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ORIGINAL PAPER

Electrochemical determinations of 6-mercaptopurine on the surface of a carbon nanotube-paste electrode modified with a cobalt salophen complex

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Abstract A mixture of multi-walled carbon nanotube/graphite paste electrode modified with a salophen complex of cobalt was prepared and was applied for the study of the electrochemical behavior of 6-mercaptopurine (MP) using cyclic and differential pulse voltammetry (DPV). An excellent electrocatalytic activity toward the oxidation of MP was achieved, which led to a considerable lowering in the anodic overpotential and remarkable increase in the response sensitivity in comparison with unmodified electrode. Utilizing DPV method, a linear dynamic range of 1–100 μM with detection limit of 0.1 μM was obtained in phosphate buffer of pH 3.0. The electrochemical detection system was very stable, and the reproducibility of the electrode response, based on the six measurements during 1 month, was less than 3.0% for the slope of the calibration curves of MP. The electrochemical method as a simple, sensitive, and selective method was developed for the determination of MP in pharmaceutical dosage form and human plasma without any treatments.

Keywords 6-Mercaptopurine · Cobalt (III) salophen complex · Multi-walled carbon nanotube · Carbon-paste electrode · Modified electrode

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Introduction

Mercaptopurine, 3,7-dihydropurine-6-thione, also called 6-mercaptopurine (MP) is an immunosuppressive drug [1]. It interferes with nucleic acid synthesis by inhibiting purine metabolism and is used as an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug to treat acute lymphoblastic leukemia. It is also used for pediatric non-Hodgkin's lymphoma polycythemia vera, psoriatic arthritis, and inflammatory bowel disease (such as Crohn's disease and ulcerative colitis) [2]. Some in vitro effectiveness has been demonstrated against *Mycobacterium paratuberculosis* using MP [3].

Numerous analytical methods have been developed for the determination of MP in pharmaceutical and biological preparations including high-performance liquid chromatography [4, 5], capillary electrophoresis (CE) [6, 7], CE with laser-induced fluorescence [8], capillary zone electrophoresis with amperometric detection at a carbon electrode [9], spectrophotometry [10, 11], spectrofluorimetry [12], chemiluminescence [13], flow injection chemiluminescence determination of 6-mercaptopurine based on a new system of potassium permanganate–thioacetamide–sodium hexametaphosphate [14], and an integrated microfluidic device with

online labeling and chemiluminescence [15]. Some electrochemical methods, e.g., polarography [16], voltammetric determination at silver microdisk electrodes [17], amperometric detection at a functionalized multi-walled carbon nanotube (MWCNT)-modified electrode in liquid chromatography coupled with microdialysis [18], voltammetric determination using a graphite electrode modified with $[Co(phen)_3]^{3+}$/MWCNT [19], determination of 6-mercaptopurine using modified multiwall carbon nanotubes TiO_2 as a voltammetric sensor [20], and cathodic stripping voltammetric determination at a silver electrode [21], have been reported for the determination of MP. Recently, CE–electrochemical detection (EC) determination in a polymethylmetacrylate biochip with on-chip gold nanoelectrode ensemble working and decouple electrodes was reported for MP [22]. Furthermore, the interaction of 6-MP with DNA was studied by voltammetry [23].

Electrochemical techniques in the field of pharmaceutical analysis have developed due to their simplicity, reasonable accuracy and precision, low cost, and rapidity. There is no need for derivatization or time-consuming extraction steps in comparison with other techniques because of less sensitivity of electroanalytical methods to the matrix effects. Carbon-paste electrodes (CPEs) are widely utilized to perform the electrochemical determinations of a variety of biological and pharmaceutical species owing to their low residual current and noise, ease of fabrication, wide anodic and cathodic potential ranges, rapid surface renewal, and low cost. Moreover, chemically modified electrodes (CMEs) can be easily prepared by adding different substances to the bulk of CPEs in order to increase sensitivity, selectivity, and rapidity of determinations [24–30]. Application of transition metal Schiff base and phthalocyanine complexes (e.g., cobalt and iron) in preparation of CMEs has shown excellent electrocatalytic properties owing to structural characterization of modifiers, in which the steric and electronic effects of substituent groups can affect the catalytic activity of the complex [25–35].

In the present study, a composite mixture of MWCNT/fine graphite powder/Nujol was modified with a salophen complex of cobalt (III) perchlorate containing chlorine and methyl substituent groups. It is shown in the previous studies that the presence of MWCNT on the surface of the electrodes, due to the large specific surface area and more edge sites, has shown a considerable enhancement in electrochemical signals leading to improvement of the detection limit in the voltammetric measurements [30, 35–38]. The prepared modified electrode was utilized in electrochemical evaluation and determination of MP in pharmaceutical and clinical preparations without expensive and time-consuming pretreatments. The prepared complex modifier showed high stability in the matrix of the paste electrode, due to high its hyrophobicity, leading to long-time stability in the response of the electrode.

Experimental

Chemical and reagents

Multi-walled carbon nanotube (purity more than 95%) with OD between 10 and 20 nm, ID between 5 and 10 nm, and tube length from 0.5 to 200 nm was prepared from Nanostructured & Amorphous Materials (USA). MP (Fig. 1a), graphite powder, and spectroscopic mineral oil (Nujol) were purchased from Merck. All other chemicals, which were of analytical reagent grade, were provided from Merck. The procedure of preparation and structural identification of the bromine and methyl substituted salophen complex of cobalt (III) perchlorate is previously reported [39]. The structure of this complex is shown in Fig. 1b. Double-distilled deionized water was used for preparation of all aqueous solutions.

Stock solutions of MP were freshly prepared as required in phosphate buffer solutions (PBS) (pHs 3, 6, 7, 8; 0.1 M) or acetate buffer solutions (pHs 4, 5; 0.1 M) as supporting electrolytes. All voltammetric investigations were per-

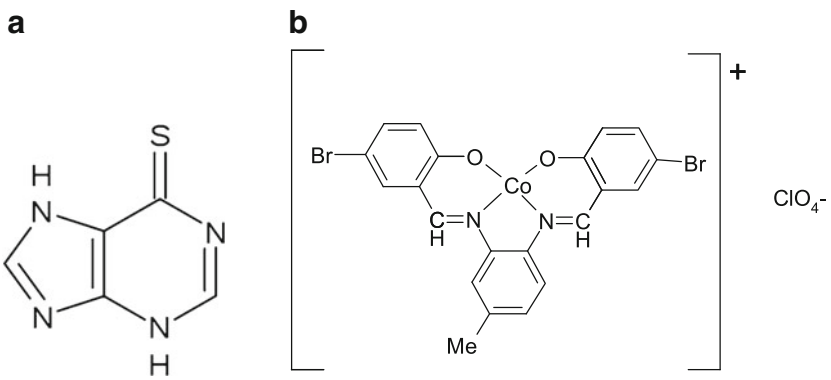

Fig. 1 Chemical structure of **a** 6-mercaptopurine and **b** cobalt (III) salophen perchlorate complex

formed in deoxygenated solutions by purging the pure argon (99.999% from Roham Gas Company). Pharmaceutical formulations of MP (Purinethol 50 mg tablets, Glaxo Smith Kline Pharmaceutical Co., Germany) were purchased from the local pharmacy. Fresh frozen plasma was prepared from Iranian Blood Research and Fractionation Holding Company.

Instrumentation

Voltammetric experiments were performed using a Metrohm Computrace Voltammetric Analyzer model 757 VA. The system was operated using 757 VA Computrace software (Metrohm) that runs under windows XP. The three-electrode cell system consisted of carbon-paste working electrode (modified or unmodified), a saturated Ag/AgCl reference electrode, and a Pt wire as the counter electrode. The pH measurements and preparation of buffer solutions were made using a digital pH/mV/Ion Cyberscan model 2500.

Procedures

Preparation of modified and unmodified electrodes

In order to prepare the unmodified electrode, graphite powder was mixed with appropriate amount of mineral oil (Nujol) (~75:25%, *w*/*w*) through hand mixing in a mortar and pestle, and the composite mixture was packed in the end of a Teflon tube (ca. 2.5 mm i.d.). Electrical contact was made by forcing a copper pin down into the Teflon and into the back of the composite.

In order to remove the probable amorphous carbon and catalytic impurities, as previously reported [35], a 500 mg of the MWCNT was heated in an oven with 400°C in nitrogen atmosphere for 2 h. The heat processed MWCNT was dispersed in 50 mL of 6.0 M HCl for 2 h under ultrasonic agitation under the nitrogen atmosphere, filtered on a Wattman 42 filter paper, and washed with doubled-distilled water until the pH of the solution was neutral. This MWCNT was dried under the IR lamp. Based on our previous study [35], a 10 wt.% of the treated MWCNT was mixed with fine graphite powder in an appropriate volume of dichloromethane. After evaporation of the solvent in room temperature, a portion of it was mixed with Nujol (~75:25%, *w*/*w*) in a mortar and pestle. The modified electrode was prepared by mixing unmodified composite with the prepared cobalt (III) salophen complex (3%, *w*/*w* in optimum value). After dispersing the composite in dichloromethane, the mixture was stirred and the solvent evaporated, completely. After air drying of the composite, the modified electrode prepared the same as the unmodified electrode. This electrode is presented in the following parts as modified carbon nanotube-paste electrode (MCNTPE).

Validation procedure

The linear range, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision, recovery, and selectivity were evaluated in the voltammetric determination of MP. The linear range was determined by the differential pulse voltammetric analysis in solutions with various concentrations of MP. The LOD and LOQ were calculated from the linear calibration curve. Repeatability (intra-day) and intermediate (inter-day) precision were assessed at three different concentrations of MP. To assess the repeatability, three replicate measurements of each solution were made in a short period of time. To determine intermediate precision, the solutions were each analyzed three times per day for three consecutive days. The accuracy of the procedure was verified by performing recovery assays in triplicate.

Analytical applications

Pharmaceutical analysis Content of 20 tablets of Purinethol was transferred into 100-mL volumetric flasks and was diluted with double-distilled water. The content of the flask was sonicated for 15 min to complete dissolution and then reached to the volume. The solutions were filtered through 0.45-μm membrane filter, and suitable aliquots of solutions were taken and diluted with appropriate supporting electrolyte to prepare final concentration. Solutions recovery and precision studies in assay procedure were evaluated in lower, middle, and higher level concentrations of pharmaceutical preparation. Quantitations were performed using the calibration curve method.

Analysis of MP in spiked human plasma Aliquot volumes of plasma sample was fortified with MP and, after 1 min vortexing, were diluted up to final concentrations in the range of 1×10^{-6}–1×10^{-4} M without extraction or further treatments. Quantitations were performed using the calibration curve method from the related calibration equations.

Results and discussion

Electrochemical behavior of MP and modification effect

Cyclic voltammetric studies of 0.1 mM of MP were performed in 0.1 phosphate buffer solution of pH 3.0 at the unmodified and MCNTPE at a scan rate of 100 mV s^{-1}. The cyclic voltammograms, which are illustrated in Fig. 2,

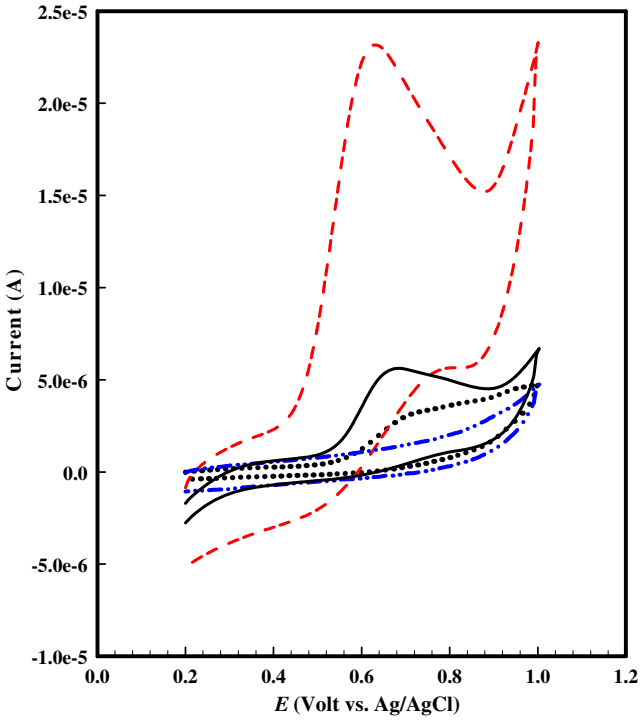

Fig. 2 CVs of 0.1 mM MP on the surface of bare CPE (*dotted line*), CoSal-CPE (*solid line*), and MCNTPE (*dashed line*) in 0.1 M PBS (pH 3.0). *Curve with dot-dot-dash line* shows the CV of the MCNTPE in buffer supporting electrolyte. Scan rate was 100 mV s^{-1}

showed that on the surface of CPE, a very broad and weak anodic peak was obtained. However, on the surface of MCNTPE, a well-defined irreversible oxidation peak with a negative shift of 166 mV was observed for MP in the potential window of 0.2–1.0 V. Moreover, the anodic peak current showed a considerable enhancement on the surface of the modified electrode. On the basis of these observations, it can be affirmed that cobalt (III) complex acts as an effective electron transfer mediator in the electrocatalytic oxidation of MP, leading to the reduction of the anodic overpotential and a remarkable enhancement of the anodic peak current. In the other hand, presence of MWCNT in the matrix of the modified electrode improved the microscopic surface area and, by increasing the edge sites, estimated more catalytic activity in the electrochemical oxidation of MP. For this sulfhydryl compounds, at all potential sweep rates, no cathodic peak is observed on the reverse scan, which confirms the EC mechanism with coupled irreversible chemical reactions hindered to the electron transfer step.

pH effect on voltammetric responses

In order to study the effect of the pH of the buffer solution on the electrochemical behavior of MP, cyclic voltammograms of 0.1 mM of it were recorded in the pH range of 2.0–8.0. As can be seen in Fig. 3a, by increasing the pH, a negative shift was resulted in the anodic peak potential. Such a behavior indicates a deprotonation step in the mechanism prior to the electron transfer step, which has been reported for the electrocatalytic oxidation of other biological sulfhydryl compounds on the surfaces of various modified electrodes [25–28, 34, 35]. In these studies, a linear relationship was observed between the anodic peak potential with the pH of the buffer solution regarding following equation (Fig. 3b):

$$E_{p,a}(\mathrm{mV}) = -50.2\mathrm{pH} + 741.6\left(R^2 = 0.998\right) \quad (1)$$

A result of a slope of −50.2 mV/pH indicates that equal numbers of electrons and protons are involved in the electrode process of MP on the surface of the modified electrode. The anodic peak current first increased pH from 2.0 to 3.0 and then decreased by increasing pH from 3 to 8 (Fig. 3c). Therefore, phosphate buffer with pH=3.0 was chosen as an optimum condition in order to obtain the best sensitivity in all voltammetric measurements. The observed electrochemical behavior can be related to instability of MP in higher pH values, like the other sulfhydryl compounds, e.g., captopril [25], cysteine [26], and penicillamine [28].

Effect of sweep rate

The cyclic voltammograms of 0.1 mM MP in pH 3 (0.1 M phosphate buffer solution) were recorded at MCNTPE in the various potential sweep rates of 10–150 mV s^{-1}. As can be seen in Fig. 4a, a positive shift in the peak potential of MP ($E_{p,a}$) was obtained, which confirms the irreversibility of the electro-oxidation process. On the other hand, a linear relationship between the peak current (i_p) and the square root of potential sweep rate ($\upsilon^{1/2}$) was resulted regarding following equation, which reveals the diffusion-controlled process for the electro-oxidation of MP on the surface of MCNTPE in the studied range of the potential sweep rate (Fig. 4b).

$$i_p = 0.228\upsilon^{1/2} + 0.007\left(R^2 = 0.999\right) \quad (2)$$

The plot of peak potential ($E_{p,a}$) versus the logarithm of the scan rate (υ) was linear and is expressed as Eq. 3:

$$E_p = 46.66\log(\upsilon) + 513.2\left(R^2 = 0.997\right) \quad (3)$$

Comparing the results with the following equation for an irreversible electrochemical process [40]:

$$E_p = b/2\log(\upsilon) + (\mathrm{constant}) \quad (4)$$

(where b=2.303RT/(1−α)n_aF), values of 0.093 and 0.634 were estimated for b and (1−α)n_a, respectively. Consider-

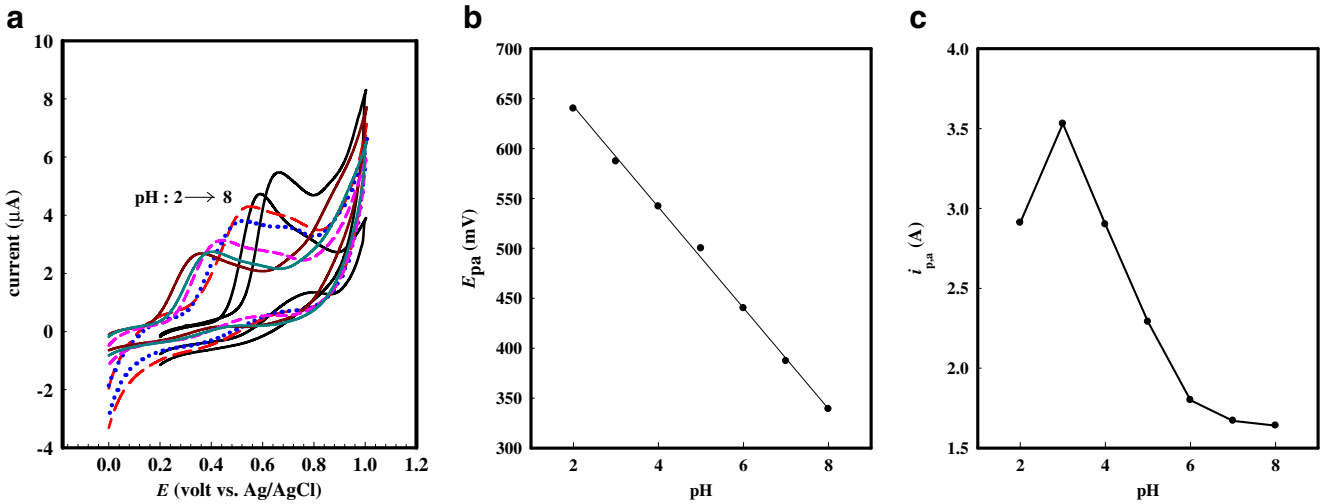

Fig. 3 **a** CVs of 0.1 mM MP at MCPE in various pHs (from 3.0 to 8.0) of PBS (υ=100 mV s^{-1}); the relationship between the pH and **b** the oxidation peak potential (E_{pa}) and **c** the oxidation peak current (i_{pa}) of MP

ing one electron mechanism in the rate determining step of the electrode process, value of 0.365 was found for α, indicating that the activation free energy curve is asymmetrical in this irreversible oxidation process.

In the light of the above-mentioned results and previous works on the electrocatalytic oxidation of other sulfhydryl compounds [25, 26, 28] at the surface of electrodes modified with Schiff base complexes as electron mediator, the following mechanisms may be suggested for the catalytic effect of cobalt salophen in the modified carbon-paste electrode:

$$RSH + H_2O \rightarrow RS^- + H_3O^+ \qquad (RSH : MP) \qquad (5)$$

$$RS^- + Co(III)Salophen \rightarrow [RS - Co(III)Sal]_{ads} \rightarrow RS_{ads}{}^{\bullet} + Co(II)Sal \qquad (6)$$

$$2RS_{ads}{}^{\bullet} \rightarrow RSSR \qquad (7)$$

$$Co(II)Sal \rightarrow Co(III)Sal + e^- \qquad (8)$$

Differential pulse voltammetric determinations

Differential pulse voltammetric (DPV) measurements under the optimum experimental conditions were performed for MP solutions with various concentrations, and the obtained voltammograms are illustrated in Fig. 5a. The calibration curve was obtained and a linear relationship between the oxidation peak currents ($i_{p,a}$) and MP concentration in two ranges: 1×10^{-6}–1×10^{-5} M (slope=0.192) and 1×10^{-5}–1×10^{-4} M (slope=0.139) with a detection limit of 1×10^{-7} M

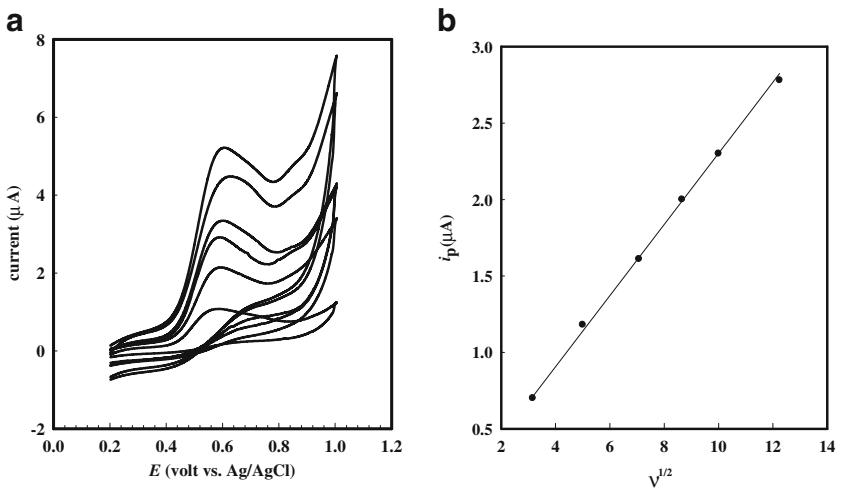

Fig. 4 CVs of 0.1 mM MP at different potential scan rates (down to up—10 to 150 mV s^{-1}) in PBS (pH 3.0)

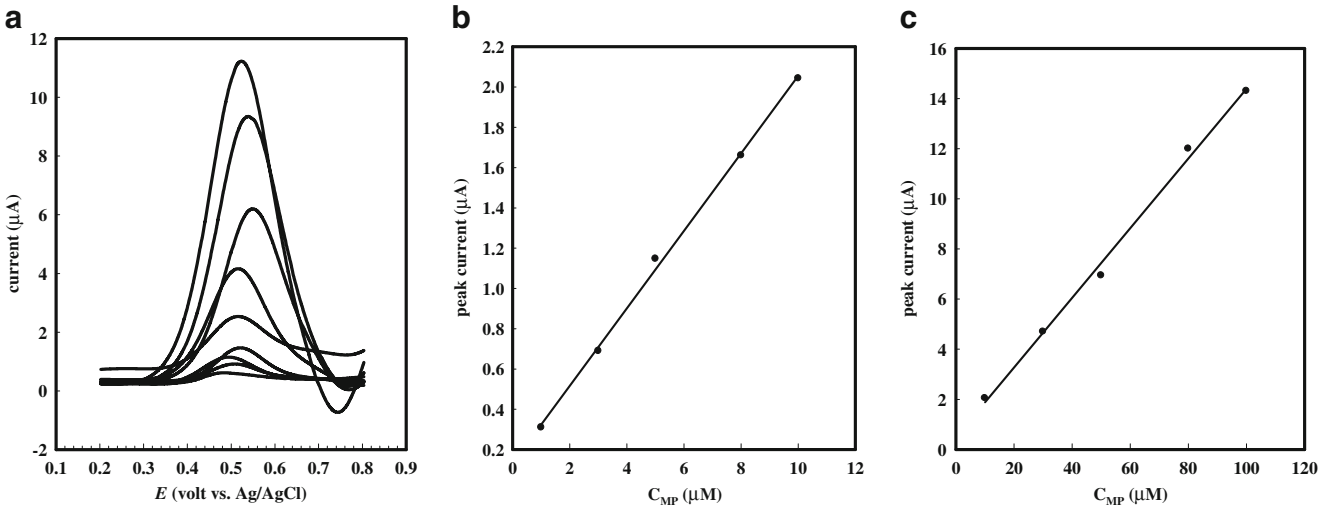

Fig. 5 **a** DPVs of various concentrations of MP (down to up—1, 3, 5, 8, 10, 30, 50, 80, and 100 μM) in 0.1 M PBS as supporting electrolyte (pH 3.0); corresponding linear calibration curve of oxidation peak current versus MP concentration **b** 1 to 10 and **c** 10 to 100 μM. Pulse amplitude was 50 mV

was resulted (Fig. 5b, c). These relationships can be described with the following linear regression equations in the mentioned concentration ranges:

$$i_{p,a}(\mu A) = 0.013 C_{MP}(\mu M) + 0.499 \left(R^2 = 0.998\right) \quad (9)$$

Analytical applications

The carbon nanotube-paste electrode modified with cobalt salophen complex was utilized for the electrochemical measurements of MP using DPV method. The method validity was tested according to the International Conference for Harmonization guidelines [41]. The developed method was applied as a very sensitive electrochemical method with sub-micromolar detection limit and high precision for the determinations of MP in a wide concentration range. Repeatability (intra-day) for the proposed sensor was tested with each three DPVs of three sample solutions containing lower, middle, and higher concentrations in the linear range. Intermediate precision (inter-day) of the method was evaluated by considering lower, middle, and higher concentrations in the linear range in 3 days. The precision results, which are presented in Table 1, express excellent precisions for the determination of MP using MCPE. The accuracy of the proposed method was studied by recovery experiments in lower, middle, and higher concentrations in the linear range (1–100 μM, n=9). The results showed very good recoveries between 97.36% (±3.51%) and 103.66% (±7.04%) with an average of 101.11% (±1.73%). The selectivity of the method was assessed by adding known quantities of standard solution to the tablet solutions and human plasma samples. Considering the obtained DPVs, no interferences were found in the potential range for the voltammetric peak of MP during the analysis.

In order to evaluate the applicability of the proposed method for drug analysis, the proposed method was utilized for the determination of MP tablets. For the analysis of 20 tablets with a labeled value of 50.00 mg, an average amount

Table 1 Precision (intra- and inter-day) in standard solutions of MP

Concentrations (μM)	Intra-day (n=3)		Inter-day (n=9)	
	Mean response±SD	CV (%)	Mean response±SD	CV (%)
10	0.304±0.005	1.521	0.305±0.006	1.883
50	1.147±0.066	5.807	1.17±0.046	3.940
100	2.043±0.015	0.747	2.067±0.039	1.905
500	6.87±0.400	5.827	6.811±0.221	3.249
1,000	13.3±0.400	3.007	13.44±0.286	2.130

of 48.92 mg was found, which represents a good accuracy of 97.32% with a RSD of 3.89% (n=5). Recovery and precision studies were evaluated in the lower, middle, and higher level concentrations of the spiked MP to the pharmaceutical solutions. For the added concentration of MP in the range of 58.75–176.25 μM, the recovery results between 97.44% (±3.14%) and 98.13% (±3.66%) were resulted.

Human plasma samples were spiked with standard concentrations of MP in the range of 10–100 μM, and the prepared modified electrode was applied for direct analysis of these samples without further pretreatment and extraction steps. Recovery and precision studies were evaluated in lower, middle, and higher levels of the concentration range. The recovery results were obtained in the range of 96.58% to 105.8% with an average of RSD 3.66%, which are in acceptable level according to USFDA [42].

Our investigations showed that the anodic peak of the AA oxidation appeared in potentials less than 0.200 V on the surface of MCNTPE. Also, the uric acid (UA) has a slight solubility in buffer solution of pH 3.0. However, it showed a weak anodic oxidation on the surface of this electrode, and its peak potential is seen near 0.300 V. These results revealed that the corresponding anodic peaks for AA and UA are completely resolved from the anodic peak of MP. Therefore, it can be concluded that the modified electrode in this work can be successfully applied for MP determinations in the presence of such compounds.

Conclusions

The electrocatalytic oxidation of MP at the carbon nanotube-paste electrode modified with a newly synthesized salophen complex of cobalt (III) perchlorate was studied, and a reasonable selectivity and sensitivity was achieved in comparison with previous studies. The proposed methodology, with respect to the presence of cobalt complex and carbon nanotube on the surface of the electrode, showed very good catalytic effect toward the electro-oxidation of MP, since it enhances the oxidation peak currents and lowers the oxidation overpotential. Therefore, a simple and effective electrochemical sensor utilizing DPV method allowed the successful determination of MP in pharmaceutical and clinical preparations and proved that this method can be a good alternative and advantageous over the reported methods.

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