

**FULL PAPER**

# Selective and sensitive voltammetric sensor for methocarbamol determination by molecularly imprinted polymer modified carbon paste electrode

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A molecularly imprinted polymer modified carbon paste electrode for determination of methocarbamol drug was constructed and used in aqueous and biological samples. The MIP was synthesized by methacrylic acid as monomer, azobis isobutyronitrile as initiator and methocarbamol as analyte with precipitation polymerization process. The oxidation voltammograms of analyte using MIP modified nickel ferrite catalyst electrode with cyclic voltammetry (CV) and differential pulse voltammetry (DPV) by potentiostat – galvanostat device were obtained. The methocarbamol drug has amine groups in its structure, thus it is affected by the medium pH, and the effect of this factor on the electrode response was investigated. In optimum conditions, 4% MIP, 8% nanoparticles catalyst, and pH 3,  $1.0 \times 10^{-5}$  M standard solution of methocarbamol was prepared and studied by DPV and CV mode. The dynamic calibration ranges,  $3.0 \times 10^{-8}$  -  $3.0 \times 10^{-6}$  M, detection limit of the method  $1.3 \times 10^{-8}$  M and RSD of the method for two measurements 3.33% and 1.00% was obtained.

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**KEYWORDS**

Carbon paste electrode; voltammetry; methocarbamol; MIP; CV; DPV.

**Introduction**

Methocarbamol (Scheme 1) is a drug with aryl glycerol ether structure that is used for muscle pain and has a calming effect [1]. This drug is absorbed through the intestine and reaches all body tissues. Due to its side effects, it is on the list of high-risk medications for the elderly. Determination of methocarbamol in biological fluids using voltammetric methods has advantages such as low cost, high sensitivity and short analysis time. Using the MIPs and nano catalysts can also increase selectivity of the method. In this method, MIPs can bond to analyte molecules and improves the electrochemical reaction condition.

Drugs are important analytes in analytical chemistry, because of its effects on human health and quality control methods in manufacturers. Thus, the development of sensitive and selective, simple and rapid method for the determination of drugs is of great importance and interest. Various methods, such as high performance liquid chromatography, HPLC, [2-3], HPLC-MS [4], reverse phase liquid chromatography, RPLC, [5], supercritical fluid chromatography, SFC, [6], HNMR spectroscopy [7], derivative spectrophotometry [8] and electrochemical methods [9] have been used for determination of methocarbamol. The cost of these methods is very high. Further, because of the high

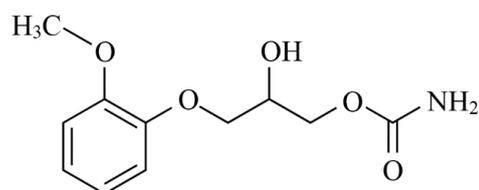
sensitivity of the electrochemical analysis, its development has been done by researchers. For example, determination of methocarbamol to buffer solution by CPE [10], rationally designed MIP for selective extraction of methocarbamol from human plasma [11], electrochemical behaviors of methocarbamol at an acetylene black-ionic liquid modified CPE and its electrochemical determination by square wave voltammetry (SWV) [12], electrochemical oxidation and determination of methocarbamol at multi-walled carbon nanotubes-modified glassy carbon electrode [9], and survey on the integration of MIPs as artificial receptors in potentiometric transducers for pharmaceutical drugs [13] are among such cases.

Voltammetry has been used to primarily study redox processes in different environments, surface adsorption processes, and electron transfer mechanisms at the surface of chemically modified electrodes [14-20]. Voltammetry has been used as a detector for high performance liquid chromatography. High sensitivity, selectivity to electrophoretic species, wide linear range, cheapness and availability, and the possibility of using a wide range of electrodes are the advantages of voltammetry, drawing attention to other analytical electrochemical methods.

Carbon paste electrode is a mix of fine graphite powder as electrical conducting and a pasting liquid, and has been used as working electrode in different electroanalytical methods. The CPE has exhibited many advantages, such as a wide potential range, easy and fast preparation, porous and high working surface, low cost, and small residual current. However, the sensitivity of simple CPE for determination of organic analyte is relatively poor. To improve the sensitivity, modification of simple CPE has been used by mixing with some other unique substances, such as MIPs and suitable nano catalyst [21].

In this study, the carbon paste electrode modified by MIP and NiFe<sub>2</sub>O<sub>4</sub> nano catalyst

have been used as a sensitive and selective method for determination of methocarbamol in plasma, urine and tablets samples solution by differential pulse voltammetry. The MIP synthesis in chloroform solvent resulting in optimal properties accompanied by nano catalyst is the best choice for sensitive and selective voltammetric sensor construction.



**SCHEME 1** Molecular structure of methocarbamol

## Experimental design

### Materials and solutions

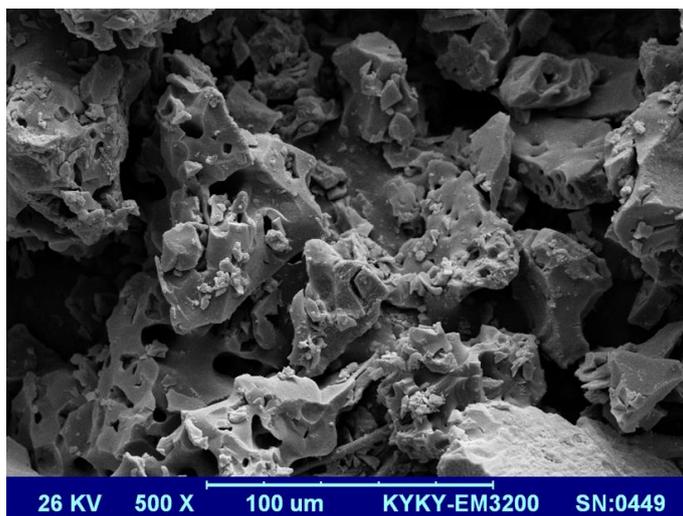
Methacrylic acid (MAA) as functional monomer, azobis isobutyronitrile (AIBN) as initiator, ethylene glycol dimethacrylate (EGDMA) as crosslinker and chloroform as solvent for MIP synthesis was used from Merck. All other solvents such as methanol, ethanol, acetone, acetic acid, ammonia solution, hydrochloric acid solution and phosphoric acid were purchased from Merck and used without any purification. Other materials for buffer solution preparation and construction of carbon paste electrode, such as sodium hydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide, sodium acetate, boric acid, graphite powder and paraffin oil were purchased from Merck. Methocarbamol powder with high purity was used from Daropakhsh drugs manufacture, Tehran, Iran. NiFe<sub>2</sub>O<sub>4</sub> nano catalyst was synthesized by Ni(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O and Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O that were purchased from Merck.

Standard stock solutions (1×10<sup>-2</sup> M) of methocarbamol powder was prepared in methanol and stored at 4 °C. The desired working solution (1×10<sup>-5</sup> M) was prepared by

appropriate dilution of the standard stock solutions with methanol.

Buffer solutions with pH 5.0, 6.0, 7.0, 8.0 and 9.0 were prepared by mixing of 50 mL 1.0 M disodium hydrogen phosphate and appropriate volume of 1.0 M sodium hydroxide solution and diluted to 100.0 mL. Buffer solutions with pH 3.0 and 4.0 were prepared by mixing 5.7 mL 1.0 M sodium acetate solution and appropriate volume of 1.0 M acetic acid solution and diluted to 100.0 mL. Britton-Robinson (BR) universal buffer was prepared by mixing appropriate volume of 0.2 M phosphoric acid, 0.2 M boric acid and 0.2 M acetic acid and tested as supporting electrolytes.

#### *Synthesis of NiFe<sub>2</sub>O<sub>4</sub> nano catalyst*



**FIGURE 1** The SEM image of NiFe<sub>2</sub>O<sub>4</sub> nano catalyst

#### *Synthesis of MIP*

The synthesis of the MIP was performed using the previously reported work [11]; the only change was that chloroform solvent was used here. 0.1 mmol methocarbamol template and 10 mL chloroform solvent was poured into a test tube and the tube was placed in ultrasonic device for 10 minutes. Then, 0.9 mmol methacrylic acid monomer was added to the mixture and was re-sonicated for 10 minutes. The solution was then placed in the

14.4 g Ni (NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O was solved in 10 mL deionized water. Then the solution of 26.9 g Fe (NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O in 10 mL deionized water was added to it. The mixture was agitated and heated to 50-60 °C on heater magnet for 1 hour. Next, under nitrogen gas, 20.0 mL concentrated ammonia solution and 40 mL deionized water was added slowly. The paste product was placed in 40 °C oven at 72 hours and finally was treated in 350 °C for 1 hour. The magnetic properties of nano catalyst and their morphology were respectively tested by magnet and scanning electron microscopy (SEM). From the SEM image shown in Figure 1, it is clear that the nanoparticles are agglomerated. The resulting nanoparticles were used to prepare the modified electrode.

refrigerator at 4 °C for 30 minutes to form a bond between the monomer and the template. Then, about 0.8 mL ethylene glycol methacrylate and 0.024 g of the azobis isobutyronitrile were added to the tube and placed in ultrasonic again for 10 minutes. The dissolved oxygen was replaced by nitrogen gas for 10 minutes and the tube was placed in oil bath at 60 °C for 20 to 24 hours. The non-imprinted polymer (NIP) was also prepared by the mentioned method without methocarbamol drug. To remove

methocarbamol, the synthesized MIP was washed two times and each time by 3.0 mL deionized water and then two times by 2.0 mL methanol.

#### *Modified carbon paste electrode preparation*

0.07 g graphite powder and 0.03 g paraffin oil was mixed using porcelain pestle and mortar. Then appropriate amounts of MIP and nano catalyst were added to obtain a series of modified carbon pastes. Then a portion of the resulting paste was packed into one end of a plastic tube with about 3.5 mm inner diameter and a simple copper wire was inserted through the opposite end to produce electrical contact. The surface of carbon paste was polished by suitable paper and used as working electrode.

#### *Real sample preparation*

To evaluate methocarbamol determination in real samples, human serum and urine samples and commercial tablets samples at various concentrations were used. In tablet sample, one 500 mg methocarbamol tablet was powdered, dissolved by deionized water and diluted to 500 mL. The aliquot of this solution was diluted to 1000 and 1500 times, respectively, and three solutions were tested by voltammetric methods.

The plasma sample was prepared from the medical diagnostic laboratory and was buffered by sodium phosphate buffer. It was then diluted to 100 mL before methocarbamol measurements. Urine sample was diluted 70 times by appropriate electrolyte solution, then 10 mL of it was transferred to a voltammetric cell and voltammogram was drawn.

#### *Apparatus*

All electrochemical measurements, such as cyclic voltammetry and differential pulse voltammetry were carried out in a one-compartment three-electrode cell potentiostat-galvanostat, model 12-30-302

manufactured by Metrohm-Autolab, Netherlands with Nova 1.8 software. The carbon paste electrodes (modified and unmodified) was used as working electrode. The platinum as auxiliary electrode and saturated Ag-AgCl as reference electrode constructed by Metrohm, Netherlands were employed and all measurements were performed at  $25\text{ }^{\circ}\text{C} \pm 1$ .

A Metrohm 713 pH meter was used for pH adjustments. The FTIR spectra of MIP and NIP were performed on a Jasco 4200 (Japan) spectrometer using KBr discs.

#### *General analytical Method*

Molecularly imprinted polymer with methocarbamol template was synthesized and the MIP accompanied by nano catalyst was used for modified carbon paste electrodes (MCPEs). Then, voltammetric methods by these MCPEs were applied for determination of methocarbamol in aqueous and biological samples. The time-dependent potential applied to an electrochemical cell and the electrical current through the cell was measured as a function of this potential.

In typical voltammetric procedure, 10 mL of buffered solution was transported to cell and the smooth MIP-NiF<sub>2</sub>O<sub>4</sub> modified carbon paste electrodes were placed to the electrolyte. Then, for determination of the low baseline current, several CV scans were done. Next, the desired volume of the methocarbamol solution was added to the cell until the  $1.0 \times 10^{-5}$  M concentration was obtained. As the next step, the voltammogram of analyte was obtained in the range of 0.9 to 1.5 Volts, while the modified carbon paste electrode was applied as working electrode.

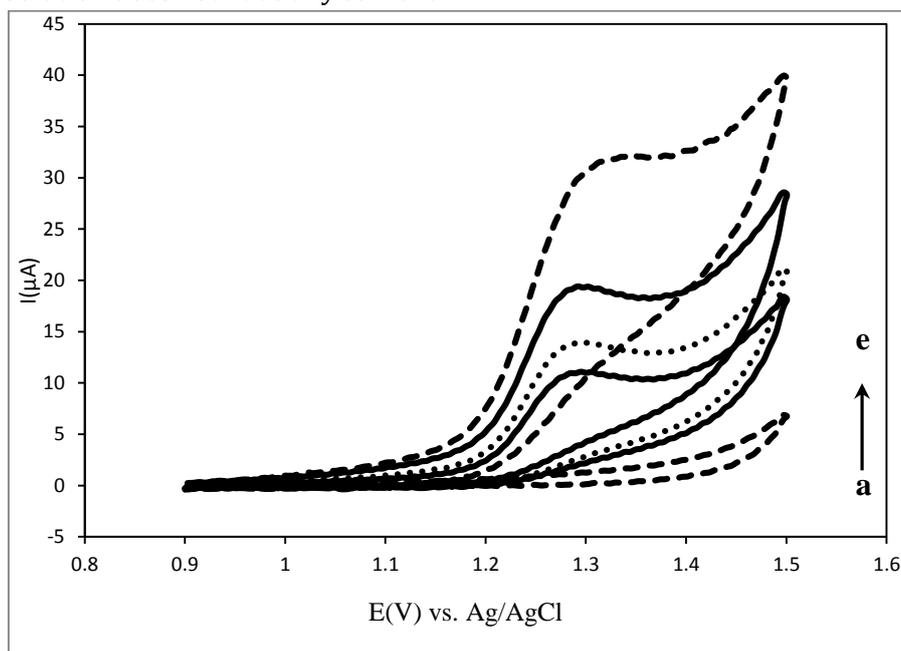
## **Results and discussion**

#### *Electrochemical behavior*

Initially the cyclic voltammogram of blank electrolyte solution without methocarbamol was obtained by MIP-modified carbon paste

electrode (MIP-MCPE) was obtained. Then, the cyclic voltammograms of  $1.0 \times 10^{-5}$  M methocarbamol solution were obtained by unmodified carbon paste electrode, MIP-MCPE,  $\text{NiFe}_2\text{O}_4$ -modified carbon paste electrode ( $\text{NiFe}_2\text{O}_4$ -MCPE) and by MIP- $\text{NiFe}_2\text{O}_4$  modified carbon paste electrode (MIP- $\text{NiFe}_2\text{O}_4$ -MCPE). In all solutions, the optimum pH was adjusted to 3.0 by phosphate buffer. The all cyclic voltammograms are shown in Figure 2. As shown in Figure 2, the CV of blank solution does not have any current

peak (curve a), but in the presence of analyte using a CPE an oxide peak is seen at a potential of about 1.27 V (curve b). Using the MIP-MCPE, we can observe increasing peak current (curve c), and using of  $\text{NiFe}_2\text{O}_4$ -MCPE, we can see a significant increase in the oxide current (curve d). As shown in Figure 2, using the MIP- $\text{NiFe}_2\text{O}_4$ -MCPE as hybrid electrode of two modifiers, we can see the highest rise of the peak current (curve e) comparison to b, c and d curves, is the best.



**FIGURE 2** Cyclic voltammogram of a) MIP-MCPE in the absence of methocarbamol, b) unmodified CPE, c) MIP-MCPE, d)  $\text{NiFe}_2\text{O}_4$ -MCPE and e) MIP- $\text{NiFe}_2\text{O}_4$ -MCPE, in the presence of  $1.0 \times 10^{-5}$  M methocarbamol concentration. In all cases, the pH of solution was adjusted to 3.0

There exist studies on the differential pulse voltammetric behavior of the MCPDs for better understanding and more accurate use of them. The DPV voltammograms of blank electrolyte solution without analyte by MIP-MCPE (Figure.2. curve a), in the present of  $1.0 \times 10^{-5}$  M methocarbamol were obtained by unmodified CPE (Figure. 3. Curve b), MIP-MCPE (Figure. 3. Curve c),  $\text{NiFe}_2\text{O}_4$ -MCPE (Figure. 3. Curve d) and MIP- $\text{NiFe}_2\text{O}_4$ -MCPE (Figure. 3. Curve e) (Figure. 3). In all solutions, the optimum pH was adjusted to 3.0 by phosphate buffer.

As can be seen in Figure 3, the DPV of blank solution does not show current peak (curve a), but in the presence of analyte, a peak is seen at a potential of about 1.27 V, using a CPE (curve b). Using the MIP-MCPE, we can observe increasing peak current (curve c), and using  $\text{NiFe}_2\text{O}_4$ -MCPE, we can observe a significant increase in the current (curve d), and using MIP- $\text{NiFe}_2\text{O}_4$ -MCPE shows the highest increase of the peak current (curve e), when compared with other curves.

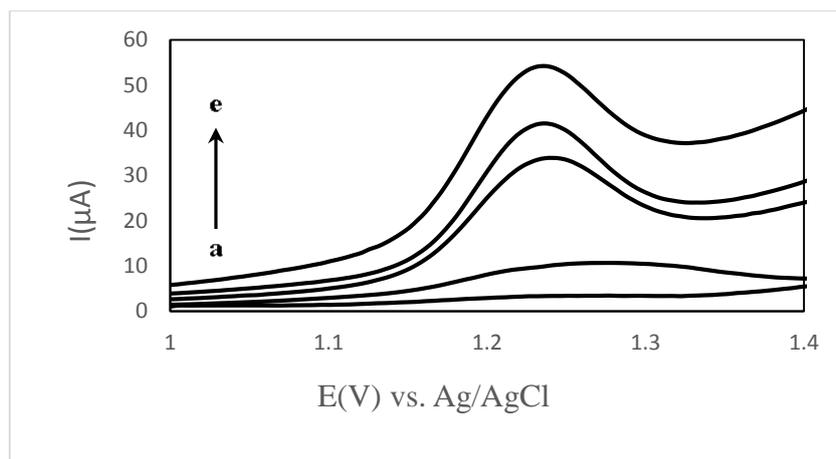
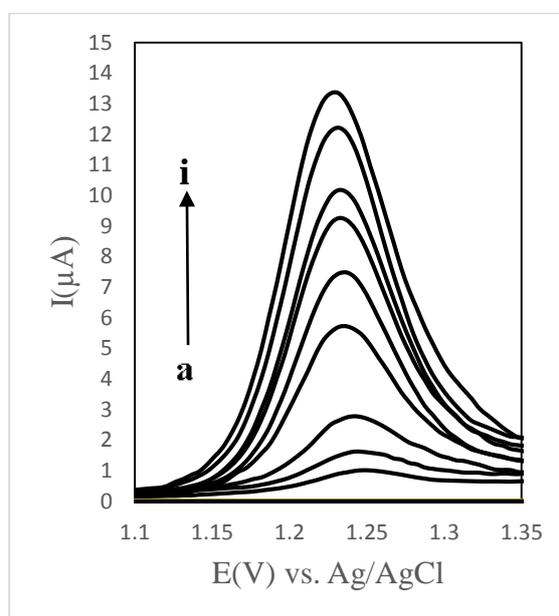


Figure. 3. Differential pulse voltammetry of a) MIP-MCPE in the absence of methocarbamol, b) unmodified CPE, c) MIP-MCPE, d) NiFe<sub>2</sub>O<sub>4</sub>-MCPE and e) MIP-NiFe<sub>2</sub>O<sub>4</sub>-MCPE, in the presence of  $1.0 \times 10^{-5}$  M methocarbamol concentration. In all cases, the pH of solution adjusted to 3.0

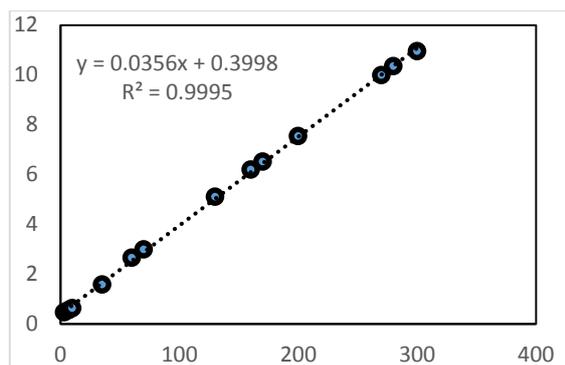
As shown in Figures 1 and 2, using MCPEs as working electrode for determination of methocarbamol causes the anodic current to increase and so does the sensitivity of the method. The chemical reaction on the surface of MCPEs is oxidation and the mechanism has been reported [9], indicating the ability of MIP and MIP-NiFe<sub>2</sub>O<sub>4</sub> to more adsorption response to methocarbamol analyte.

#### Calibration curve

After optimization of all the effective parameters on fabrication and electrochemical behavior of the MCPEs, we studied the calibration curve and determined its linear range. Using the optimum value of MIP (4%) and NiFe<sub>2</sub>O<sub>4</sub> nano-catalyst (8%) in carbon paste and at the optimum buffer value (pH = 3.0), we prepared standard solutions of varying concentration and plotted the differential pulse voltammogram (Figure 4). The calibration curve for methocarbamol was then plotted using voltammograms (Figure 5). As shown in Figure 5, the calibration curve was linear in the range of  $3.0 \times 10^{-8}$ – $3.0 \times 10^{-6}$  M.



**FIGURE 4** Differential pulse voltammograms of methocarbamol solutions at surface of the CPE modified by 8% NiFe<sub>2</sub>O<sub>4</sub> and 4% MIP at pH=3.0. Different concentrations of methocarbamol are  $3.0 \times 10^{-8}$ ,  $7.0 \times 10^{-8}$ ,  $1.0 \times 10^{-7}$ ,  $6.0 \times 10^{-7}$ ,  $1.3 \times 10^{-6}$ ,  $1.6 \times 10^{-6}$ ,  $1.9 \times 10^{-6}$ ,  $2.0 \times 10^{-6}$  and  $3.0 \times 10^{-6}$  M (a to i, respectively)



**FIGURE 5** DPV calibration curve for methocarbamol determination by MIP-NiFe<sub>2</sub>O<sub>4</sub>-MCPE as working electrode

#### Detection limit

Detection limit (*DL*) of the method was calculated using  $DL=3 S_b/m$  equation, where *m* is slope of calibration curve (0.0356) and *S<sub>b</sub>* is standard deviation of blank solution signal. For determination, the *S<sub>b</sub>*, 10.0 mL of blank electrolyte was introduced to the cell and its voltammogram was drawn five times. The peak current was recorded 3.46, 3.38, 3.79, 3.42 and 3.41 μA, thus the average was calculated 3.35 μA, and *S<sub>b</sub>* for five data  $2.6 \times 10^{-7}$  was obtained. Therefore, the detection limit of the method by  $1.3 \times 10^{-8}$  M was obtained.

#### Accuracy and precision

For determination of accuracy, two solutions of methocarbamol with  $5.0 \times 10^{-9}$  and  $2.0 \times 10^{-7}$  M were prepared and at optimum conditions

five DPV measurements were done separately by MIP-NiFe<sub>2</sub>O<sub>4</sub>-MCPE as working electrode. The relative standard deviation percent (RSD%) was calculated by  $RSD\% = (S/a) \times 100$ , where *S* signifies standard deviation of five repetition and *a* is the average of them. Therefore, the relative RSD% was calculated by 3.33 and 1.00, respectively. The results show that this method has good accuracy.

#### Interferences

A  $1.0 \times 10^{-7}$  M methocarbamol solution was introduced to the cell and DPV anodic oxidation peak current was measured. Then, some interference such as guaifenesin, theophylline, citric acid and glucose with 100 times concentration ( $9.0 \times 10^{-7}$  to  $1.0 \times 10^{-6}$ ) was added to the early methocarbamol solution and the DPV anodic peak currents were measured. Then, percent of interferences was calculated by  $B = [(I - I_o)/I] \times 100$  equation, where *I* symbolizes the measurement current at present of interfering species, *I<sub>o</sub>* is the peak current at absent of interfering species and *B* denotes the percent of interference. The data of this investigation was listed in Table 1. The data show that the interfering species has no significant interference, because the *B* value is less than 5%. Table 1 shows that these interfering species carry no significant effect, thus the proposed method exhibits good selectivity.

**TABLE 1** The effect of some interfering species on methocarbamol determination using the proposed method

Interfering species	Concentration added, M	B%
Guaifenesin	$9.0 \times 10^{-7}$	+ 4.05
Theophylline	$1.0 \times 10^{-6}$	+ 4.25
Citric acid	$1.0 \times 10^{-6}$	- 2.84
Glucose	$1.0 \times 10^{-6}$	+ 4.23

#### Real samples analysis

The application of the DPV proposed method by MIP-NiFe<sub>2</sub>O<sub>4</sub>-MCPE as working electrode was investigated with some real samples, such as human plasma and urine and commercial

methocarbamol tablets from drugstores. To do so, the solutions of the human serum and urine, and tablets were prepared following the procedures explained in the experimental section, and the DPV anodic oxidation currents were obtained without standard addition and

after addition of appreciate concentration of methocarbamol standard solution. The results are given in Table 2. The measurements were repeated five times by DPV method. For this purpose, certain concentrations of methocarbamol standard solution were repeatedly added to the solution each time using standard addition method and after

three times measurement of each sample; recovery percentage (R%) of the analyte was calculated using  $R\% = (C_2/C_1) \times 100$  equation, where  $C_2$  stands for the determined concentration of methocarbamol after the addition of standard solution and  $C_1$  is the standard concentration.

**TABLE 2** The analysis of the real samples by the DPV proposed method with MIP-NiFe<sub>2</sub>O<sub>4</sub>-MCPE as working electrode

Samples	Standard solution added (M)	Determined (M)	Recovery (R%)	RSD%
Human plasma,				
1	0.0	0.0	---	---
2	$1.0 \times 10^{-7}$	$1.005 \times 10^{-7}$	100.5	2.21
3	$4.0 \times 10^{-7}$	$3.992 \times 10^{-7}$	99.5	3.26
4	$7.0 \times 10^{-7}$	$6.985 \times 10^{-7}$	99.7	2.24
5	$1.0 \times 10^{-6}$	$1.021 \times 10^{-6}$	100.21	1.43
Human urine, 1	0.0	0.0	---	---
2	$1.0 \times 10^{-7}$	$9.45 \times 10^{-7}$	94.5	4.21
3	$4.0 \times 10^{-7}$	$4.012 \times 10^{-7}$	100.3	1.26
4	$7.0 \times 10^{-7}$	$7.085 \times 10^{-7}$	101.02	4.32
5	$1.0 \times 10^{-6}$	$1.097 \times 10^{-6}$	100.97	4.43
500 mg tablet,				
1	0.0	---	---	---
2	$7.0 \times 10^{-7}$	$7.050 \times 10^{-7}$	100.71	2.50
3	$4.7 \times 10^{-7}$	$4.705 \times 10^{-7}$	100.10	4.30
4	$2.3 \times 10^{-7}$	$2.342 \times 10^{-7}$	101.82	4.39

## Conclusion

The new selective and sensitive modified carbon paste electrode for methocarbamol determination in complex matrix was proposed. Chemical modification of the electrodes with a suitable modifier provides many advantages such as high sensitivity and high electrode capability in the measurement. The use of this MIP-NiFe<sub>2</sub>O<sub>4</sub>-MCPE as a sensor for electrochemical determination of methocarbamol offers merits, such as high stability, selectivity sensitivity and repeatability and low detection limit. Easy and inexpensive to make, the modified electrode has become an ideal and ideal proposition for measurement in real samples. The proposed method introduces itself as a novel and precise method that can be a good substitute for other quantitative and qualitative methods.

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