Multivariate optimization of mebeverine analysis using molecularly imprinted polymer electrochemical sensor based on silver nanoparticles

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ABSTRACT

Thin film of a molecularly imprinted polymer (MIP) based on electropolymerization method with sensitive and selective binding sites for mebeverine (MEB) was developed. This film was cast on pencil graphite electrode (PGE) by electrochemical polymerization in solution of pyrrole (PY) and template MEB via cyclic voltammetry scans and further electrodeposition of silver nanoparticles (AgNPs). Several parameters controlling the performance of the silver nanoparticles MIP pencil graphite electrode (AgNPs-MIP-PGE) including concentration of PY(mM) concentration of mebeverine (mM), number of cycles in electropolymerization, scan rate of CV process (mV. s⁻¹), deposition time of AgNPs on to the MIP surface (s), stirring rate of loading solution (rpm), electrode loading time (min), pH of Britton–Robinson Buffer (BRB) solution were examined and optimized using multivariate optimization methods such as Plackett–Burman design (PBD) and central composite design (CCD). Two dynamic linear ranges of concentration for the MIP sensor were obtained as $1 \times 10^{-9}$ to $1 \times 10^{-6}$ and $1 \times 10^{-5}$ to $1 \times 10^{-3}$ M with the limit of detection (LOD) of $8.6 \times 10^{-9}$ M (S/N = 3). The proposed method was successfully intended for the determination of MEB in real samples (serum, capsule). The sensor was showed highly reproducible response (RSD 1.1%) to MEB concentration.

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1. Introduction

MEB belongs to a category of antispasmodics known as musculotropic drugs and is used largely in treatment of irritable bowel syndrome and gastrointestinal spasm secondary to organic disorder [1,2]. Several methods have been reported in literature for the determination of MEB (Scheme I) either in bulk powder or in pharmaceutical formulations. The following techniques have been described: spectrophotometric methods [3,4], electrochemical methods [5,6] and chromatographic methods [7,8]. MIP technique is becoming a more
commonly accepted and useful method for the selective recognition and isolation of key biological target molecules. The preparation of polymeric materials with tailor made molecular recognition sites owes the capability to distinguish target molecules through their size, shape and functional group distribution. MIP is a field of fast growing interest and has been applied in variety of applications, especially in sensors [9]. Conducting polymers offer a wide platform for chemical sensing and can be used as solid contact electrodes. Efficient electron transfer ability, manageable and homogeneous film character, reproducibility and easy production, availability of various types of monomers, stability and biocompatibility, and ability to modify physical and optical properties are some of many reasons for the use of conducting polymers in biosensor design [10,11]. Among different conducting polymers, polypyrrole (PPy) has particularly attracted attention. Pardieu et al. [12] designed molecularly imprinted conducting polymer (MICP) sensor for atrazine with poly (3,4-ethylene dioxythiophene, co-thiophene acetic acid) film coated on platinum electrode. Meanwhile, the emergence of nanotechnology is opening new horizons for highly sensitive electrochemical assays [13,14]. By incorporation with nanoparticles (NPs), electrochemical sensors have shown great promise for diagnosis of trace molecules because the nanoparticle based amplification platforms and amplification processes have been reported to dramatically enhance the intensity of the electrochemical signal and lead to ultrasensitive assays. Two approaches have been developed for NPs-based electrochemical sensors: (1) NPs are directly used as an electroactive reporter. For example, metal NPs [15,16] and semiconductors have been used as electroactive labels to amplify electrochemical detection. (2) NPs are used as carriers to load a large amount of electroactive species, e.g., ferrocene, as reporter’s markers for amplifying detection of molecules [17,18]. In this work, MEB sensor based on pyrrole as functional monomer was fabricated by electropolymerization. Further deposition in AgNPs solution, a bi-layer of AgNPs and imprinted polypyrrole (AgNPs-PPY) film was constructed. We examined the formation of specific and selective binding sites for MEBin the AgNPs-PPY matrix. Recently, some statistical designs of experiment methods have been employed in optimization of electrochemical sensors [19,20]. Using statistical design of experiment imparts advantages, as it allows one to obtain the optimum conditions through relatively smaller numbers of experiments. A proper design matrix can lead to obtain a regression equation which highlights effect of individual factors and their relative importance in given operation process. The possibility of evaluating the interaction effect between the variables on the response can also be known which are not readily possible in a classical method [21]. Until now, a few reports of the optimization of voltammetric response using response surface methodology (RSM) have been published [22]. Nevertheless, there is lack of reports on optimization of voltammetric response of modified electrode for determination of MEB using RSM.

2. Experimental

2.1. Reagents and chemicals

Deionized double distilled water was used throughout the experiment. Boric acid, acetic acid sodium hydroxide, phosphoric acid, nitric acid, hydrochloric acid, potassium chloride, potassium nitrate and PY, were purchased from Merck (Darmstadt, Germany). Donpezile chloroquine (Pars DarouCompany, Iran), levetiracetam, venlafaxine, MEB(Samisaz Company, Iran) ketorolac (SajadDarouCompany, Iran), naproxen (Pars DarouCompany, Iran) mequinol (DarouPakhshCompany, Iran), and other reagents were commercially available as analytical grade. Britton-Robinson buffer solution (BRB) was used as a supporting electrolyte. Stock solutions of MEB and buffer solution were prepared in deionized double distilled water.

2.2. Apparatus

Differential pulse voltammetry, cyclic voltammetry and chronoamperometry experiments were performed using a three electrode cell assembly consisted of a MIP-modified PGE, a platinum wire, and an Ag/AgCl (saturated KCl) as working, counter, and reference electrodes, respectively. All measurements were carried out both at bare and modified PGEs, using an Autolab PGSTAT 12 potentiostat–galvanostat controlled by GPES 4.9 software (Ecochemie, The Netherlands). Morphological images of MIP–modified PGE surfaces were obtained using field emission scanning electron microscope (FESEM) (Tescan –Mira (ui) Czech Republic).

2.3. Software

Minitab 16 softwares was used for experimental designs, statistical evaluation and model fitting in this work.
2.4. Fabrications of the imprinted AgNPs-MIP-PGE

A Noki pencil model 2000 (Japan) was used as a holder for graphite leads (HB 0.5 mm diameter, Japan). PGEs were washed with water and methanol to remove the impurity and dried at room temperature before the experiments. Then, 2 cm of PGE was immersed into a solution containing supporting electrolyte containing KCl, 0.1 M, BRB solution (0.04 M of boric acid, acetic acid and phosphoric acid) pH 6.5, functional monomer (pyrrole, 50 mM) and template (MEB, 5 mM). The CV technique was performed from −0.75 V to +1.30 V for 20 cycles at a scan rate of 16 mVs⁻¹ to obtain the polymer-modified -PGE. The electrode was rinsed thoroughly with ethanol, and then immersed in a mixture solution of KNO₃ (0.1 M) and AgNO₃ (3 mM). AgNPs were formed on the imprinted electrode by potentiostatic electrodeposition with deposition potential of −0.4 V and duration time of 400 s. Subsequently, the embedded MEB were extracted by scanning the potential between 0.3 and 0.8 V at a scan rate of 16 mVs⁻¹ in BRB solution (pH 6.5) for several cycles until no obvious oxidation peak for MEB could be observed; this process gave AgNPs-MIP-PGE. Thus, the obtained sensor was used for the further experiments.

2.5. Electroanalytical measurements

DPV measurements were carried out in a three-electrode cell, in BRB at pH 6.5. The current measurements were performed using DPV in a potential range between 0.3 and 0.8 V. To record differential pulse voltammograms, the following conditions were used: step potential 8 mV, modulation amplitude 50 mV and scan rate 16 mVs⁻¹. All electroanalytical measurements were made at room temperature.

2.6. The interferences

The selectivity of the Ag-MIP-PGE in this work was evaluated in the presence of different interfering molecules like donpezile, chloroquine, levetiracetam, venlafaxine, ketorolac, naproxen and mequinol. To evaluate the selectivity of the sensor, the modified electrode was used at the concentrations of 10, 20 and 50 μM for each interferences in the presence of 10 μM of MEB.

2.7. Real sample analysis

MEB capsule (Iran Darou Company) was chosen. The contents of five MEB capsule was evacuated and precisely weighed in order to get the average weight of each capsule. An equivalent quantity of the powder including a known amount of active material was weighed and placed into a glass vial containing water to stir for 20 min. Finally, it was filtered to make a sample solution of MEB. A 0.5 mL human serum sample (from a local clinical laboratory) was spiked with analyte to give a working concentration of mebeverine (20, 40 and 60 μM). This sample was placed into a 1.5 eppendorf Safe-Lock microcentrifuge tubes including 0.5 mL of acetonitrile and diluted to 1.5 mL with water then vortexes for 10 s and centrifuged at 2500 rpm for 20 min to eliminate serum protein [23]. A 1.0 mL of supernatant layer was placed into an other eppendorf Safe-Lock microcentrifuge tubes 0.5 mL acetonitrile was added and centrifuged again at 2500 rpm for 20 min. A 0.50 mL of the supernatant was diluted to 10 mL with water. After 19 min adsorption, the AgNPs-MIP-PGE was washed by distilled water to wash out unwanted materials, which were chemically close to the analyte or the analyte molecules adsorbed to nonspecific binding point of MIP. Eventually, voltammetric detection was applied to record analyte current at 0.63 V.

3. Results and discussion

3.1. Preparation and characterization of AgNPs-MIP-PGE

PGE was immersed in the polymerization solution. The pre–polymer mixture made from template/functional mono- mer (molar ratio, 1:10), 50 mM of PY, 5 mM of MEB in 15 mL BRB (pH 6.5) was taken in a voltammetric cell. The resulting mixture was electropolimerized at the tip of PGE using CV technique for 20 cycles at 40 mV s⁻¹ (Fig. 1). Furthermore, NIP-PGE was fabricated following the same procedure, but in the absence of template molecules. The polymerization initially started with the electro-oxidation of PY that was conducted on to the PGE surface. Fig. 1 shows representative cyclic voltammograms related to electropolymerization of PY on the PGE surface in the presence of MEB. An oxidation peak at about 1.05 V was clearly observed on the first scan. Then, this peak decreased slightly under continuous cyclic scan. After six cyclic scan, the peak current started to be stable. Moreover, the oxidation peak potential of PY slightly shifted to more negative direction values (Fig. 1). Results indicate that PY was successfully electropolymerized onto the surface of PGE. The decrease of the peak current by increasing scan cycle seems to be related with the continual formation of PPY films that hinders PY monomer further access to the surface of modified electrode. Similar voltammograms was also obtained for aminothiophenol [24] and hydroxyphenol [25]. On the other hand, similar cyclic voltammograms for electropolymerization of PY on PGE surface had also been obtained in the absence of template indicating that MEB does not submit any effect on the electropolymerization of PY. It is well known that the sensitivity of the imprinted sensor is dictated by the amount of effective imprinted sensor imprinted sites on the sensor surface. Although the amount of the imprinted sites...

Fig. 1 – The voltammetric cycles for the preparation of AgNPs-MIP-PGE in a BRB solution (pH 6.5) containing 50 mM PY and 5 mM MEB.
increases with the increase of the imprinted film thickness, thick imprinted films can lead to slow diffusion of the analytes to the recognition sites and to inefficient communication between the binding sites and transducer [26]. So the effect of scan cycles during electropolymerization of PY on the response of MEB was investigated. Best sensitive results were observed, when the thickness of MIP film was optimized by adjusting the scanning cycles to be 20 during electropolymerization process.

3.2. Electrodeposition of Ag nanoparticles

MEB has a large molecular weight (429.6 g/mol) (Scheme I). One of the main problems faced with imprinting large molecular weight compounds is attributed to the high crosslinking required to achieve recognition. With increased crosslinking, large templates such as MEB can become entrapped in the network after polymerization. If the template molecule can not be extracted, the network is rendered useless for bulk recognition applications, as template binding only occurs on surface recognition sites [27]. It is need an effective method to overcome this obstacle therefore the extraction of mebeverine template molecules were performed electrochemically. Here, silver NPs (AgNPs) exhibits a good electrocatalytic effect can effectively catalyze the oxidation of MEB are chosen to amplify the electrochemical response because they are easy to functionalize and quite stable in aqueous environment. Among several different methods for preparation of silver nanoparticles such as chemical reduction of silver cation in the presence of stabilizers [28], layer-by-layer adsorption [29], template induction [30], electrodeless preparation [31], and electrochemical deposition [32], electrodeposition is a simple, fast and inexpensive method for preparation of metal nanoparticles possessing unique properties such as high purity of the particles, higher control over the dimension and density of particles, lower particle size distribution and a very short time scale compared to other methods [33]. Thus, in this work, we used a potentialistic electrodeposition method. Imprinted film of MEB poly pyrrole was prepared by electrochemical polymerization immersed in a mixture solution of KNO$_3$ (0.1 M) and AgNO$_3$ (3 mM) then AgNPs were formed on the imprinted electrode by potentiostatic electrodeposition with deposition potential of $-0.4$ V [34] and duration time of 400 s. Mebeverine was removed from the imprinted films electrochemically by differential pulls voltammetry (DPV) scanning between 0.3 and 0.8 V in BRB solution pH 6.5, for several times until all MEB molecules were stripped from the imprinted PPy film. For comparison, a control non imprinted polymer (NIP) electrode was prepared by the same procedure, only without the addition of template molecule in the polymerization process.

3.3. SEM characterization

SEM was applied to characterize the surface of electrodes of the MIP-PPY-PGE and the AgNPs-MIP-PGE. After electrodeposition of AgNPs, a number of nanoparticles were clearly seen (Fig. 2b). The size of AgNPs was approximately 50–80 nm and some bigger particles may be possibly caused by the aggregation but after extraction of template some of silver nanoparticles were removed from the electrode surface and it can be observed few number of very small silver nanoparticles (Fig. 2d). As shown in Fig. 2d in comparision with MIP-PPY (Fig. 2a) there was many hole on the surface of AgNPs-MIP-PGE that related to removing of mebeverine molecules from MIP matrix. AgNPs-NIP-PGE revealed somewhat compact surface (Fig. 2c).

3.4. Optimization of experimental parameters

3.4.1. Screening of significant factors using Plackett–Burman design

At the beginning of a study, we generally do not know which factors have an effective influence on the responses. Large number of factors could potentially affect the AgNPs-MIP-PGE response and therefore a PB design was used as a screening method to select the most statistically significant parameters for further optimization. The PB factorial design can identify main factors affecting the response of electrode by a relatively few experiments. A PB design for 8 factors, consisted of 12 randomized runs, was carried out. Evaluated factors were 8 factors including concentration of PY (mM) ($X_1$), concentration of MEB (mM) ($X_2$), number of cycles in electropolymerization ($X_3$), scan rate of CV process (mV s$^{-1}$) ($X_4$), deposition time of AgNPs onto the MIP surface (s) ($X_5$), stirring rate of loading solution (rpm) ($X_6$), electrode loading time (min) ($X_7$), pH of BRB solution ($X_8$). The levels of the factors as low (−) and high (+), are listed in Table 1. Table 2 shows the PB experimental design matrix together with the analytical response expressed as current peak heights. The results are mean of triplicate experiments. The statistical evaluation of the results produced the standardized main effect Pareto chart as shown in Fig. 4 and offered a minimum t-value of 2.57 at a confidence level of 95.0%. In this chart bar lengths are proportional to the absolute values of the estimated effects and t-value is included as a vertical reference line. The variables which exceeded this reference line were considered as statistically significant factors [35]. The Pareto plot shows that $X_7, X_4, X_8$ and $X_6$ are most important to the process, therefore should be studied at a greater depth. The other factors i.e., $X_1, X_2, X_3$ and $X_5$ were not significant. Furthermore, the positive and negative signs in Fig. 3 corresponding to a grey and diagonal bar filling, respectively showed that whether the response would be improved from the low to high level or not.

3.4.2. Optimization using central composite design

Based on the results of the screening design, a CCD optimization procedure was performed. Four variables including $X_1, X_2, X_3$ and $X_0$ which all significantly influenced the analytical response were simultaneously optimized using CCD and the effects as well as their mutual interactions were studied. This three level fractional factorial design allows estimation of a second order (quadratic) model with linear, quadratic and interaction terms. The CCD design with 30 experiments was carried out, consisting $2^3$ full factorial design, augmented with $2 \times 3$ axial (or star) points ($k = 1.682$) and 6 replicates of the center point. Table 3 shows the selected factors and the irdomain.e.low, central, high and axial levels. The complete design matrix and the corresponding analytical response of each run are presented in Table 3. To find the most
suitable fitting with the experimental data, a response surface model was developed using the regression analysis by considering different combinations of the linear, squared, and interaction terms in polynomial equation. The adequacy of each model was checked using the analysis of variance (ANOVA). A maximum p-value for lack-of-fit (LOF) of 0.076 (>0.05) was obtained for the following equation suggesting that the quadratic model was significant. By applying the regression analysis, a second-order polynomial equation was established to express a semi empirical model for the MIP response:

\[ I = \frac{C_0^{3.31}}{X_8^{2}} \cdot \frac{C_0^{0.03}}{X_7^{2}} + 39.35X_8 + 0.47X_7 + 0.03X_6 - 0.06X_4 + 0.12X_5X_7 + 0.03X_5X_4 - 137.45 \]

Table 1 – The experimental field definition for PBD.

| Variable                                      | Symbol | Low (–) | High (+) |
|-----------------------------------------------|--------|---------|----------|
| Concentration of PY (M)                       | X1     | 0.01    | 0.05     |
| Concentration of Mebeverine (M)               | X2     | 0.001   | 0.005    |
| Number of cycles in electro-polymerization   | X3     | 10      | 20       |
| Scan rate of CV process (mV s⁻¹)              | X4     | 50      | 100      |
| Deposition time of AgNPs onto the MIP surface (s) | X5   | 200     | 400      |
| Stirring rate of loading solution (rpm)       | X6     | 400     | 800      |
| Electrode loading time (min)                  | X7     | 5       | 15       |
| pH of BRB solution                            | X8     | 6.5     | 7.5      |

The ANOVA test was performed to verify the statistical significance of the terms. The small resulted p-values (<0.05) for the most of the squared and interaction terms, indicated their significance at 95% confidence level and suggested the presence of curvature in the response surface. The determination coefficient (R²) of 96% also confirmed that the model was well fitted to the experimental data. They were used for determination of the optimum conditions and the interactions between the investigated factors. The optimum conditions for Ag NPs-MIP-PGE synthesis, extraction and

Fig. 2 – SEM images of: (a) MIP-PPY-PGE, (b) AgNPs-MIP-PGE before template extraction (c) AgNPs-NIP-PGE and (d) AgNPs-MIP-PPY after template extraction.
determination of MEB were obtained for every parameter by using toolbox in Minitab 16 when X1, X2, X3, X4, X5, X6, X7 and X8 were 50 mM, 5 mM, 20, 40, 400 s, 930 rpm, 19 min and 6.5, respectively.

### 3.5. Cyclicvoltammetric behavior of AgNPs-MIP-PGE

The electrochemical behavior of different electrodes were studied in KCl (0.1 M) containing 10 mM K$_3$Fe(CN)$_6$, served as a probe (Fig. 4). A couple of typical redox peaks of K$_3$Fe(CN)$_6$ appeared at bare PGE electrode (curve b), while weak peak was observed at the unremoved mebeverine MIP-PGE (curve a). Since the formed MEB-PPY film by electropolymerization was very compact, and there were almost no channels for the active probe to approach the electrode surface, only very small background response was observed. However, the layer of AgNPs directly affected the current response of the sensor. After AgNPs was electrodeposited on the surface, the voltammogram of unremoved MEBAgNPs-MIP-PGE in K$_3$Fe(CN)$_6$ displayed a clear redox peaks although it was lower than that of bare PGE electrode and the peak potential shifted positively (curve c). This phenomena was due to the presence of AgNPs, which possessed inherent conductive properties, thus it can

### Table 2 – The results of PB experimental design matrix.

| Run order | X1   | X2   | X3  | X4   | X5  | X6  | X7  | X8  | response |
|-----------|------|------|-----|------|-----|-----|-----|-----|-----------|
| 1         | 0.05 | 0.001| 20  | 100  | 200 | 800 | 5   | 6.5 | 5.5       |
| 2         | 0.01 | 0.001| 20  | 100  | 400 | 400 | 15  | 7.5 | 3        |
| 3         | 0.05 | 0.005| 10  | 100  | 400 | 400 | 15  | 6.5 | 9        |
| 4         | 0.01 | 0.001| 10  | 50   | 200 | 400 | 5   | 6.5 | 4.7      |
| 5         | 0.01 | 0.001| 10  | 100  | 400 | 800 | 5   | 7.5 | 0.5      |
| 6         | 0.05 | 0.005| 20  | 50   | 400 | 800 | 5   | 7.5 | 7.5      |
| 7         | 0.01 | 0.005| 20  | 50   | 400 | 800 | 5   | 7.5 | 7.5      |
| 8         | 0.05 | 0.005| 20  | 50   | 400 | 800 | 5   | 7.5 | 7.5      |
| 9         | 0.01 | 0.005| 20  | 100  | 200 | 800 | 5   | 7.5 | 11.50    |
| 10        | 0.01 | 0.005| 10  | 50   | 200 | 800 | 5   | 7.5 | 13.00    |
| 11        | 0.05 | 0.001| 10  | 50   | 400 | 800 | 5   | 7.5 | 17.00    |
| 12        | 0.05 | 0.005| 10  | 100  | 200 | 400 | 5   | 7.5 | 0.75      |

### Table 3 – The CCD matrix and the experimental results.

| Run order | X8  | X7  | X6  | X4  | IMIP (µA) | INIP (µA) | Iadj (µA) |
|-----------|-----|-----|-----|-----|-----------|-----------|-----------|
| 1         | 6   | 20  | 600 | 70  | 7.16      | 3.81      | 3.35      |
| 2         | 6   | 10  | 600 | 30  | 10.30     | 6.90      | 3.40      |
| 3         | 7   | 10  | 1000| 30  | 17.00     | 13.72     | 3.28      |
| 4         | 7   | 10  | 600 | 70  | 12.00     | 12.00     | 0.00      |
| 5         | 6   | 10  | 1000| 70  | 8.00      | 5.60      | 2.40      |
| 6         | 7   | 20  | 1000| 70  | 14.00     | 8.87      | 5.13      |
| 7         | 6   | 20  | 1000| 30  | 10.20     | 3.10      | 7.10      |
| 8         | 6.5 | 15  | 800 | 50  | 9.01      | 2.28      | 6.78      |
| 9         | 6.5 | 15  | 800 | 50  | 8.78      | 2.04      | 6.74      |
| 10        | 7   | 20  | 600 | 30  | 13.00     | 8.48      | 4.52      |
| 11        | 6.5 | 15  | 800 | 50  | 9.15      | 2.38      | 6.77      |
| 12        | 7   | 10  | 600 | 30  | 5.82      | 5.04      | 0.78      |
| 13        | 6.5 | 15  | 800 | 50  | 9.20      | 2.45      | 6.75      |
| 14        | 6   | 20  | 600 | 30  | 9.90      | 3.65      | 6.25      |
| 15        | 6   | 10  | 1000| 30  | 5.17      | 0.63      | 5.80      |
| 16        | 6   | 10  | 600 | 70  | 8.50      | 7.27      | 1.23      |
| 17        | 7   | 20  | 1000| 30  | 11.00     | 4.67      | 6.33      |
| 18        | 7   | 10  | 1000| 70  | 15.11     | 13.91     | 1.20      |
| 19        | 6   | 20  | 1000| 70  | 9.30      | 2.75      | 6.65      |
| 20        | 7   | 20  | 600 | 70  | 7.72      | 2.79      | 4.93      |
| 21        | 6.5 | 15  | 1000| 50  | 5.7       | 1.25      | 6.95      |
| 22        | 6.5 | 15  | 800 | 70  | 9.10      | 3.15      | 5.95      |
| 23        | 6.5 | 15  | 800 | 50  | 9.50      | 2.67      | 6.83      |
| 24        | 6.5 | 10  | 800 | 50  | 6.70      | 1.85      | 4.85      |
| 25        | 7   | 15  | 800 | 50  | 7.93      | 1.61      | 6.32      |
| 26        | 6.5 | 15  | 600 | 50  | 0.63      | 6.01      | 6.64      |
| 27        | 6.5 | 15  | 800 | 50  | 9.35      | 2.57      | 6.78      |
| 28        | 6.5 | 20  | 800 | 50  | 6.50      | 1.70      | 8.20      |
| 29        | 6.5 | 15  | 800 | 30  | 4.50      | 2.70      | 7.20      |
| 30        | 6.5 | 15  | 800 | 50  | 8.92      | 2.18      | 6.74      |
provide a conductive pathway to electron transfer and promote electron transfer reactions of K₃Fe(CN)₆ probe [35]. Furthermore, after removing of MEB, the AgNPs-MIP-PGE showed an increase of redox peak currents (curve d). This may result from the cavity of the removed MEB molecule, forming a electron transfer channels for K₃Fe(CN)₆. The imprinted AgNPs-MIP-PGE electrode exhibited high affinity and selectivity to MEB molecules. By incubation of the electrode in MEB solution (10 mM) for 19min, the peak current of K₃Fe(CN)₆ on AgNPs-MIP-PGE decreased remarkably (curve e). Comparing with the MIP-PGE without AgNPs, the AgNPs-MIP-PGE exhibited more sensitive response.

3.6. Electrochemistry of MEB hydrochloride

Cyclic voltammograms of MEB at different electrodes in BRB (pH 6.5) were shown in Fig. 5. It can be seen that the MEB oxidation peak (0.72 V) at MIP-PGE was weak and broad due to slow electron transfer (Fig. 5 curve a). In comparison, at AgNPs-MIP-PGE, the anodic peak was observed at the less positive potential of 0.066 V and clearly showed an increased peak current compared with the MIP-PGE (Fig. 5 curve b). The increased current as well as the negative shift of the anodic peak demonstrated an efficient catalytic oxidation of MEB on the Ag-MIP-PGE. It also showed that no reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. The electro deposition of silver nanoparticles on MIP-PGE resulted in an observable increase in the peak current, which indicated an improvement in the electrode kinetics and a decrease in the potential of oxidation substantial, where Ag-MIP-PGE acts as a cation exchange [36] that attracts the positively charged MEB.

Fig. 6 shows the cyclic voltammograms of MEB at different values of pH on the AgNPs-MIP-PGE. By increasing the pH the oxidation peak potential of MEB shifts to less positive potentials which indicates the participation of protons in the electrode process and the electrocatalytic oxidation of MEB is a pH-dependent reaction. The formal value $E^\text{c}$ is linear with pH in BRB, with a slope 52.6 mV/pH. This value is close to the theoretical value of 59 mV/pH [37] indicating the participation of the same proton and electron numbers in the electrochemical process. Moreover, MEB oxidation is a one-electron process, which may be attributed to the oxidation of tertiary amine group [38,39].

Fig. 7 shows the cyclic voltammograms of MEB at the AgNPs-MIP-PGE when the scan rate ($\gamma$) varies from 10 to 110 mVs⁻¹. As is shown in Fig. 7, the anodic peak current of MEB is proportional to the sqar root of scan rate, which indicates that the electrode process is diffusion-controlled [40] and by increasing the scan rate the peak potential is shifted to positive potential because of irreversible electrode process of the oxidation reaction of MEB [41].

3.7. Analytical application of AgNPs-MIP-PGE

Differential pulse voltammetry has a much higher current sensitivity than cyclic voltammetry used for the determination of MEB. Under optimal conditions (BRB solution, pH 6.5) the oxidation peak current of MEB yields well-defined concentration dependence. At the optimal conditions, several different concentration of MEB was analyzed by DPV (Fig. 8). The calibration curve showed two dynamic linear ranges including $1 \times 10^{-8}$ to $1 \times 10^{-6}$ and $1 \times 10^{-5}$ to $1 \times 10^{-3}$ M with correlation coefficients ($R^2$) of 0.9880 and 0.9880, respectively. The limit of detection was calculated as $8.6 \times 10^{-9}$ M (based on S/N = 3). This value of the detection limit and the linear dynamic range for MEB observed at AgNPs-MIP-PGE are comparable and even better than those obtained for other modified electrodes (Table 4).

3.8. The selectivity of sensor

The selectivity of AgNPs-MIP-PGE as an important characteristic was estimated. Hence, the effect of some interfering factors was investigated.
species such as donpezile, chloroquine, levetiracetam, Phexophenadine, naproxen, mequinol were investigated. There was no obvious signal towards the some interferences such as chloroquine, levetiracetam, venlafaxine, naproxen, mequinol and good recoveries for MEB were achieved but donpezile and ketorolac decreased peak current of MEB obviously (Table 5).

### 3.9 Reproducibility

Intra-electrode and inter-electrode coefficients of variation were used in order to investigation of the reproducibility. The relative standard deviation (RSD) of reproducibility was 5.9% for 3 measurements of 1 mM MEB with the different sensors (Inter-electrode). Also for three times the reproducibility of the sensor was estimated by determining 1 mM MEB with one sensor (Intra-electrode) and RSD was calculated at 1.1%.

### 3.10 Application of the sensor in real sample analysis

In order to investigate the possibility of the developed sensor for real sample analysis, the AgNPs-MIP-PGE was applied for the determination of MEB in spiked human serum samples. Several samples were prepared by adding different concentrations of MEB (20, 40, 60 μM) into the blood serum, and were analyzed by the proposed sensor. To test the efficiency and selectivity of the proposed analytical method to pharmaceutical formulations, the sensor was applied to determine MEB in tablets according to the recommended method under...
optimal conditions. The results (Table 6) showed that there was no serious interference occurred from the classical additives of MEB Iran Darou capsule.

4. Conclusion

In this study, an economic, simple and rapid MIP-based electrochemical sensor was developed for the selective and sensitive detection of MEB in pharmaceutical and biological samples. Ag NPs-MIP-PGE formed by bulk electrochemical polymerization technique, and electrodeposition of AgNPs on the MIP surface. Ag NPs-MIP-PGE presents several attractive analytical features such as excellent electrocatalytic effect, good reproducibility, high conductivity, and high selectivity as well as quick response to MEB. Using this method, a rigid and uniform molecularly imprinted film with controlled thickness was synthesized on the surface of the electrode. Experimental designs (PBD and CCD) were applied to find a model for optimizing the technique. The results show electrode loading time, scan rate of CV process, stirring rate of loading solution, pH of BRB solution have most important effects on the sensor efficiency. Under the optimum conditions, the oxidation peak current was proportional to the MEB concentrations. The proposed method was successfully applied to the MEB detection in real samples such as drug and human serum sample. Finally, the recommended sensor provides the foundation for designing portable MEB sensor due to its easy and fast preparation and low cost.

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