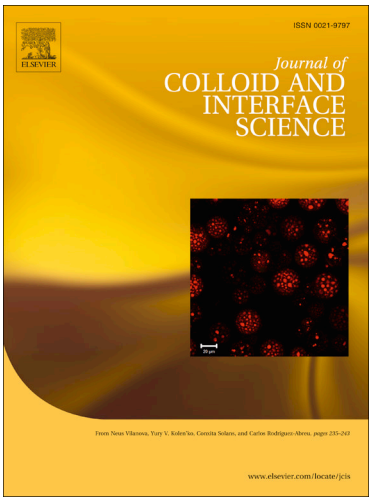

Regular Article

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Mani Govindasamy, Akilarasan Muthumariappan, Shen-Ming Chen, Yi-Hui Cheng, Veerappan Mani, Kogularasu Sakthivel, Fahad M.A. Al-Hemaid, M. Ajmal Ali, Xiaoheng Liu

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One-pot synthesis of three-dimensional Mn_3O_4 microcubes for high-level sensitive detection of head and neck cancer drug nimorazole

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Abstract

We described a three-dimensional Mn_3O_4 microcubes (3D–Mn_3O_4MCs) synthesised via a facile hydrothermal route for the determination of nimorazole (NMZ), an important drug that used in the treatment of head and neck cancer. The 3D–Mn_3O_4 MCs possess large active area and high conductivity, and 3D–Mn_3O_4 MCs film modified screen-printed carbon electrode (3D–Mn_3O_4MCs/SPCE) was fabricated which displayed excellent electrocatalytic ability towards NMZ. Under optimized working conditions, the modified electrode responded linearly to NMZ in the 0.025-8060µM concentration range and the detection limit was6 nM. A rapid, sensitive, selective, reproducible, and durable sensor was described. The practical feasibility of the sensor was demonstrated in human serum and NMZ tablet samples. The obtained results revealed the potential real-time applicability of the sensing device in biological analysis and pharmaceutical formulations.

Keywords: Drug analysis; Nimorazole; anti-cancer drug; biosensor; electrochemical biosensor; modified electrode; transition metal oxides

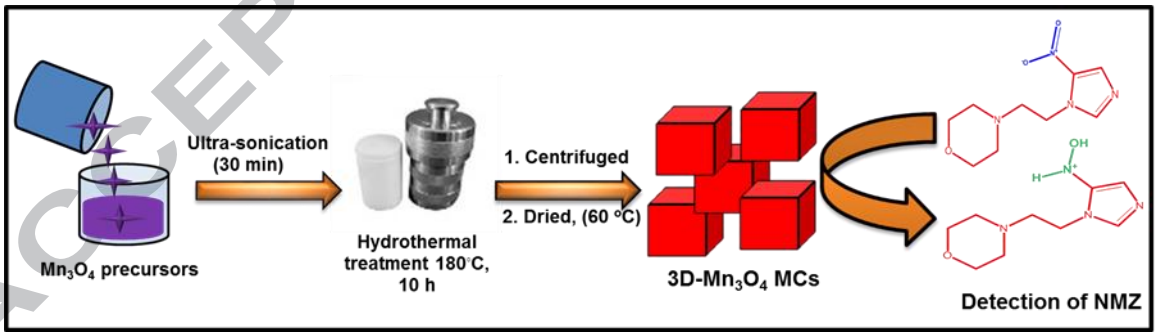
1. Introduction

Drug analysis is an important step used to perform during various phases of pharmaceutical evaluation, including active drug monitoring through regular status and control, toxicology, stability, and other pharmacological studies [1, 2]. The study mainly involves the analysis of patient samples for oral drug evolution [3] and pharmacokinetic studies [4]. In order to achieve such purposes and for measuring drugs in different media, reliable and validated analytical methods are desirable that offers direct, simple and quick determination platform requiring a minimal sample volume with purifications or pre-sampling free steps [5]. Nimorazole(4-[2-(5-nitro-1H-imidazol-1-yl)ethyl]morpholine, NMZ) is a synthetic nitroimidazole derivative having anti-cancer and antibacterial properties [6]. For NMZ determination, several analytical methods have been developed which includes, spectrophotometry [7], liquid chromatography [8], liquid chromatography–mass spectrometry [9-11], liquid chromatography–tandem mass spectrometry [10], high performance liquid chromatography (HPLC) [12], and electroanalytical methods [13]. However, some of these methods suffer from several problems, such as high cost, bulky and complex, long analysis time, cumbersome extraction or pre-sampling procedure, which prevent their use for routine sample analysis [14]. On the other hand, electrochemical methods are low-cost, portable, easy-to-use, fast, direct digital signal, miniaturizable and less power consumption. The electroanalytical techniques are efficient methods to study the drug reaction mechanisms at an electrode surface, which can provide insight into their metabolic fate, *in vivo* redox processes and biological activity as well [15]. However, there are no reliable electrochemical sensors based on low-cost electrode for the NMZ sensing.

Recent developments in materials chemistry research are accompanied by the synthesis of new structured metal oxide materials with novel properties [16, 17]. These metal oxide materials offer excellent electronic, conductivity and catalytic properties, and electrocatalytic activity, which accelerate electron transfer between the electrode surface and redox species. These unique structured metal oxide materials have been applied to electroanalysis of drugs in various preparation methods [18-20]. The design and synthesis of unique structured metal oxide on electrode surfaces were performed to improve the sensitivity of electrochemical measurements by increasing the surface area or using the

catalytic activity of metal oxide materials and the transition metal oxides have been used for the anticancer activities [21-27]. Among the transition metal oxides Mn_3O_4 materials have been also utilized as a drug in the treatment of cancer [21]. Mn_3O_4 (hausmannite) have an outstanding theoretical capacity of electrochemical activity, low-cost and have high surface area; as a result, it has been extensively employed as an electrode material in batteries and supercapacitor [28-31]. It's an important metal oxide that is widely used as an active catalyst for the oxidation of methane [32], carbon monoxide[33] and the reduction of nitro groups [34]. Although, Mn_3O_4 can be prepared by many methods, Mn_3O_4 with three-dimensional(3D) structure can be prepared by altering methods or reaction conditions. Owing to the various versatile characteristic features, including large surface area and the folded architecture of 3D network had promoted it as an attractive material for electrochemical sensor and biosensor applications.

The main objective of this work is to prepare 3D–Mn_3O_4 microcubes as a novel electrocatalyst for the electrocatalytic reduction of NMZ. Because of low-cost, easy fabrication, flexibility, and reproducibility, we adopted screen-printed carbon electrodes (SPCE) modified using 3D–Mn_3O_4 to prepare NMZ sensor. The developed amperometric sensor have displayed highly enhanced electrocatalytic activity, good sensitivity and detection limits in sub-nanomolar levels. The other advantages of the method are low over potential, enhanced signal, and quick response time.



Scheme 1. Schematic representation for the synthesis of 3D–Mn_3O_4MCs and its application towards the determination of NMZ in biological and pharmaceutical samples.

2. Experimental

2.1 Apparatus

The electrochemical measurements were carried out using CHI 1205a workstation with a conventional three-electrode cell using SPCE as a working electrode (Area= 0.2 cm^2), Ag|AgCl (saturated KCl) as a reference electrode and Pt wire as an auxiliary electrode. Amperometric measurements were conducted using analytical rotator AFMSRX (PINE instruments, USA). EIM6ex ZAHNER (Korach, Germany) was used for performing electrochemical impedance spectroscopy (EIS) studies. Surface morphological studies were analysed using Hitachi S-3000 H Field-Emission Scanning Electron Microscope (FE-SEM). Energy-dispersive X-ray (EDX) spectra were recorded using HORIBA EMAX X-ACT (Sensor + 24V=16 W, resolution at 5.9 keV). Powder X-ray diffraction (XRD) studies were explored using XPERT-PRO (PAN alytical B.V. The Netherlands) diffractometer using Cu Kα radiation. Fourier Transform Infrared spectroscopy (FT-IR) measurements were completed using Perkin Elmer spectrum RXI. Raman spectral studies were executed using Raman spectrometer (Dong Woo 500i, Korea) equipped with a charge-coupled detector.

2.2 Reagents and materials

Manganese(II) sulphate monohydrate ($MnSO_4 \cdot H_2O$), thiourea and other chemicals were purchased from Sigma-Aldrich and used as received. SPCEs were purchased from Sensor R&D Co., Ltd., Taipei, Taiwan. All the reagents used were of analytical grade and used without any further purification. The supporting electrolyte used for the electrochemical studies was 0.1 M Phosphate buffer (PB), prepared using Na_2HPO_4 and NaH_2PO_4 and the pH was adjusted either using H_2SO_4 or NaOH. Prior to each experiment, the electrolyte solutions were deoxygenated by passing nitrogen gas for 10 min.

2.3 Synthesis of 3D–Mn_3O_4Microcubes and fabrication of modified SPCE

The 3D–Mn_3O_4 MCs were synthesised via a simple hydrothermal route as represented in **Scheme 1**. Briefly, 30mg of thiourea and 67.6 mg of $MnSO_4 \cdot H_2O$ were dissolved in distilled water (40 mL) separately. After being fully dispersed by ultrasonic agitation, the solution of $MnSO_4$ was added dropwise into the solution of thiourea. The obtained mixture was stirred for 30 min to acquire a homogeneous solution. The resultant solution was transferred to a Teflon-lined stainless steel autoclave and hydrothermally treated at180°C for 10 h. After the completion of reaction, the reddish product was collected and washed with ethanol and dried at 60^o C for overnight. The product was transferred to a tube furnace, heated to 400° C at a heating rate of 2° C min^{-1}, and kept at that temperature for 2 h. Finally, the product three-dimensional Mn_3O_4 microcubes (3D–Mn_3O_4 MCs) were dispersed in ethanol via ultrasonication to obtain 1 mg mL^{-1}. Next, 6 µL dispersion of 3D–Mn_3O_4 MCs were dropped at the SPCE surface and dried at room temperature.

3. Results and discussions

3.1 FESEM, EDX and mapping of 3D–Mn_3O_4 MCs

The FESEM images of 3D–Mn_3O_4 MCs were shown in **Fig. 1A, B.** The overall view of 3D–Mn_3O_4 MCs revealed the presence of several 3D cube-shaped particles and the side view of a microcube exposed the smooth and clean surface. The size of the microcubes were in the range of micrometre. The microcubes were well separated from each other and there were no aggregations between cubes and the 3D structure, rough surface and high crystallinity jointly furnished large surface area, which is highly beneficial in electrochemical sensing, due to the large surface area of 3D–Mn_3O_4 MCs it achieves the abundant catalytic sites on its microcubes. The EDX profile of 3D–Mn_3O_4 MCs exhibited elemental signals of Mn and O with weight percentages of 41.26, and 58.74, respectively, while their corresponding atomic percentages were of 35.20, and 64.80, respectively (**Fig. 1C**). The EDX mapping of 3D–Mn_3O_4 MCs displayed the distribution profile of Mn and O elements on the surface (**Fig. 1 D, E, and F**).

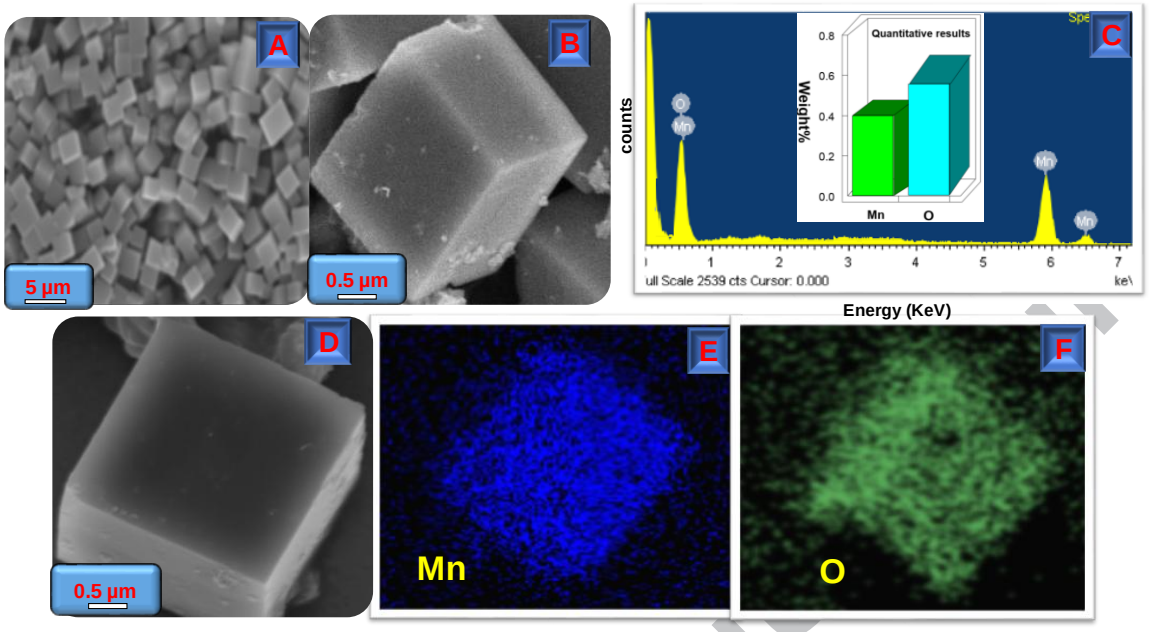


Fig. 1 FESEM images of 3D–Mn_3O_4MCs overall image (A) and side view of a microcube (B). EDX profile and quantitative result (insert)(C), and mapping of 3D–Mn_3O_4MCs (D, E and F)

3.2 XRD patterns, Raman, FT-IR and impedance spectral studies

Fig. 2A presented the XRD pattern of 3D–Mn_3O_4MCs which sketched peaks at 2θ of 18.7º (101), 28.4º (112), 32.3º (200), 32.7º (103), 35.6º (211), 39.4º (004), 43.7º (220), 50.9º (105), 52.6º (513), 57.1º (303), 58.3º (321), 62.2º (224), and 64.7º (440). These peaks were consistent with the standard XRD data of Mn_3O_4 MCs (JCPDS no. 24-0734) [35-39], hence confirmed the crystal structure of as-prepared microcubes [40].

Raman spectrum of the synthesized sample exhibited by **Fig. 2B**. Three peaks at 317 cm^{-1}, 366 cm^{-1}, and 653 cm^{-1} can be observed. The Raman spectrum of bulk Mn_3O_4 MCs ($MnMn_2O_4$ in spinel notation) was characterized by a very sharp peak at 653 cm^{-1}, this peak is responsible for the Mn-O breathing vibration of trivalent manganese ions in tedrahedral coordination. Two smaller peaks at 317 $cm^{-1}$and 366 cm^{-1} were also observed, similar results were found for Mn_3O_4 nanoparticles [41, 42].

Fig. 2C shows the FT-IR spectrum of Mn_3O_4 MCs. Mainly, two sharp peaks at 562 cm^{-1} and 761 cm^{-1} were observed , which corresponds to the Mn–O bending and stretching vibrations in the spectrum is indicative of a tetragonally distorted cubic lattice. These peaks may be due to the coupling mode between Mn–O stretching modes of tetrahedral and octahedral sites, which confirms the presence of Mn_3O_4 MCs. Similar reports were found for Mn_3O_4 MCs [43].

EIS analysis has been carried out to study the electrical and interfacial properties of the fabricated films modified electrodes [44]. Fig.2D displays the EIS curves obtained at SPCE (a), 3D–Mn_3O_4MCs/SPCE (b) in 0.1 M KCl containing 5 mM $Fe(CN)6^{3-/4-}$. Randles equivalent circuit model was adopted to fit the experimental data wherein, R_s, R_{ct}, C_{dl} and Zw are stands for electrolyte resistance, charge transfer resistance, double layer capacitance and Warburg impedance (inset to Fig.2D). The results were exemplified as Nyquist plots. The observed semicircles portions of the EIS curves indicates the parallel combination of R_{ct} and C_{dl} at electrode surface resulting from electrode impedance and the linear portions of the curves represents diffusion limited process. The charge transfer resistance (R_{ct}) values obtained at unmodified SPCE, 3D–Mn_3O_4MCs/SPCE were 91.45Ω and 4.23Ω, respectively. The R_{ct} values are in the following order; unmodified SPCE > 3D–Mn_3O_4MCs/SPCE, respectively. Obviously, the EIS results revealed that the 3D–Mn_3O_4MCs/SPCE material interface has high electrical conductivity.

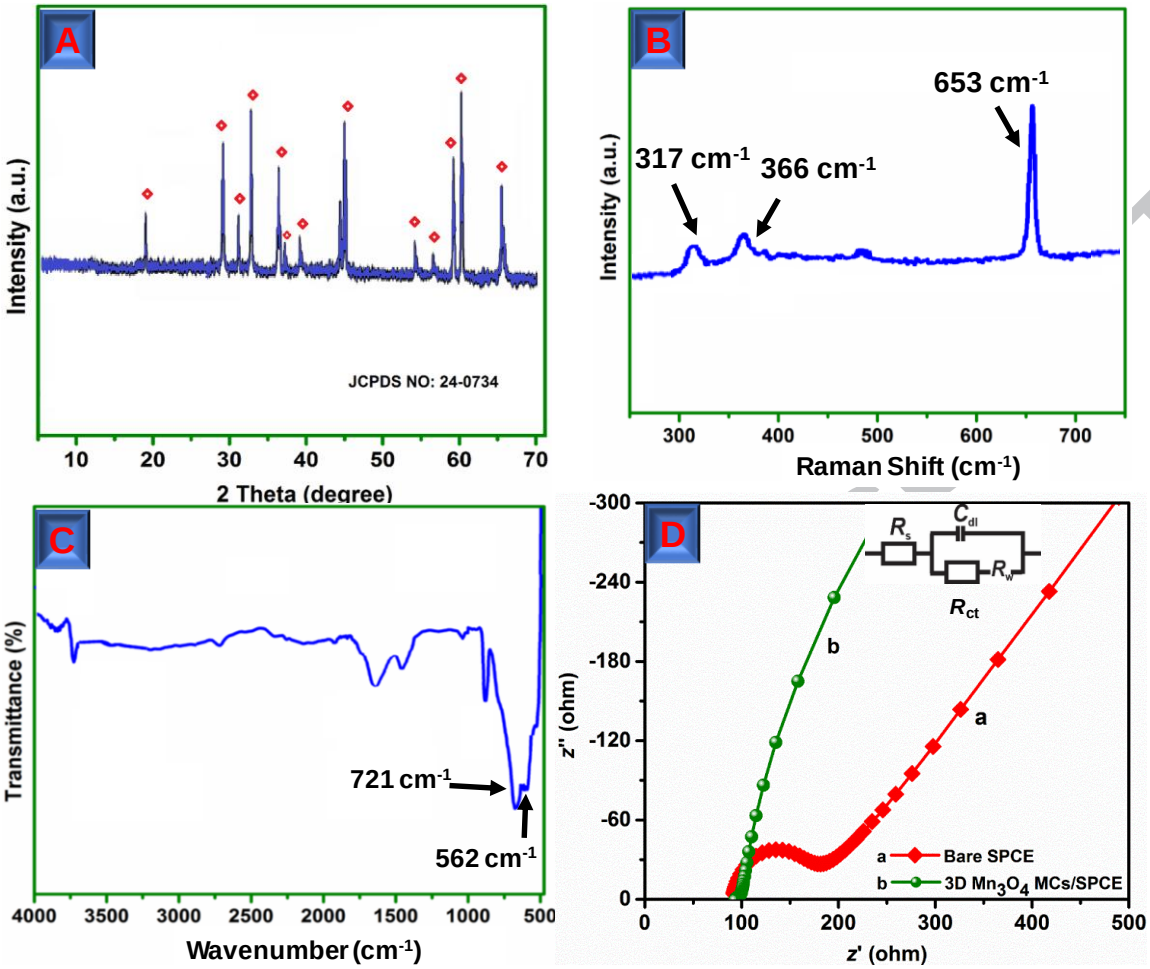


Fig. 2(A) XRD, (B) Raman and (C) FTIR spectra of 3D–Mn_3O_4MCs, and (D) EIScurves of unmodified SPCE (a), 3D–Mn_3O_4MCs/SPCE (b)in 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$. Amplitude= 5 mV, Frequency: 0.1 Hz to 100 kHz.Inset: Randles equivalent circuit used to fit the data; R_s, R_{ct}, C_{dl}, and Z_w are electrolyte resistance, charge transfer resistance, double layer capacitance and Warburg impedance, respectively.

3.3 Electrocatalytic activity of 3D–Mn_3O_4 MCs/SPCE towards NMZ reduction

Next, the electrochemical activity of 3D–Mn_3O_4 MCs was investigated. **Fig. 3A** illustrated the cyclic voltammograms (CVs) of SPCE (a), and 3D–Mn_3O_4 MCs (b) over a potential range of –0.38 V to 0.48 V (vs. Ag/AgCl) at a scan rate of $2mV.s^{-1}$. The CV of 3D–Mn_3O_4 MCs exhibited enhanced background current in comparison with control electrode. Two redox couples,–0.24 V/–0.28 V (I/II) and –0.04 V/–0.15 V (III/IV)

corresponding to reversible reactions of Mn_3O_4/MnOOH and MnOOH/MnO_2 were observed and those are characteristic voltammetric behaviour of electrochemically active 3D–Mn_3O_4 MCs [45].

Electrocatalytic ability of the 3D–Mn_3O_4/SPCE towards electrocatalysis of NMZ (50 µM) reduction is investigated in phosphate buffer (pH 7.0) (**Figure 3B)**. The forward segment of first cycle displayed a sharp cathodic peak at –0.60V, which is manifested to the reduction of NO_2–NMZ to NHOH–NMZ (**Eq. 1)**. An oxidation peak is observed at + 0.1 V in the backward segment, which is related to the oxidation of NHOH–NMZ to NO–NMZ and this reaction is reversible and hence corresponding reduction is also observed at –0.32 V during the second cyclic run. This type of electrocatalytic behaviour is consistent with the previous reports [14, 46-50].

$$\mathbf{NO_2\text{–}NMZ + 4e^- + 4H^+ \rightarrow NHOH\text{–}NMZ - 2e^- - 2H^+ \leftrightarrows NO\text{–}NMZ + 2e^- + 2H^+} \quad \text{Eq.(1)}$$

In this study, we focused on the reduction peak of NMZ (NO_2–NMZ to NHOH–NMZ). Accordingly, the NMZ reduction at 3D–Mn_3O_4/SPCE was monitored and compared with control electrodes. In comparison with control electrodes (bare SPCE, and 3D–Mn_3O_4/SPCE), the 3D–Mn_3O_4/SPCE is shown significantly enhanced reduction peak current. The observed over potentials at bare SPCE, and 3D–Mn_3O_4 MCs/SPEC are –0.78 V, and –0.6 V, respectively. The over potential at 3D–Mn_3O_4/SPCE is 180 mV lower than the bare SPCE. The drastic shift in the over potential at 3D–Mn_3O_4/SPCE is due to the electrocatalytic activity of 3D–Mn_3O_4, which greatly accelerates and catalyzes the reduction process. Thus, the 3D–Mn_3O_4 MCs/SPEC exhibited excellent electrocatalytic ability towards the reduction of NMZ, which is obvious from the observed high faradaic current and minimised over potential. This can be attributed to the large surface area, high conductivity, the3D structure of Mn_3O_4 and abundant catalytic sites on the cubes.

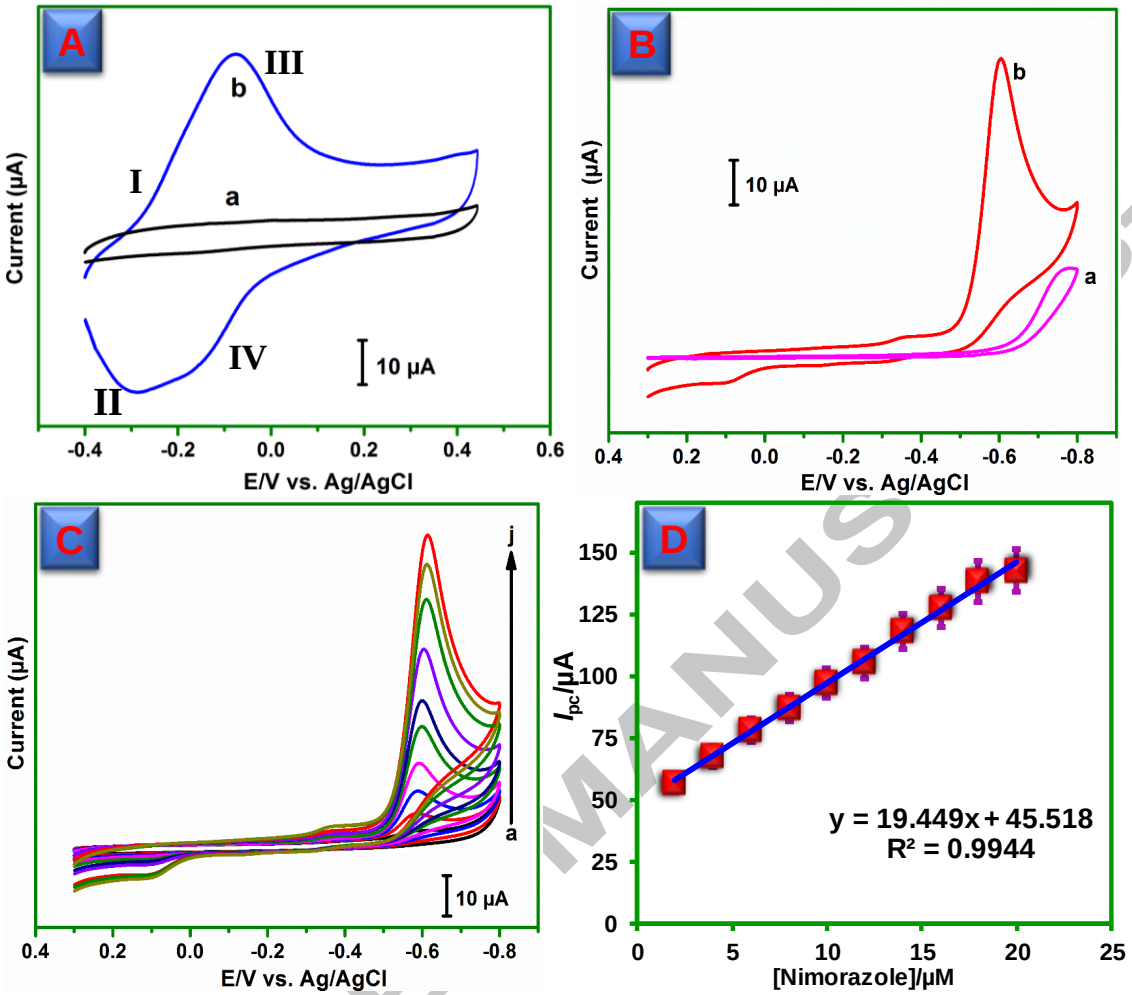


Fig. 3(A) CVs obtained at Bare SPCE (a), and 3D–Mn_3O_4(b), films modified SPCEs in 0.1 M NaOH at scan rate of 2 mV s^{-1}**.** (B) Cyclic voltammograms obtained at bare SPCE (a), and3D–Mn_3O_4MCs/SPCE(b) in PB (pH 7.0) containing 50 µM NMZ at the scan rate of 50 mV s^{-1}. (C) Cyclic voltammogram obtained at 3D–Mn_3O_4/SPCE in PB (pH 7) in different concentrations of NMZ (a=50, b=100, c=150, d=200, e=250, f=300, g=350, h=400, i=450, and j=500 µM). (D) Calibration plot, cathodic peak current (I_{pc})/µA vs. [NMZ]/µM

Fig. 3C displayed the CVs obtained at 3D–Mn_3O_4 MCs/SPCE towards different concentrations of NMZ in PB (pH 7). As can be seen from the figure, the peak current was increased linearly as the concentration of NMZ increased over a linear range of 50–500 µM with a correlation coefficient of 0.994**(Fig. 3D)**. Thus, the modified electrode is

capable of providing linear responses towards NMZ and the obtained good linearity is indicating anti-fouling nature of the electrode surface.

3.4 Effect of scan rate and pH dependence

Next, the effect of scan rate on the reduction of NMZ was studied by applying different scan rates (**Fig. 4A**). The cathodic peak current was linearly increased as the scan rate increased over the studied range from 0.02 to 0.2 Vs^{-1}. The plot between reduction peak current and the square root of scan rate exhibited linear relationship, which revealed the diffusion controlled electrocatalytic process of NMZ at the modified electrode (**Fig. 4B**). The influence of buffer pH on the electrochemical response of 3D–Mn_3O_4/SPCE towards 50 µM NMZ was investigated (**Fig. 4C).** As the pH of supporting electrolyte varied, the peak current and potential corresponding to NMZ reduction were changed. The NMZ reduction peak current increased as the pH increases from 3.0 to 7.0 and reached maxima at pH 7. From pH 7.0 to pH 11.0, the peak current gradually decreased. Thus, the reduction of NMZ at modified electrode was more favourable at pH 7.0; therefore, we set this pH as optimum pH for established NMZ sensor. The plot between different pH and peak potential was also exhibited a linear behavior (**Fig. 4D**).

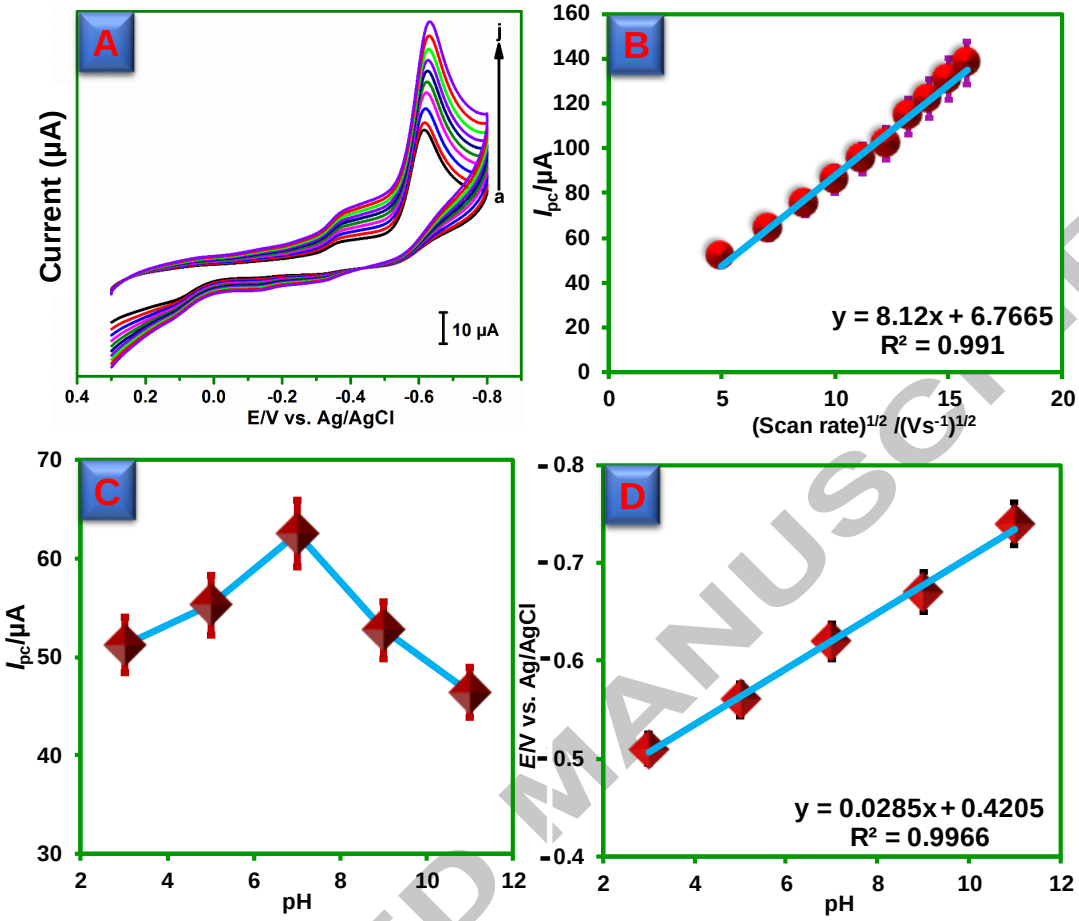


Fig. 4(A) Cyclic voltammograms obtained at 3D–Mn_3O_4MCs/SPCE in PB (pH 7.0) containing 50 μM NMZ at different scan rates (a=0.02, b=0.04, c=0.06, d=0.08, e=0.10, f=0.12, g=0.14, h=0.16, i=0.18 and j=0.20 V s^{-1}. (B) (Scan rate)$^{1/2}$/(V.s^{-1})$^{1/2}$ vs. peak currents (μA),(C) Plot of peak current (μA) vs. pH, and (D) Plot between peak potential (V) vs. pH For pH studies, the cyclic voltammograms were carried out in supporting electrolyte of different pH containing 50 μM NMZ at a scan rate of 0.50 V s^{-1}.

3.5Amperometric determination of NMZ

Fig. 5A displayed the amperometric responses of 3D–Mn_3O_4MCs film modified rotating electrode upon following successive injections of aliquots of NMZ into PB (pH 7.0) at regular intervals of the 50s. The rotation speed of the electrode was set at 1200

RPM and the electrode potential (E_{app}) was applied as –0.54 V. Clearly defined and steady amperometric responses were observed for each addition of NMZ. The steady state current was reached in less than 5s revealing quick sensor response. The response current was increased linearly as the concentrations of NMZ. The working range of the sensor was 0.025–8060 µM and the corresponding regression equation was, I_{pc}/µA=0.4958 [NMZ]/ µA μM^{-1}; with the correlation coefficient of 0.9945 (**Fig. 5B**). The limit of detection (LOD, S/N= 3) of the sensor was calculated to be 6 nM, while the limit of quantification (LOQ, S/N=10) was estimated to be 20 nM. Such a low detection limit in nanomolar level and wide linear range were clearly revealing the excellent sensing attributes of the 3D–Mn_3O_4MCs film towards the determination of NMZ. The sensitivity of the sensor was 3.80µA$\mu M^{-1}cm^{-2}$. The electroanalytical parameters achieved at 3D-Mn_3O_4 MCs were compared with previous reports as a **table 1.**

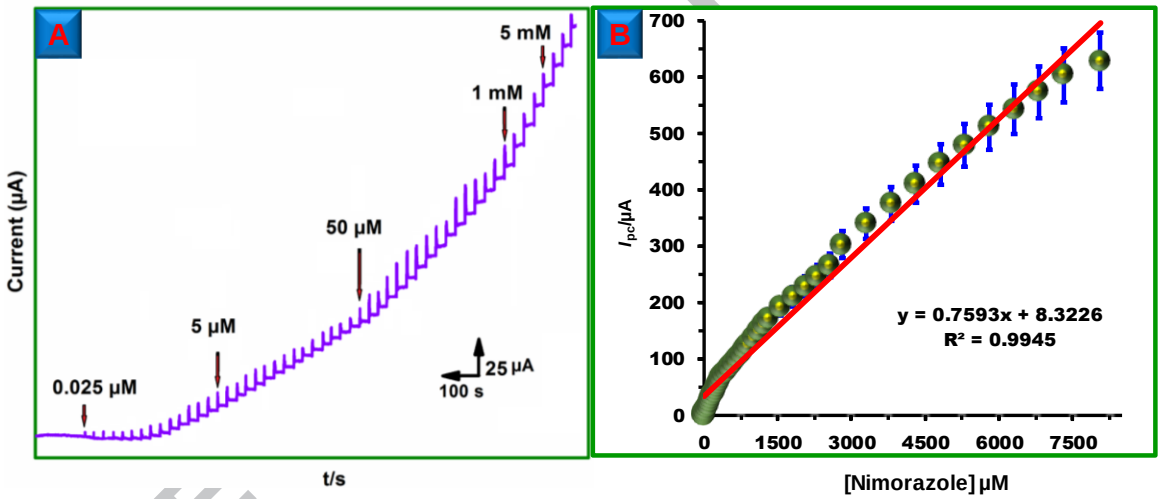


Fig. 5(A) Amperometric response of 3D–Mn_3O_4MCsfilm modified rotating disk electrode towards each sequential additions of NMZ into PB (pH 7.0). The rotation speed of the electrode was 1200 RPM and the electrode potential was– 0.54 V (vs. Ag/AgCl). (B) the plot between [NMZ]/µM vs. current (µA).

Table 1 Comparison of analytical parameters for the determination of NMZ at 3D-Mn_3O_4 MCs film modified SPCE with previously reported works

Methods	**Linear range**	**LOD**	**Ref.**
Differential pulse polarography	0.005-10 µM	2 µM	[14]
Liquid chromatography–mass spectrometry	0.25–200 ng/mL	0.25 ng/mL	[51]
Reversed-phase high-performance liquid chromatography	20 – 60µg/mL	20.62 ng/mL	[52]
Amperometry	0.025-8060 µM	6 nM	This work

3.6 Selectivity, reproducibility, repeatability, and stability

The selectivity of the modified electrode was assessed by performing amperometric analysis in presence of likely interfering compounds. **Fig. 6A** presented the amperometric responses of 3D–Mn_3O_4MCs/SPCE towards 5µM NMZ (a) and 0.5 mM of NADH (b), dopamine (c), folic acid (d), uric acid (e), cysteine (f), ascorbic acid (g), guanine (h), epinephrine (i), pyridoxine (j), acetaminophen (k), benperidol (l), and 5-Fluorouracil (m). The electrode quickly responded to NMZ, but it was insensitive to all the other tested species; thus, the electrode capable of recognising NMZ specifically in presence of many similar species and this situation usually exists in biological sample analysis.

The storage stability of the described 3D–Mn_3O_4MCs/SPCE incorporated sensor has been evaluated. The electrode retained its 95.2% initial current response after 3 weeks of its storage, which validated good storage stability of the modified electrode. The good stability of the electrode could be correlated to the strong interaction of 3D-Mn_3O_4 MCs film to the electrode surface. For the reproducibility and repeatability study, sensor response of the electrode towards 50 µM NMZ was monitored. The sensor

displayed good reproducibility with a relative standard deviation (R.S.D) of 3.68 % for five individual measurements performed on a single modified electrode. The R.S.D for five repetitive measurements using five modified electrodes was 4.58% indicating good repeatability.

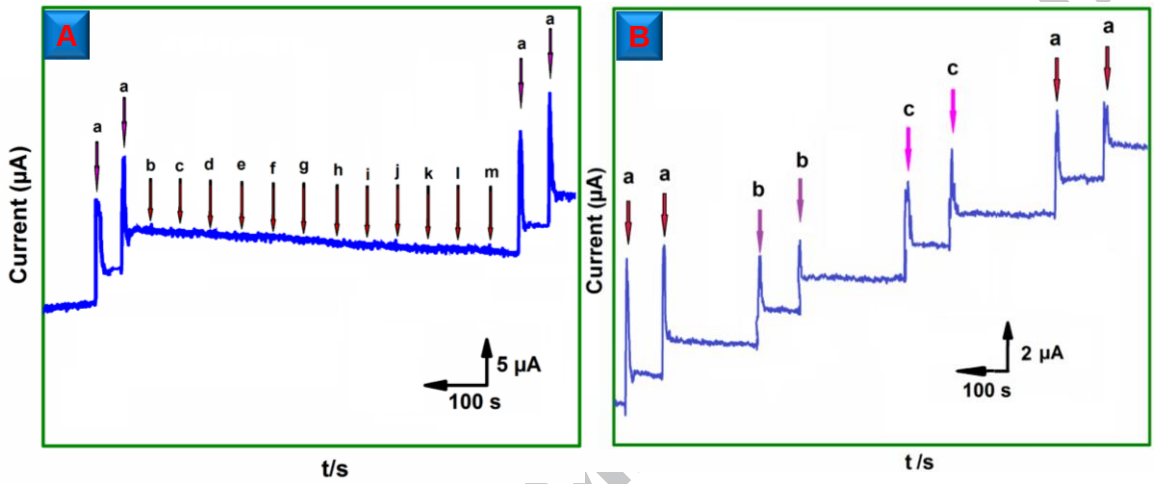


Fig. 6(A) Amperometric response of 3D–Mn_3O_4MCs/SPCE towards 50 μM NMZ (a) and 0.5 mM of NADH (b), dopamine (c), folic acid (d), uric acid (e), cysteine (f), ascorbic acid (g), guanine (h), epinephrine (i), pyridoxine (j), acetaminophen (k), benperidol (l) and 5- Fluorouracil (m). (B) Real sample analysis: Amperometric responses of 3D–Mn_3O_4MCs/SPCE towards 1 μM NMZ, lab sample (a), NMZ tablets (b), and spiked human blood serum (c)

3.7 Real sample analysis

All the excellent sensor features of 3D–Mn_3O_4MCs/SPCE were indicating that this electrode could be suitable for real-time drug analysis in pharmaceutical and biological samples. We have performed real sample study in drug and human serum samples. The drug sample was purchased from local pharmacy, grinded and dissolved in ethanol to prepare stock solution. Human serum sample was spiked with appropriate amount of NMZ. Then, amperometric experiments was performed to detect NMZ present in the aforementioned samples using 3D–Mn_3O_4 MCs/SPCE as electrode. Aliquots of known concentrations of lab sample, NMZ tablet sample and human serum sample were

injected and their corresponding amperometric responses were monitored. The signal sensitivity and response time of real samples were consistent with that of lab sample. The recoveries were calculated and found to be in the acceptable range of 96–97.5% (**Table 2**) and hence we believe the method can be used as reliable NMZ sensor towards real-time drug analysis.

Table 2 Determination of NMZ in drug and human blood serum samples

Real Samples	Added/μM	Found/μM	Recovery/%	*RSD/%
NMZ drug	1.0	0.96	96.0	3.75
	2.0	1.94	97.0	4.50
Human blood	1.0	0.97	97.0	3.53
serum	2.0	1.95	97.5	3.82

* Related standard deviation (RSD) of 3 independent experiments

4. Conclusions

We described the synthesis of novel 3D-Mn_3O_4MCs and characterised it through SEM, EDX, mapping, XRD, Raman, FTIR and electrochemical studies. The characterization results revealed the successful formation of high crystalline microcubes. The electrocatalytic studies revealed the superior electrocatalytic activity of 3D-Mn_3O_4 MCs/SPCE towards NMZ reduction. The effect of concentration, kinetics, and pH were optimised in order to obtain maximum sensor performance. The fabricated NMZ amperometric sensor displayed nanomolar level detection of NMZ with LOD of 6 nM and linear range of 0.025μM to 8060μM. Moreover, the fabricated sensor acquired good selectivity, repeatability, reproducibility and durability. In addition, the sensor was successfully analysed the NMZ amount present in NMZ drug and NMZ spiked human serum sample. The application of this material with simple preparation steps and exceptional properties such as 3D structure, large surface area, high conductivity, long-term stability on this metal oxide. In future, 3D-Mn_3O_4 MCs can be paired with immobilized antibodies for the biosensoric detection of food allergens.

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GRAPHICAL ABSTRACT

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One-pot synthesis of three-dimensional Mn_3O_4 microcubes for high-level sensitive detection of nimorazole (Head and neck cancer drug)

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