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Voltammetric determination of Molnupiravir used in treatment of the COVID-19 at magnetite nanoparticle modified carbon paste electrode

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A R T I C L E   I N F O

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A B S T R A C T

To reduce the progression of the viral process in patients infected with COVID-19, new treatments and drug active substances are needed. One of these drugs is Molnupiravir (MNP) which has a direct antiviral effect and has also proven to be highly effective in reducing the azopharyngeal SARS-CoV-2 infectious virus and viral RNA. Due to the importance and frequent use of this drug in the treatment of COVID-19, its accurate, quick, and cheap detection in pharmaceutical or biological samples is crucial. In this work, electrochemical behavior and sensitive voltammetric determination of MNP are described using a magnetite nanoparticle modified carbon paste electrode (Fe₃O₄@CPE) for the first time. Fe₃O₄ nanoparticles (NPs) were characterized by recording their transmission electron microscopy (TEM) images, energy dispersive X-ray (EDX), and X-ray diffraction (XRD) spectra. Cyclic voltammetric measurements showed that MNP was irreversibly oxidized at Fe₃O₄@CPE at 760 mV in pH 2.0 Britton Robinson buffer solution (BRBS). The peak current of MNP was increased approximately threefold at Fe₃O₄@CPE compared to bare CPE due to a good electrocatalytic efficiency of Fe₃O₄ NPs. According to differential pulse voltammetric studies, the fabricated electrode exhibited a linear range (LR) between 0.25 and 750 µM with sensitivity and limit of detection (LOD) of 4591.0 µA mM⁻¹ cm⁻² and 0.05 µM, respectively. On the other hand, although lower sensitivity (327.3 µA mM⁻¹ cm⁻²) was obtained from CV compared to DPV, a wider linear calibration curve between 0.25 and 1500 µM was obtained in CV. Studies performed in tablet samples confirmed that the Fe₃O₄@CPE exhibits high applicability for selective and accurate voltammetric determination of MNP in real samples.

1. Introduction

The new type of coronavirus (COVID-19, also known as SARS-CoV-2) was first detected in December 2019, in Wuhan, China, and has an ongoing impact all around the globe, especially because of the recently emerged variants such as Delta, Omicron, and Ba.2. On 11th March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak to be a global pandemic [1]. More than 630 million cases and more than 6.5 million deaths were reported by WHO on 9th November 2022 [2]. While vaccine studies are being conducted to combat COVID-19, several existing antiviral drugs that have previously proven effective and safe against other viruses have been urgently used for the treatment of COVID-19.

One of these drugs is Molnupiravir (MNP) known as N-Hydroxy-5'-O-isobutryl-3,4-dihydrocytidine [(2R,3S,4R,5R)-3,4-Dihydroxy-5-[4-(hydroxyamino)-2-oxopyrimidin-1-yl]oxolan-2-1]-methyl 2-methylpropanoate with commercial cod (EIDD-2801 and MK-4482) (Scheme 1 in the oxidation mechanism of MNP) [3]. It was originally developed by the university’s drug innovation company (Drug Innovation Ventures at Emory (DRIVE)) at Emory University for the treatment of influenza [4]. Several synthesis routes, along with the synthetic route proposed by Emory University in 2019 to obtain MNP, are given in a review article [5]. To develop this drug, Merck & Co and Ridgeback Biotherapeutics companies made a partnership and a new oral anti-viral drug was launched [6,7]. Owing to favorable results obtained from placebo-controlled double-blind randomized clinical trials; MNP was affirmed for medical treatment in the UK in November 2021 [8]. In December 2021, the U.S. Food and Drug Administration (FDA) granted emergency use authorization of MNP for use in certain populations where other treatments are not available [9]. MNP, an oral ribonucleoside analog...
with broad-spectrum antiviral activity, is a prodrug of the synthetic nucleoside derivative N4-hydroxycytidine (NHC) and exerts its antiviral effect through the insertion of transcription errors during viral RNA replication. MNP blocks the replication of SARS-CoV-2 in the lines of effect through the insertion of transcription errors during viral RNA polymerase (RdRp) causes viral mutations and lethal mutagenesis. In this way, MNP shows potent antiviral activity against SARS-CoV-2.

The development of sensitive, accurate, low-cost, reliable analytical methods is required for the monitoring and determination of this important drug in different matrices such as biological fluids and pharmaceutical preparations. The determination of this newly developed drug in both pharmaceutical samples and body fluids is very important, and thus studies on detection methods have recently been started. In the first studies, various chromatographic methods have been developed for the determination of MNP such as reversed phase-high performance liquid chromatography (RP-HPLC) with a photodiode array detector (UV measurement at 240 nm) [10], HPLC with UV detector [11] and LC-MS/MS [12]. Although chromatographic methods offer selective and simultaneous determination of MNP with together other drugs or its metabolites, these methods need some requirements such as time-consuming sample preparation steps, highly trained personnel, highly cost instrumentation, high solvent consumption, and complicated procedures. Therefore, the development of simple, selective, and sensitive new alternative methods in drug analysis has always been of interest.

Among the various analytical methods, electroanalytical methods have been widely used for the determination of various compounds in pharmaceutical samples because they offer great advantages such as high simplicity, easy operation, portability, low cost, miniaturization, rapid response, acceptable sensitivity, accuracy, precision, and selectivity [13–18]. In electrochemical measurements, both bare and modified electrodes have been extensively used in pharmaceutical analysis. However, bare electrodes show poor sensitivity and selectivity due to high background current and overpotentials, heterogeneous and passive surface, and slow redox kinetics in electrochemical reactions of many compounds. These limitations have been overcome by modifying the electrode surfaces with new synthetic materials and modifiers [19]. In this direction, many researchers have recently reconstructed the electrode surfaces by modifying them with nanomaterials to improve the sensitivity and selectivity and to provide excellent electrocatalytic potentials owing to their beneficial properties such as large active surface area, good electrical conductivity, high surface/volume ratio [19–22]. Carbon paste electrodes (CPEs) are one of the most commonly used electrodes in the fabrication of modified electrodes because they offer some advantages such as ease of fabrication and modification, renewable surface, low residual current, low cost, porous structure, chemical inertness, good reproducibility, and stability [23,24]. Due to their important properties such as a high ratio of surface area to volume, strong adsorption ability, and electrocatalytic activity, metal or metal oxide nanomaterials have been frequently preferred in the preparation of CPE-modified electrodes [23–28]. Metal oxides are active and durable electrocatalysts for the construction of sensors and biosensors [29]. Among the nanomaterials, magnetite nanoparticles (Fe3O4 NPs) have attracted growing interest in recent years in electroanalysis because they have advantages over other oxides in terms of their high theoretical capacity of 922 mAh g–1, eco-friendliness, natural abundance, low particle size, low-cost production, controllable and large active surface area, ease of preparation, strong superparamagnetic, nonpoisonous, electrical conductivity, biocompatibility, and catalytic properties [29–31]. Therefore, Fe3O4 NPs have been widely used as modification material for electrode surfaces in fabricating sensors/biosensors [19,23–37]. In particular, the modification of CPE with Fe3O4 NPs, which involves a very simple preparation procedure, yields an efficient surface area and good electrocatalytic activity on the bare electrode surface. Thus, an efficient mass transfer is achieved by mediating electron transfer between the electroactive species and the electrode surface by using a graphite/Fe3O4 system, as well as an easily prepared modified CPE with a low-cost and renewable surface is obtained [23].

According to our literature search, the first electrochemical study on MNP determination using a reduced graphene oxide modified glassy carbon electrode (rGO/GCE) was reported by Nemutlu et al [38]. Although rGO/GCE [38] offers high sensitivity, reproducibility, and stability for MNP analysis, a time-consuming modification procedure was used with expensive electrode materials. In addition, a theoretical electrochemical study has been reported for MNP and the possibility of electrochemical determination of MNP was evaluated as theoretical [39]. Electrochemical oxidation of MNP on a composite material containing poly(1,2,4-triazole-co-(squaraine dye)) and cobalt(III) oxyhydroxide was theoretically examined in this published study. In our study, Fe3O4@CPE used provides an easily prepared renewable electrode surface and includes a simple and low-cost modification procedure. Moreover, the electrode used has a competitive detection limit compared to the rGO/GCE [38]. Thus, the novelty of this study is the development of a sensitive, selective, and stable electrochemical method for MNP analysis with a simple, easily prepared, low-cost electrode (Fe3O4@GCE) whose electrode surface can be easily regenerated. The feasibility of the fabricated electrode was investigated by performing the determination of MNP in a pharmaceutical sample and recovery studies in pharmaceutical samples spiked with MNP. The fabricated electrode exhibits highly sensitive, selective, repeatable, and reproducible results with high recovery values for the determination of MNP in real samples.

2. Materials and methods

2.1. Chemicals and instrumentation

Molnupiravir (C13H19N5O7, 98%, 329.31 g/mol) and graphite (fine powder extra pure, particle size ≤ 50 µm, and bulk density 20–30 g/100 mL) were supplied from Merck. Mineral oil with spectroscopic grade was supplied from Sigma-Aldrich. All other chemicals used in this study are of analytical grade.

Scheme 1. A possible mechanism for the electrocatalytic oxidation of MNP.
All electrochemical experiments were performed by using an interface compactstat device with Ivium technologies (from Netherlands) and an electrochemical cell with a classical three-electrode system: (i) working electrode: BASI carbon paste electrode (Geometric area (GA): 0.065 cm²; diameter: 2.87 mm), (ii) reference electrode: BASI-RESB Ag/AgCl(sat. KCl) (diameter: 6.0 mm; length: 7.5 cm) and (iii) counter electrode: BASI-MW1032 Pt wire (diameter: 0.5 mm; length: 7.5 cm). An Elga Option-Q7B device was utilized to get ultra-pure water (18.2 MΩ cm⁻¹) for the preparation of all solutions. The pH values of buffer solutions were measured with a Hanna HI-221 pH-meter with a combined glass electrode. TEM micrograph, EDX, and XRD spectra of Fe₃O₄ NPs were recorded using a JEOL-JEM-1400 PLUS, JEOL SEM-7100-EDX, and XRD PANalytical Empyrean devices in the Applying Science and Technology Center of Çanakkale Onsekiz Mart University (ÇOBILTUM). A stock MNP solution (0.01 M) was prepared by dissolving 0.033 g of MNP in 10 mL of methanol. Britton Robinson buffer solutions (BRBSs) were prepared in the pH range between 2.0 and 10.0 from 0.10 M KCl and DPVs were recorded based on the standard addition method. In all experiments, highly pure Ar was purged from supporting electrolytes for 5 min.

3. The results and discussions

3.1. Optimization of the amount of Fe₃O₄ in modified CPE

To see the effect of the amount of Fe₃O₄ in the modified CPE on the voltammetric response of the MNP, modified carbon paste electrodes containing different ratios of Fe₃O₄ in the range from 0.0 to 10.0 % were prepared. Then CVs of each modified electrode were recorded in pH 2.0 BRBS containing 0.10 M KCl in the presence of 0.5 mM MNP at a scan rate of 50 mV s⁻¹. According to the recorded voltammograms (Fig. S1A) and the curve of peak current versus %Fe₃O₄ in the modified CPE (Fig. S1B), the oxidation current of MNP increased proportionally with increasing Fe₃O₄ amount from 0.0 % to 5.0 % and did not change much after 5.0 % Fe₃O₄. Thus, a 5.0 % Fe₃O₄ amount in the modified electrode was determined as the optimum value, and modified CPEs prepared at this ratio were used in all following studies.

3.2. Characterization of the Fe₃O₄@CPE

Electrochemical impedance spectroscopy (EIS) gives valuable information about the surface conductivity of the modified electrode surface. Thus, EIS spectra of both bare and modified electrodes were recorded in the solution of 0.10 M KCl + 5.0 mM Fe(CN)₆³⁻/⁴ (Fig. S2A). It is seen from the Nyquist diagram of bare CPE (Fig. S2A-a) that it has a very high charge transfer resistance (Rct = 1330 Ω), while this Rct value is considerably reduced by modifying of the CPE surface with Fe₃O₄ NPs (Rct = 853 Ω, Fig. S2A-b). The decrease in Rct value is attributed to the increase in the effective surface area of the electrode, and the electroactive sites due to the good conductive properties of Fe₃O₄ NPs. Therefore, it was concluded that the Fe₃O₄ NPs improved the surface conductivity properties of the CPE. Fig. S2B shows the CVs of the bare and modified electrodes in the same redox probe solution. Compared to bare CPE (Fig. S2B-a), the increases observed in the peak currents of the redox couple of ferro/ferri cyanides at Fe₃O₄@CPE support the results of the EIS analysis.

The morphological characterization studies were carried out by recording the TEM micrographs, EDX, and XRD spectra of Fe₃O₄ NPs. As can be seen from the TEM micrographs, the dispersion of the Fe₃O₄ NPs is nearly spherical and the size of the nanoparticles differs between 12 and 18 nm (Fig. 1A). The recorded EDX spectrum also reflects the elemental components (33.7 % Fe and 66.1 % O) of Fe₃O₄ NPs (Fig. 1B). The crystal structure of Fe₃O₄ NPs was examined by XRD and the diffraction patterns of Fe₃O₄ are given in Fig. 1C. As can be seen from the figure, the characteristic peaks observed at 2θ degree of 30.45, 35.77, 43.41, 53.69, 57.52 and 63.16 are attributed to the (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0) crystal planes of Fe₃O₄ NPs which are in good agreement with standards (JCPDS19-0629) and the Fe₃O₄ NPs show spinel structure of cubic inverse [40]. All results obtained from TEM, EDX, and XRD analyses support the successful synthesis of magnetite nanoparticles.

3.3. Determination of the electroactive surface area

The electroactive surface area of the bare CPE and Fe₃O₄@CPE was further examined by evaluating the current response of Fe(CN)₆³⁻/⁴ redox probe from Fig. S2B. As can be seen from the Fig. S2B, Fe₃O₄@CPE displays an improvement in the current response, in other words, the electrochemically active sites of bare CPE were increased by the modification of the Fe₃O₄ NPs onto the surface. As a result, the Fe₃O₄@CPE gives a higher current response (Ipa = 33.1 μA) compared with the bare CPE (Ipa = 12.7 μA) due to the electrocatalytic activity of the Fe₃O₄ NPs. The electroactive surface area of the electrodes was estimated according to the Randles–Sevcik Eq. (1) [23,38,41].
\[ I_{pa} = 2.69 \times 10^5 n^{3/2} A C_0 D^{1/2} v^{1/2} \]  

(1)

“I_{pa}” is oxidation current (A), “n” is the electron number (which is equal to one), “A” is the area of the active surface (cm²), “C_0” is the concentration of the Fe(CN)_6^{3/4} (5.0 \times 10^{-6} mol cm^{-3}), “D”: diffusion coefficient of Fe(CN)_6^{4-} (6.23 \times 10^{-6} cm^2 s^{-1}) [23]. The electrochemically active surface areas were calculated as 0.044 cm² and 0.017 cm² for Fe_3O_4@CPE and CPE, respectively. In the literature, very close specific surface area values have been obtained in Fe_3O_4/CPEs prepared for use in the determination of various phenolic compounds [23] and simultaneous determination of ascorbic acid and folic acid [28]. The results indicate that the modification of Fe_3O_4 NPs enhanced (~3 times) the electrochemically active area of the electrode surface.

3.4. Electrochemical behavior of MNP at Fe_3O_4@CPE

The electrochemical response of both bare and modified CPEs in a pH 2.0 BRBS containing 0.10 M KCl towards 0.10 mM MNP was further investigated for the suitability of the respective modified electrode for the determination of MNP. As can be seen in Fig. 2, the MNP oxidized at about +760 mV on both bare CPE and Fe_3O_4@CPE surfaces. Although the capacitive current of Fe_3O_4 NPs modified CPE is higher than that of bare CPE, the peak current attributed to MNP oxidation at the modified electrode is found to be around three times higher than that obtained with bare CPE. In the literature, there are similar studies showing that both capacitive current and faradaic current of analyte increases as a result of the modification of CPE with Fe_3O_4 NPs [26–28]. Moreover, Fe_3O_4@CPE shows a better electrochemical signal than bare CPE due to obtaining a well-defined peak shape at the modified electrode. As a result, a remarkable enhancement was seen in the peak current of MNP at Fe_3O_4@CPE compared to the bare CPE. This enhancement can be attributed that Fe_3O_4 NPs showing good electrocatalytic activity towards the oxidation of MNP. As reported literature that the loading of magnetite nanoparticles to bare electrode surfaces acts as a promoter by increasing the electro-active surface area [25,28]. Therefore, the efficient electrocatalytic effect of the modified electrode is attributed to increasing the electrocatalytic surface area and even providing an active surface for the adsorption of the analyte. Due to the good electrocatalytic efficiency of Fe_3O_4 NPs, charge transfer between MNP and electrode surface is accelerated. As a result, the electrochemical characteristics of MNP were significantly improved by the modification of Fe_3O_4 NPs.

3.5. Effect of pH on the response of MNP

The electrochemical response and determination of redox-active compounds are affected by the pH conditions of the electrolyte solution. Thus, to see the effect of pH on the response of MNP, CVs of 0.10 mM MNP at Fe_3O_4@CPE in BRBSs with varying pHs between 2.0 and 7.0 were recorded separately (Fig. 3A). It was observed that the oxidation peak potential of MNP shifted towards negative potentials with the increase of pH, and the peak currents related to the electrochemical responses were found to decrease gradually. A linear curve of oxidation peak potential of MNP (E) vs pH with the equation of E(V) = −0.0491pH + 0.8345 (R^2 = 0.9983) proves that H^+ directly takes part in the electrooxidation of MNP (Fig. 3B). It can be concluded that equal
numbers of protons and electrons have participated in the irreversible oxidation of MNP at Fe₇O₈@CPE because the slope of this curve is nearly close the theoretical Nernstian slope value of 0.0592 V pH⁻¹. The BRBS with pH 2.0 where the electrochemical response of MNP oxidation is highest, was found to be the most suitable supporting electrolyte to be used. Obtaining high peak currents at acidic pH values can be explained by the protonation of MNP. At low pH values, MNP is protonated and the mass transport to the electrode surface increases resulting in the facilitation of its oxidation. The gradual decrease of the current as the pH increases from 2.0 to 7.0 is due to the decreasing protonated species and this supports the obtained result. Similar results were also obtained for the oxidation of some phenolic compounds at Fe₇O₈@CPE in the literature [23].

3.6. Effect of scan rate on the response of MNP

The effect of scan rate on the electrocatalytic oxidation peak current of MNP was examined by recording CVs of MNP at Fe₇O₈@CPE at various scan rates. Fig. S3A shows the CVs of 0.10 mM MNP in pH 2.0 BRBS containing 0.10 M KCl at increasing scan rates in the range from 10 to 1200 mV s⁻¹. It can be seen in Fig. S3B, that the oxidation peak currents of MNP increased linearly with the increasing square root of scan rate with an equation of I(µA) = 0.6798v^(1/2) + 0.1190 (R² = 0.9973). This linear curve indicates that MNP is irreversibly oxidized at Fe₇O₈@CPE surface by a diffusion-controlled process. Linear curves of logarithmic oxidation peak current versus logarithmic scan rate (Fig. S3C) support the diffusion-controlled process because the slope of 0.35 in this curve is close to the ideal slope of 0.5 reported for the diffusion-controlled process.

To calculate the electron number (n, for a totally irreversible reaction at 25 °C) that takes part in the electrocatalytic oxidation process of MNP, the following Eq. (2) [39] was used:

\[
E_p - E'_p/2 = \frac{47.7}{n} \text{mV}
\]

where \(E_p\) is the oxidation peak potential, \(E'_p\) is the formal potential of MNP, \(n\) is the electron number, and \(mV\) is millivolts.

3.7. Evaluation of the analytical performance

The response of both bare CPE and Fe₇O₈@CPE towards the oxidation of MNP was investigated by differential pulse voltammetry and cyclic voltammetry. Before analytical performance studies, pH, pulse time, and pulse amplitude were optimized by recording differential pulse voltammograms (DPVs) of 250 µM MNP at Fe₇O₈@CPE. Fig. S4A shows DPVs of MNP at BRBS with varying pH between 2.0 and 8.0. As seen in the curve of peak current versus pH (Fig. S4B), the maximum peak was obtained from pH 2.0, these results agree with the CVs of MNP at various pH media. In addition, pulse time and pulse amplitude were optimized as 2 ms and 200 mV, respectively. Related voltammograms and curves are shown in Fig. S5 for pulse time and pulse amplitude. Although the maximum peak current was obtained at a pulse time of 1 ms, 2 ms was selected to obtain well-defined peaks.

DPVs of MNP based on its concentration in the range from 0 to 1500 µM for bare CPE and Fe₇O₈@CPE were given in Fig. S6A and Fig. 4A, respectively. Current versus concentration curves of dynamic and linear ranges are given in Fig. S6B-C and Fig. 4B-C for bare CPE and Fe₇O₈@CPE, respectively. As can be seen from these curves, the peak current of MNP was linearly changed dependent on its concentration in the range between 5.0 and 250 µM for bare CPE (\(I(\mu A) = 0.045 \times [\text{MNP}] (\mu M) - 0.161, R^2 = 0.9994\)), while linearity range was found as 0.25–750 µM for Fe₇O₈@CPE (\(I(\mu A) = 0.202 \times [\text{MNP}] (\mu M) + 0.0862, R^2 = 0.9993\)). The LOD was estimated to be 0.05 µM based on 3S₀/m, where S₀ is the standard deviation of the minimum MNP concentration that gives a measurable signal and m is the slope of the linear calibration curve. Fe₇O₈@CPE offered wide linear range for MNP determination compared with bare CPE and the modified electrode give 4.5 times more sensitive responses to MNP than CPE when the ratio of the slopes was taken into consideration. From DPV studies, the sensitivity of the modified electrode was calculated as 4591.0 µA mM⁻¹ cm⁻². In addition, analytical performance studies were also performed by recording CVs of MNP, and linear ranges were found in the range from 2.5 to 750 µM for bare CPE (\(I(\mu A) = 0.0048 \times [\text{MNP}] (\mu M) - 0.0278, R^2 = 0.9991\)) and 0.25–1500 µM for Fe₇O₈@CPE (\(I(\mu A) = 0.0144 \times [\text{MNP}] (\mu M) + 0.1778, R^2 = 0.9994\)) were obtained (Fig. S7).

In the literature, only one modified electrode has been reported for the voltammetric determination of MNP based on its oxidation at pH
9.0. A comparison of the analytical performance of the fabricated electrode with previously published rGO/GCE [38] was given in Table 1. This table shows that the Fe$_3$O$_4$@CPE has a much wider linear range than rGO/GCE and the sensitivity of the Fe$_3$O$_4$@CPE is competitive with rGO/GCE. Moreover, Fe$_3$O$_4$-modified CPE has some advantages such as a simple modification procedure, renewable surface, lower cost, and wider linear range over RGO/GCE [38]. The obtained results proved that Fe$_3$O$_4$@CPE provides a favorable platform for the determination of MNP. It can be concluded that these two electrodes exhibit the first electrochemical studies on the voltammetric determination of MNP.

3.8. Precision of the Fe$_3$O$_4$@CPE

The repeatability and reproducibility of the Fe$_3$O$_4$@CPE were also examined by recording DPVs of 250 µM MNP under previously optimized experimental conditions. To investigate the repeatability, 10 replicative intra-day measurements were performed in presence of MNP at Fe$_3$O$_4$@CPE. After each measurement, the surface of the electrode was washed with deionized water. The reproducibility of the modified electrode was tested at 10 newly prepared Fe$_3$O$_4$@CPE. The RSD values for repeatability and reproducibility were calculated as 3.79 % and 4.34 %, respectively. The obtained results (RSD ≤ 5.0 %) showed that Fe$_3$O$_4$@CPE exhibits high precisions for the determination of MNP.

3.9. Selectivity

To evaluate the selectivity, the voltammetric response of 100.0 µM MNP at Fe$_3$O$_4$@CPE was recorded in presence of various molecular and ionic species. Non-electroactive groups such as some cations, anions, and monosaccharides may interact with the analyte even though they do not give any signal or oxidation peak like MNP, for example, some metal ions may form a complex with MNP, or they can participate in electrostatic or intermolecular interaction with the analyte or analyte may be chemically oxidized by some molecules. In order to elucidate all these issues, it is useful to study of interference effect of some metal ions and anions on the analyte signal in interference studies, even if they are not electroactive. Therefore, the interference results of some non-electroactive ions or molecules, as well as electroactive compounds such as ascorbic acid (AA), dopamine (DA), and uric acid (UA) are summarized in Table 2. In addition, DPVs of the electroactive molecules (AA, DA, and UA) recorded at increasing concentrations alone and in the presence of the analyte (based on increasing interferant/analyte ratios using constant concentration as MNP:100 µM) are given in Fig. S8. Results were evaluated as the relative percent change (% Change in $I_{pa}$ = [(Ip0–Ip)/Ip0] x 100, Ip0 = the oxidation current of MNP in the absence of interfering ions or molecules and Ip = oxidation current of MNP in

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**Table 1** Comparison of the analytical performance of Fe$_3$O$_4$/CPE with previously reported rGO/GCE.

| Electrode-electrochemical method | Supporting electrolyte/Oxidation potential of detected MNP | LR (µM) | LOD (µM) | Sensitivity (µA mM$^{-1}$ cm$^{-2}$) |
|---------------------------------|--------------------------------------------------------|---------|----------|-------------------------------------|
| Fe$_3$O$_4$/CPE CV$^2$ and DPV$^2$ (This work) | pH 2.0 BRBS/750 mV vs Ag/AgCl (sat. KCl) | 0.25 – 1500$^{1,3}$ | 0.07$^{1}$ 0.05$^{3}$ | 327.3$^{1}$ 4591.0$^{3}$ |
| rGO/GCE SWV [38] | pH 9.0 BRBS/260 mV vs Ag/AgCl (sat. KCl) | 0.09–4.57 | 0.03 | – |

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Fig. 4. DPVs of varying concentrations of MNP recorded at Fe$_3$O$_4$@CPE in pH 2.0 BRBS containing 0.10 M KCl at 20 mV s$^{-1}$ (pulse time: 2 ms and pulse amplitude: 200 mV) (A: 0, 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 17.5, 25, 50, 75, 100, 175, 250, 500, 750, 1000, 1250, 1500 µM MNP). Current versus concentration curves of dynamic (C) and linear (D) ranges.
the presence of interfering species in a known ratio of analyte to interfer-
ence). It can be seen that UA, DA, and AA do not give any interference
fect to 100 μM MNP in the interferant:analyte ratios of 0.1, 1, and 5, re-
pectively. As seen from the figure, significant positive interferences
cluded that common ions such as K⁺, Mg²⁺, Zn²⁺, Ca²⁺, Cl⁻, NO₃⁻, SO₄²⁻ and some molecules such as H₂O₂, glucose, sucrose, fructose,
mannose, and maltose did not show any interference effect on the
oxidation of MNP even when each interfering compound concentration
was 100 or 250 times higher than MNP. However, Cu²⁺ gave positive
ference in the 5:1 of interferant:analyte ratios due to most probably
formation of complex between Cu²⁺ and MNP. It can be concluded that
the Fe₃O₄@CPE may offer high selectivity for voltammetric deter-
mination of MNP in samples containing these interfering species in the
interferant:analyte ratios determined.

3.10. The application of Fe₃O₄@CPE on real samples

In the applicability of the electrochemical sensor, MNP with three
different concentrations (25.0, 50.0, and 100.0 μM) was spiked into
pharmaceutical tablet solutions including 50.0 μM MNP. Then, standard
addition DPVs were recorded under optimized conditions. DPVs and
standard addition calibration curves for un-spiked and spiked samples
were given in Fig. S9. The experimental results and recovery values
found between 98.2 and 100.6 % were summarized in Table 3. The MNP
content was found to be 203 ± 6 mg/tablet, which is very close to the
declared value of 200 mg MNP/tablet by a pharmaceutical company.
The obtained results illustrated that the Fe₃O₄@CPE provides a highly
accurate and precise determination of MNP in pharmaceutical samples.

4. Conclusion

According to our literature search, only one study on the electro-
chemical behavior and voltammetric determination of MNP is recently
reported using rGO/GCE [38]. As a second study, the electrochemical
study of MNP is described for the first time using highly efficient
magnetite nanoparticles modified carbon paste electrode. The prepared
Fe₃O₄@CPE shows high electrocatalytic activity towards irreversible
oxidation of MNP compared with bare CPE. The fabricated electrode
shows a quite wide linear range (between 0.25 and 750 μM) with a LOD
of 0.05 μM from DPV results. In addition, Fe₃O₄@CPE was successfully
used for the determination of MNP in real pharmaceutical samples and
satisfactory results with high accuracy and precision have been ob-
tained. It is considered to offer a good possibility as an alternative
method for use in quality control units for the qualitative and quanti-
tative analysis of MNP in samples. As a result, an accurate, sensitive, and
reproducible electrochemical method has been developed and its
applicability has been demonstrated using Fe₃O₄@CPE for the voltam-
metric determination of MNP.

Table 2

| Molecular Species | Ionic Species | Fold % Change in Ip | Fold % Change in Ip |
|-------------------|--------------|---------------------|---------------------|
| Glucose           | K⁺           | 250 –7.4 ± 0.5      | 250 –6.3 ± 0.7      |
| Sucrose           | Na⁺          | 250 –8.3 ± 0.8      | 250 –7.8 ± 0.9      |
| Fructose          | Mg²⁺         | 250 –3.8 ± 0.4      | 250 –6.1 ± 0.8      |
| Mannose           | Zn²⁺         | 250 –7.8 ± 0.6      | 250 –5.1 ± 0.7      |
| Maltose           | Ca²⁺         | 250 –4.5 ± 0.5      | 250 –2.2 ± 0.4      |
| H₂O₂              | Cl⁻          | 100 –7.7 ± 0.8      | 5 –9.0 ± 1.1        |
| Uric acid         | Cl⁻          | 0.1 + 5.9 ± 1.2     | 250 –7.8 ± 0.9      |
| Dopamine          | NO₃⁻         | 1 + 7.1 ± 1.8       | 250 –5.2 ± 0.8      |
| Ascorbic acid     | SO₄²⁻        | 5 + 6.0 ± 1.5       | 250 –4.7 ± 0.7      |

Table 3

| Sample           | Added standard MNP (µM) | Found MNP (µM) | Recovery (%) | Labeled amount (mg MNP/tablet) | Found (mg MNP/tablet) |
|------------------|------------------------|---------------|--------------|------------------------------|---------------------|
| Pharmaceutical   | 0                      | 51.4 ± 2      | –            | 200                          | 203 ± 6             |
| Tablet           | 1.6                    | 76.8 ± 100.6  | –            | –                            | –                   |
| 25.0             | 2.5                    | 101.5 ± 100.1 | –            | –                            | –                   |
| 50.0             | 148.7 ± 98.2 ± 3.7     | –             | –            | –                            | –                   |
| 100              | 148.7 ± 5.8            | –             | –            | –                            | –                   |

CRediT authorship contribution statement

Kader Vural: Software, Investigation, Validation. Serkan Kar-
akyaya: Software, Methodology, Writing – review & editing, Visualiza-
tion. Didem Giray Dilgin: Investigation, Methodology. Hatice Ismet Gökçel:
Writing – review & editing, Conceptualization. Yusuf Dilgin:
Writing – review & editing, Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial
interests or personal relationships that could have appeared to influence
the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.
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