic voltammetry-based system to determine Clarithromycin in whole blood sample.Furthermore, the high sample cleanup potential of EME in biological systems leads to enhanc the lifetime of the electrode and reducing the time consuming of sample preparation methods. A low and valuable detection limit of 1.0 ng ml^{-1} and quantification limit of 6.0 ng $ml^{-1}$are considered as a part of the sensible results of this experiment in which the P(L-Asp)/GO/PG/GCE has a critical role to optimize the quantification limit and of course it would be a promising choice for using in biological matrices. Consequently,

Asian Journal of Nanoscience and Materials

based on the obtained results and optimized parameters such as pH, time, extraction recovery, voltage and liquid membrane composition, this method could be able to be considered as a promising technique for determining such antibiotics and also similar compounds in the biological matrices.

Acknowledgement

We also acknowledge the AmirkabirUniversity of Technology for its support and help in this research.

References:

1. Lee, J., Lee, H. K., Rasmussen, K. E., & Pedersen-Bjergaard, S. (**2008**). *Anal. Chim. Acta*, 624: 253–268.

2. Oliveira, É. C., Echegoyen, Y., Cruz, S. A., & Nerin, C. **(2014)**. Talanta, 127: 59–67.

3. Mofidi, Z., Norouzi, P., Seidi, S., & Reza Ganjali, M. (**2017)**. *New J. Chem.*, 41: 13567–13575.

4. Tiwari, J. N., Vij, V., Kemp, K. C., & Kim, K. S. (**2016**). *ACS Nano*, 10: 46–80.

5. Rodríguez-Pérez, L., Herranz, M. Á., & Martín, N. **(2013)**. *Chem. Commun.*, 49: 3721.

6. Balchen, M., Gjelstad, A., Rasmussen, K. E., & Pedersen-Bjergaard, S.(**2007*)***. *J. Chromatogr. A*, 1152: 220–225.

7. Nojavan, S., & Asadi, S. (**2016**). *Electrophoresis*, 37: 587–594.

8. Seidi, S., Yamini, Y., Heydari, A., Moradi, M., Esrafili, A., & Rezazadeh, M.(**2011)**. *Anal. Chim. Acta*, 701: 181–188.

9. Goel, T., Haftka, R. T., Shyy, W., & Watson, L. T. **(2008)**. *Int. J. Numer. Methods Eng.*, 75: 127–155.

10. Gjelstad, A., Rasmussen, K. E., & Pedersen-Bjergaard, S. (**2009*)***. *Anal. Bioanal. Chem.*, 393: 921–928

11. Gjelstad, A., & Pedersen-Bjergaard, S. **(2011)**. *Bioanalysis*, 3: 787–797.

12. Restan, M. S., Jensen, H., Shen, X., Huang, C., Martinsen, Ø. G., Kubáň, P., Gjelstad, A., & Pedersen-Bjergaard, S. (**2017)**. *Anal. Chim. Acta*, 984: 116–123.

13. Román-Hidalgo, C., Martín-Valero, M. J., Fernández-Torres, R., Callejón-Mochón, M., & Bello-López, M. Á. **(2017)**. *Talanta*, 162: 32–37.

How to cite this manuscript: Navid Rabiee, Moein Safarkhani, Mohammad Rabiee*. Ultra-sensitive electrochemical on-line determination of Clarithromycin based on Poly(L-Aspartic Acid)/Graphite Oxide/Pristine Graphene/Glassy Carbon Electrode. Asian Journal of Nanoscience and Materials, 2018, 1, 63-73.