Supplementary information

Comparative insight into pristine and mixed oxides of cerium and manganese as polyphenol oxidase mimics

Anamika Arora[a], Manpreet Kaur[a]* and Mohammad Javed[b]

[a]*Department of Chemistry, Punjab Agricultural University, Ludhiana-141004, India*
[b]*Department of Mathematics, Statistics and Physics, Punjab Agricultural University, Ludhiana-141004, Punjab, India,*

Corresponding Author: * manpreetchem@pau.edu

S.1 Characterization techniques

The synthesized NPs were characterized using various techniques, including X Ray Diffraction (XRD), Fourier-Transform Infrared (FT-IR), X-Ray Photoelectron Spectroscopy (XPS), Scanning Electron Microscopy-Electron Dispersive X-Ray (SEM-EDX), Transmission Electron Microscopy (TEM), Brunaeur Emmett-Teller (BET) and Electrochemical Impeadance Spectroscopy (EIS analysis). Table S1†gives the type of characterization technique and instrument employed.

Table S1: Characterization techniques used to characterize synthesized NPs along with the model of the equipment utilized

S.No.	Instruments	Model
1.	Fourier Transform Infrared spectroscopy (FT-IR)	Bruker, Model: Tensor 27, FT-IR, Central University of Punjab, Bathinda
2.	Scanning electron microscopy with Energy dispersive spectroscopy (SEM-EDS)	Carl Zeiss Sigma 500, Bruker, Quantax 200 Thapar Institute of Engineering and Technology, Patiala.
3.	Transmission electron microscopy (TEM)	JEM 1011, Indian Agricultural Research Institute , New Delhi
4.	UV–Visible spectrophotometer	Shimadzu (UV-1800) UV spectrophotometer, CIL, Punjab Agricultural University
5.	X-ray Diffraction (XRD)	CuKα radiations (λ = 1.5404 Å) with a Panalytical X pert Pro, Indian Institute of Technology (IIT) Ropar
6.	X- ray Photoelectron Spectroscopy	PHI 5000 VersaProbe III, Indian Institute of Technology (IIT) Rookree
7.	Brunaeur Emmett Teller	Quantachrome NovaWin, Material Analysis Research Centre, Bengaluru
8.	Electrochemical Impedance Spectroscopy	Metrohm Autolab cyclic voltameter, DAV College, Chandigarh
9.	Dynamic Light Scattering (DLS) Analysis	Malvern Zetasizer, Central Instrumentation Facility, Lovely Professional University, Ludhiana

S.2.1 Synthesis of pristine oxide

For synthesis of pristine Mn_3O_4 and CeO_2 NPs to 50 ml of distilled water 0.01 moles of $M(NO_3)_2$ (M=Mn and Ce respectively) and 0.01 moles of monohydrate citric acid were added. The pH of the solution was adjusted to 7.0 by adding NH_4OH dropwise. After 6.0 hrs of stirring, the sol turned into a gel, which was then dried for 12 hrs at 100 °C in oven. The dry gel was crushed and then calcined in the muffle furnace at 500 °C for 3 hrs to obtain the final product.

S.2.2 Synthesis of doped oxides

For synthesis of doped oxides a similar method mentioned above was utilized. $Ce(NO_3)_3.6H_2O$ and $Mn(NO_3)_2$ were added in 2:1, 1:1 and 1:2 molar ratios. For synthesis of CM-1, CM-2 and CM-3 Ce: Mn moles were taken as 0.0066:0.0033, 0.005:0.005 and 0.0033:0.0066 respectively. To these solutions 0.01 moles of monohydrate citric acid were added. A similar approach mentioned in Sec S.2.1 was followed to obtain final products.

S.3 ROS Scavenging

In order to confirm the generation of radicals in the polyphenol oxidase-mimicking reaction, ascorbic acid, isopropanol alcohol (IPA) and sodium azide were employed to detect $O_2^{\bullet-}$,·OH, $^1O_2$, respectively. The typical procedure is as follows: to 0.3 mM catechol 10 mg NPs were added and scavenging agent was subsequently added and reacted for 10 min. the absorbance was noted at 390 nm.

S.4 Detection of polyphenols

The limit of detection (LOD) and limit of quantitation were calculated using equations[1]-

$$LOD = \frac{3.3\sigma}{S} \quad \text{--- (3)}$$

$$LOQ = \frac{10\sigma}{S} \quad \text{--- (4)}$$

For detection of polyphenols in green tea and wine sample a standard stock solution (10 mg/100ml) of gallic acid was prepared. Different concentration of gallic acid was prepared from the stock solution (20-100 µg/ml). The standard calibration curve was plotted as follows: to the 20 ml of 0.1mM TMB solution 100 µL of H_2O_2 (10 mmol/L) and 0.25 mg/ml of Mn_3O_4 NPs were added. To this solution different concentration of prepared gallic acid (20-100 µg/ml) was added and the change in absorbance at 652nm

was measured. Polyphenolic content in green tea sample was measured and comparatively analyzed using FC method and NPs.

S.4.1 FC method for estimation of polyphenols

For estimation of polyphenolic content using Folin-Ciocalteu method a standard calibration curve was plotted using gallic acid[2]. To different concentrations of gallic acid (20-100 µg/ml) 5000 µl of FC reagent and 4000 µl of 7.5 % sodium carbonate were added and absorbance (at 760 nm) was measured after 2 hours of incubation at 25 °C.

S.4.2 Estimation of polyphenolic content in green tea and red wine samples

For estimation of polyphenols in green tea sample 1gm of natural green tea was crushed and mixed in 20ml (80% methanol). This mixture was mixed in orbital shaker for 3hrs. The obtained mixture was centrifuged and filtered. To 20 µl of this aliquot 880 µl of methanol was added. To this solution 5000 µl of FC reagent and 4000 µl of 7.5 % sodium carbonate were added and absorbance was measured at 760nm. The total phenolic content measured by the Folin-Ciocalteu assay was statistically correlated with the results from the study by using optimized conditions of Mn_3O_4 NPs.

For estimation of polyphenols in red wine 20 µl aliquot of red wine was taken in test tube and diluted to 1 ml. To this solution FC reagent and sodium carbonate were added.

S.4.3 Interference Studies

The selectivity of the synthesized NPs towards polyphenol detection was accomplished by analyzing the effect of various interfering ions. For the purpose 100 µmol/L of interferent ions (NaCl, CTAB, KCl, Glucose, Salicylic acid and Ascorbic acid) were added to 20 ml of blank solution each consisting of 0.1 mM TMB solution and NPs. Change in absorbance on addition of these analytes was recorded.

S.5 Statistical analysis

The current study employs the Box-Behnken experimental design to investigate and optimize key factors influencing enzyme-mimic activity *viz*.: pH (A); Temperature (B); Catalyst dosage (C); Contact time (D). The coefficient of determination (R^2) and the quality and goodness of the proposed model were computed employing the equation. A graphic of actual versus expected values was also used, along with the normal distribution of the residuals. For statistical response analysis, analysis of variance (ANOVA) was employed, and a p-value of 0.05 was presumed to show that the parameters of the suggested model were statistically significant.

S.6 Recyclability

To measure reusability of the synthesized NPs to 0.3 mM catechol 10 mg of NPs were added and absorbance was noted after 15 mins. The NPs were then separated and run for subsequent four cycles to measure reusability. Change in absorbance was noted after each cycle.

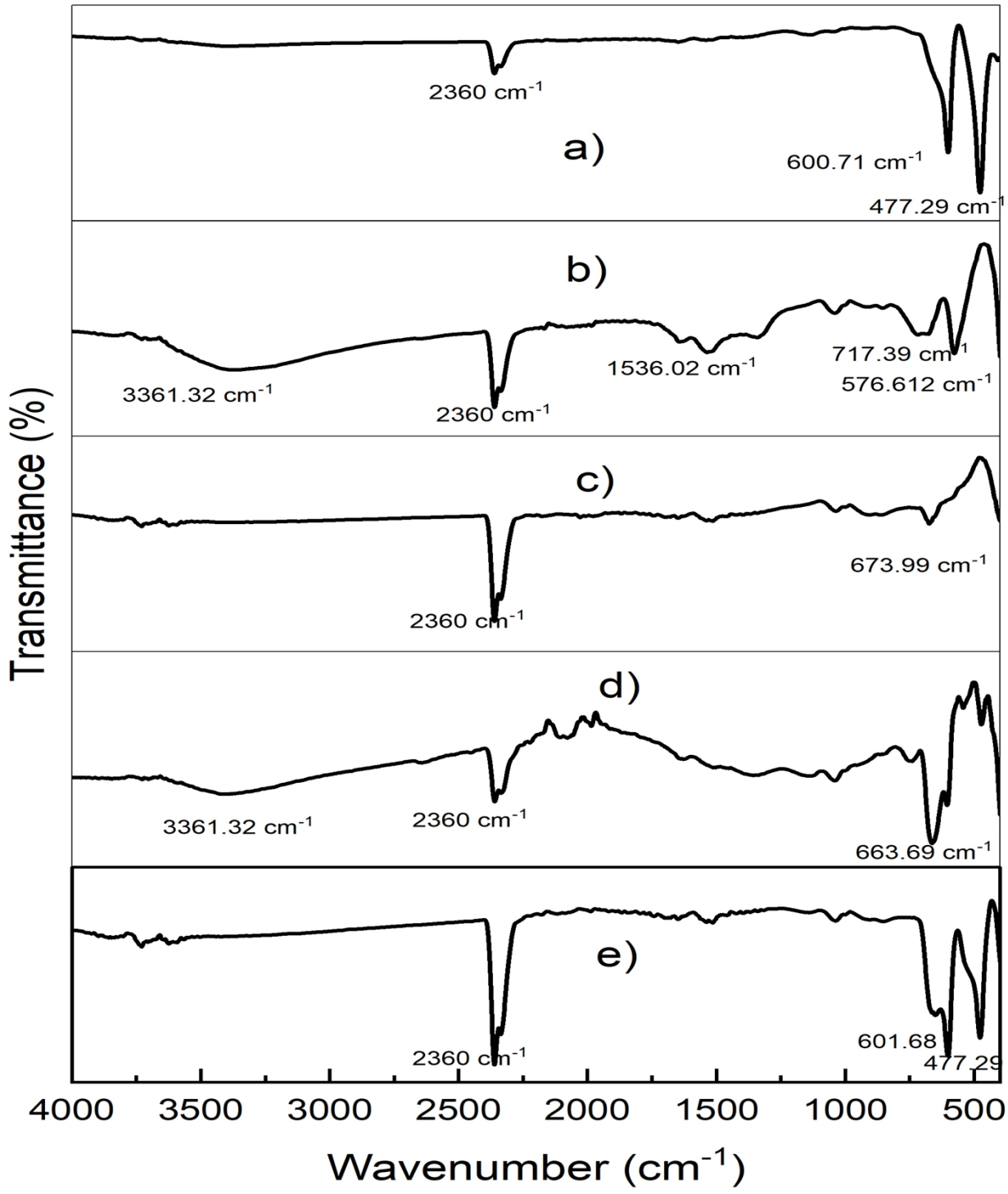

Fig. S1 FT-IR spectra of a) Mn_3O_4 b) CeO_2 c) CM-1 d) CM-2 e) CM-3

S.7 DLS analysis

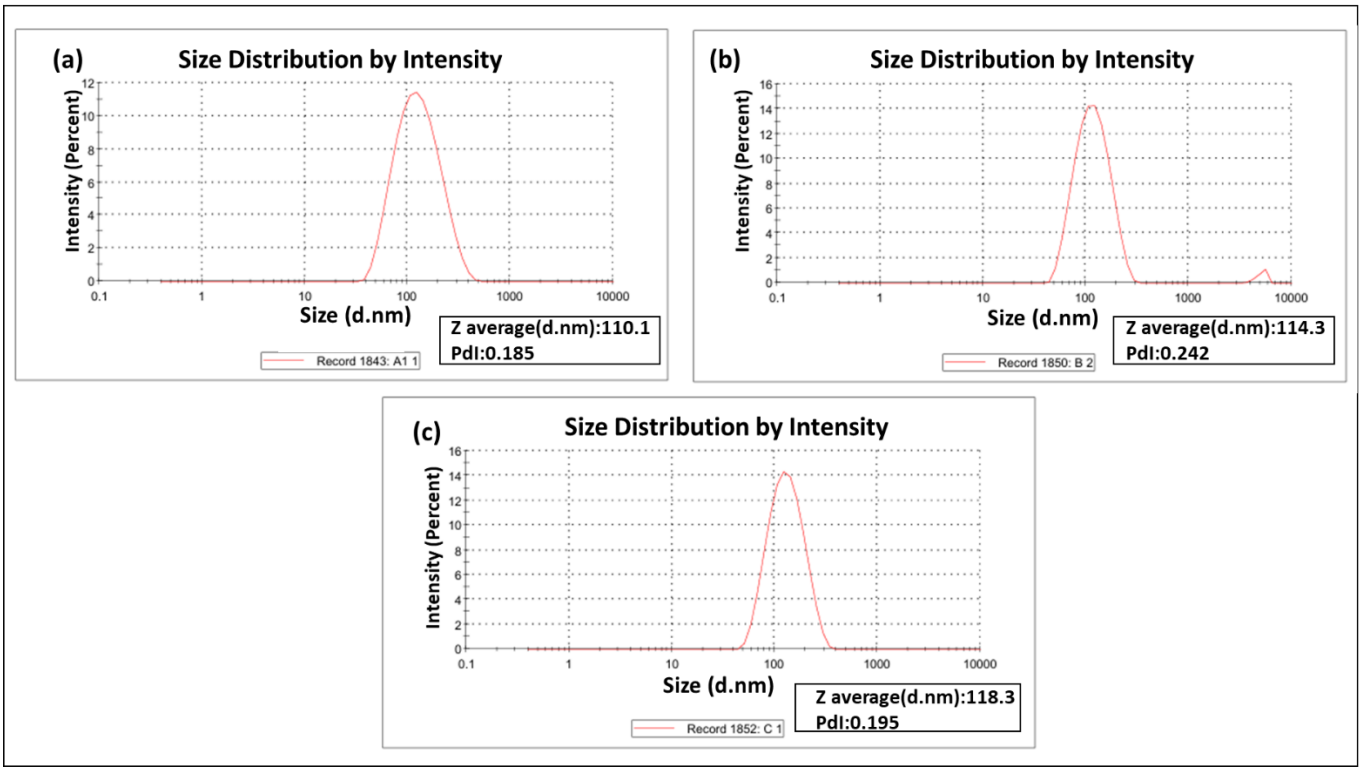

Fig. S2 Particle size distribution curve by DLS a)Mn_3O_4 b) CeO_2 and c) CM-3

DLS was used to measure average hydrodynamic size of the synthesized NPs in the aqueous media. All the synthesized NPs displayed Z average between 110 to 120 nm. Particle aggragation was observed in pristine CeO_2. The obtained hydrodynamic size is larger than the TEM particle size, which is expected since DLS measures the hydrated and potentially agglomerated particles in suspension. The low PdI of 0.185 and 0.195 indicates a high monodispersity for Mn_3O_4 and CM-3 NPs. PdI of 0.242 for CeO_2 NPs indicates toward polydispersed NPs. A low PdI attributes toward high homogeneity of NPs in aqueous media and thus is essential for stability of the synthesized NPs in solution.

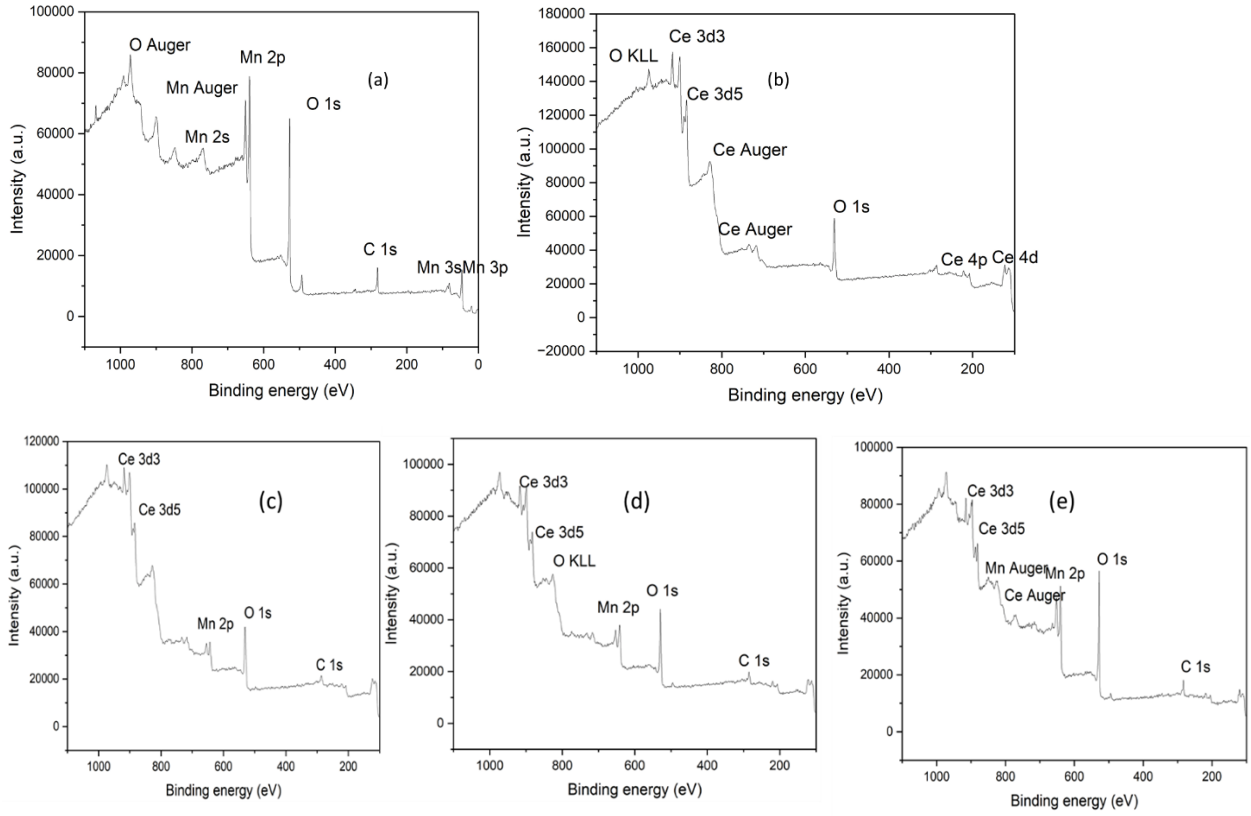

Fig.S3 XPS survey scan of a) Mn_3O_4 b) CeO_2 c) CM-1 d) CM-2 e) CM-3 (where CM-1, CM-2 and CM-3 represents Ce:Mn in ratios of 2:1, 1:1 and 1:2 respectively)

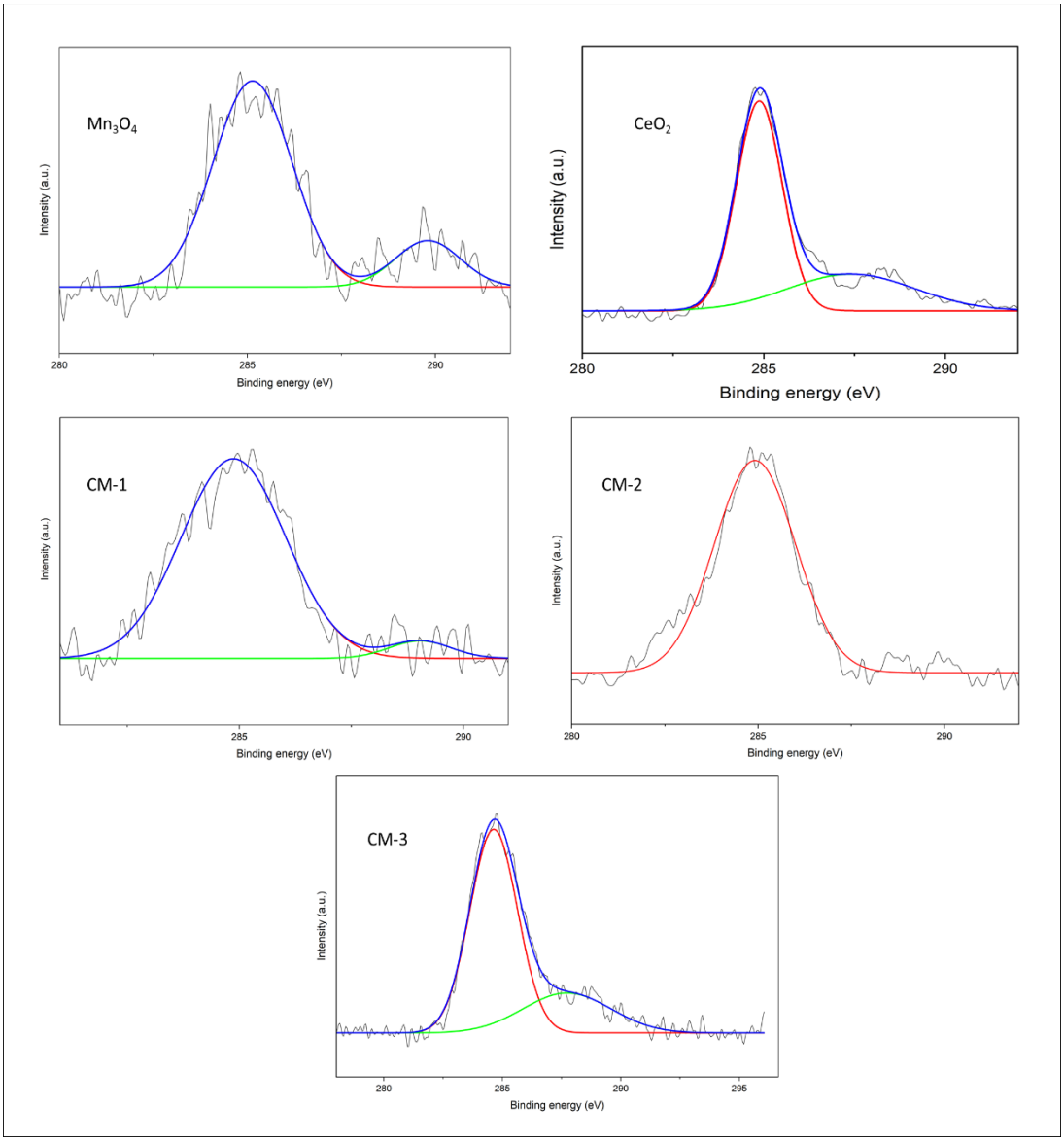

Fig.S4- C1s spectra of the synthesized catalysts

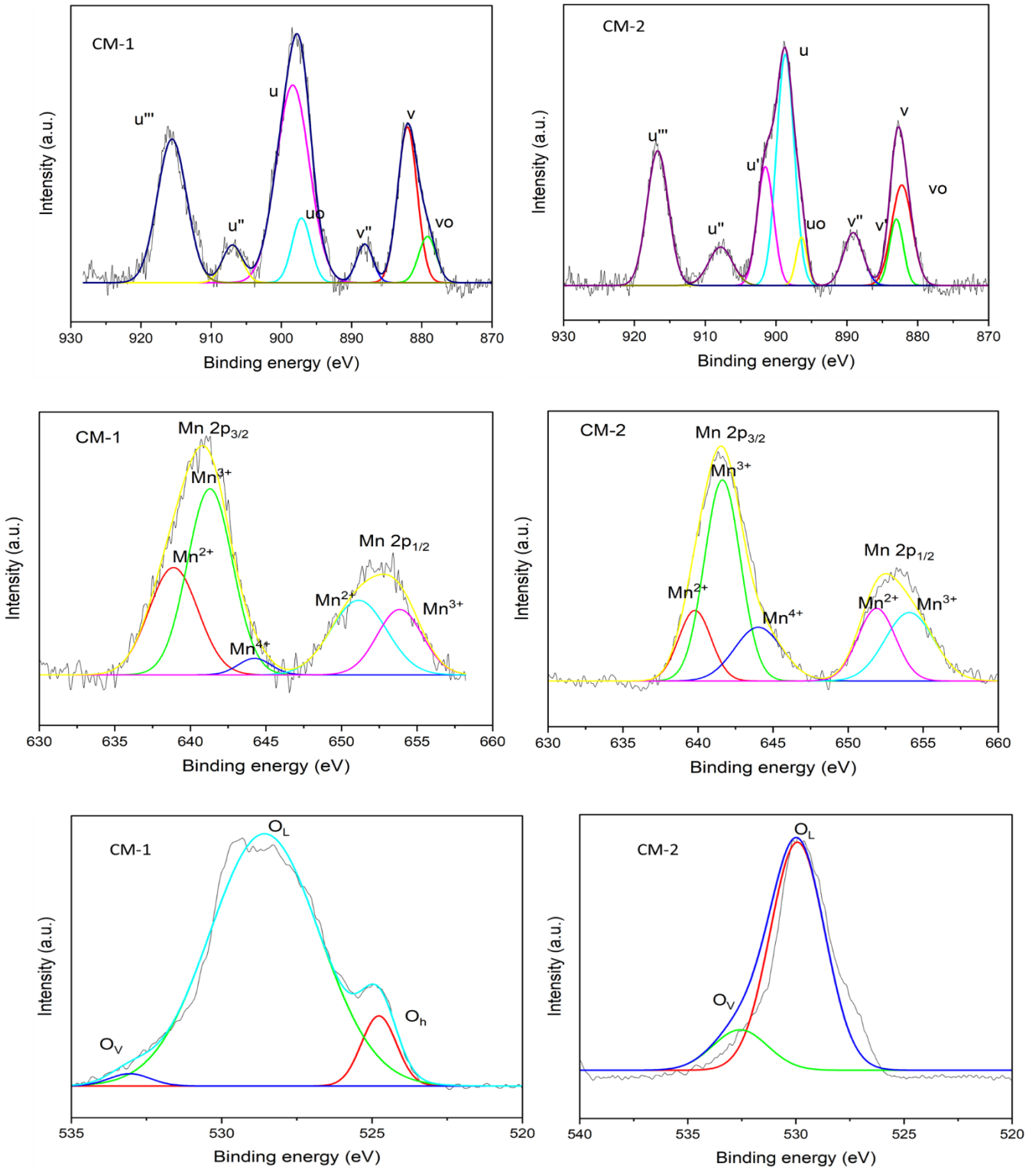

Fig. S5 Ce '3d', Mn '2p' and O '1s' spectra of CM-1 and CM-2 NPs

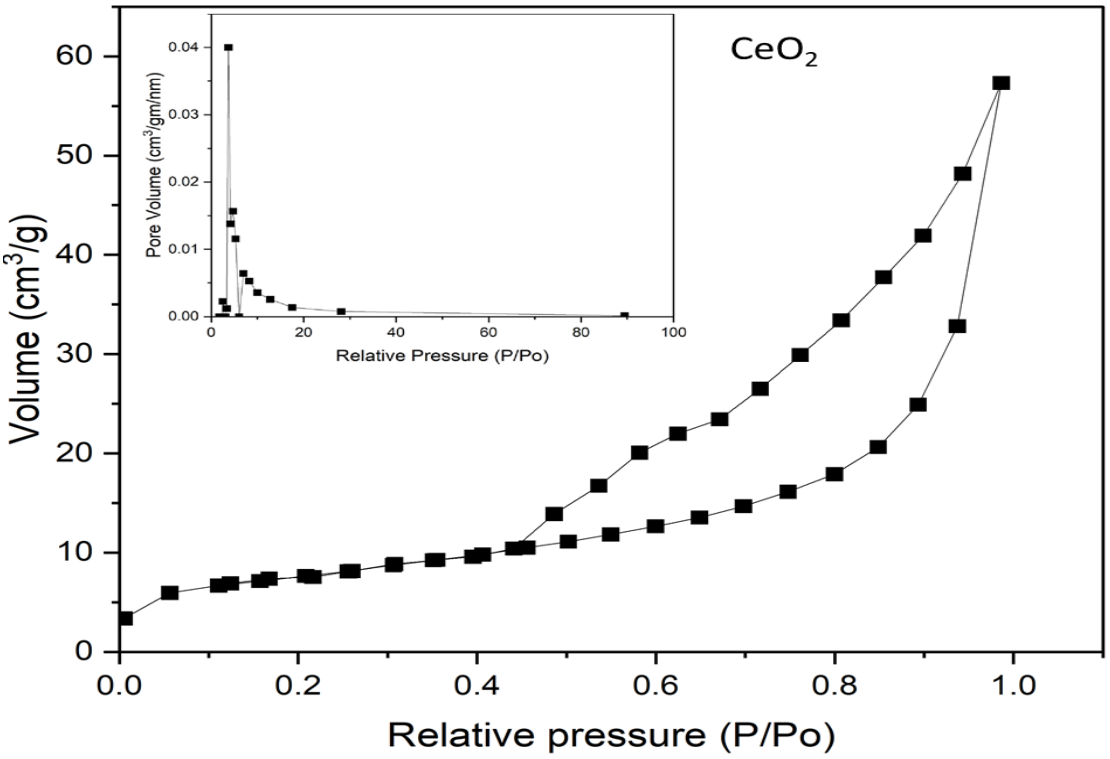

Fig. S6 BET isotherm of CeO_2 NPs

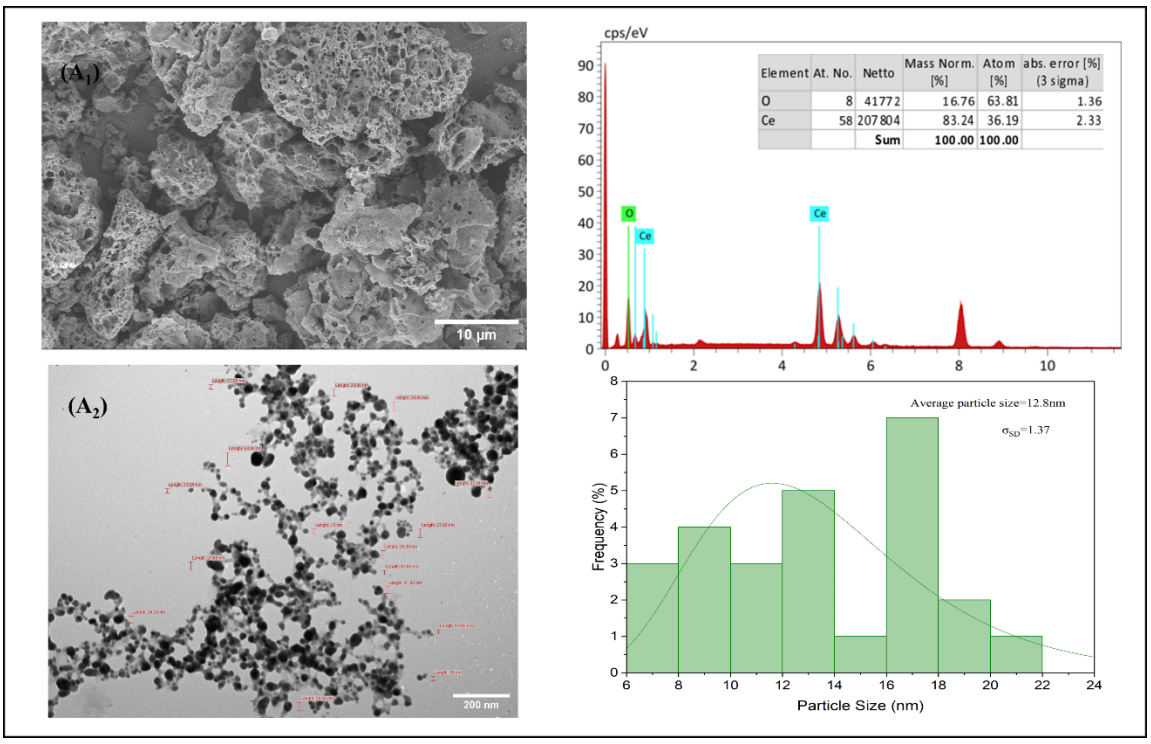

Fig.S7 SEM-EDX (A_1) and TEM micrographs (A_2) of CeO_2

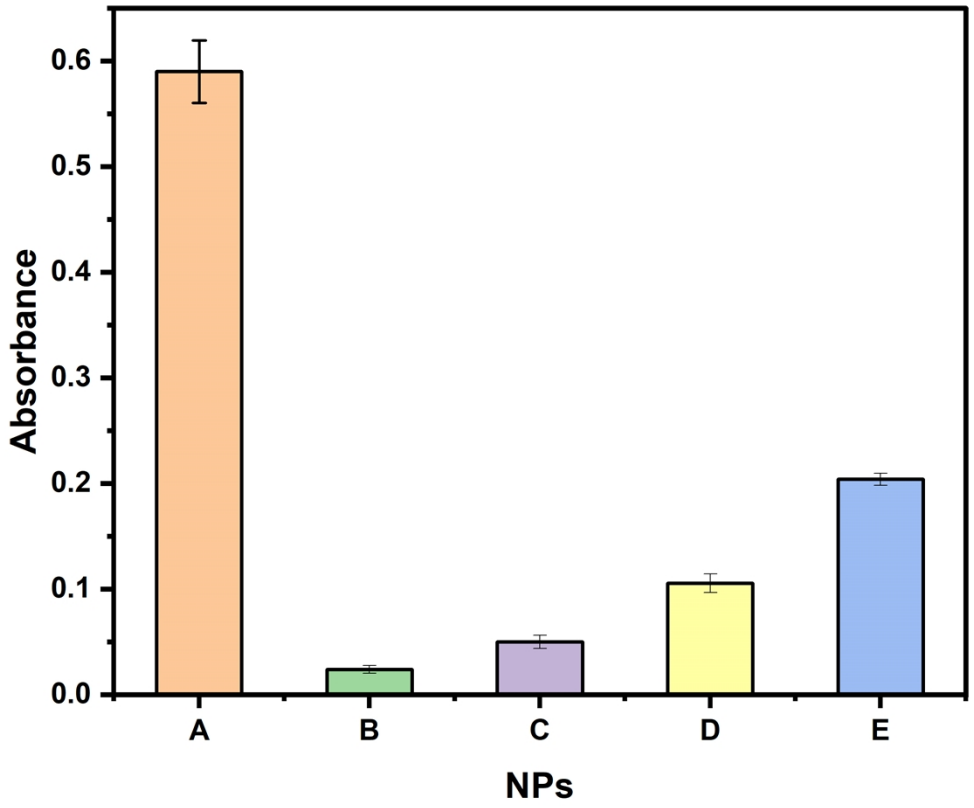

Fig. S8 Comparison of PPO mimic activity of (A) Mn_3O_4, (B) CeO_2, (C) CM-1 D) CM-2 E) CM-3

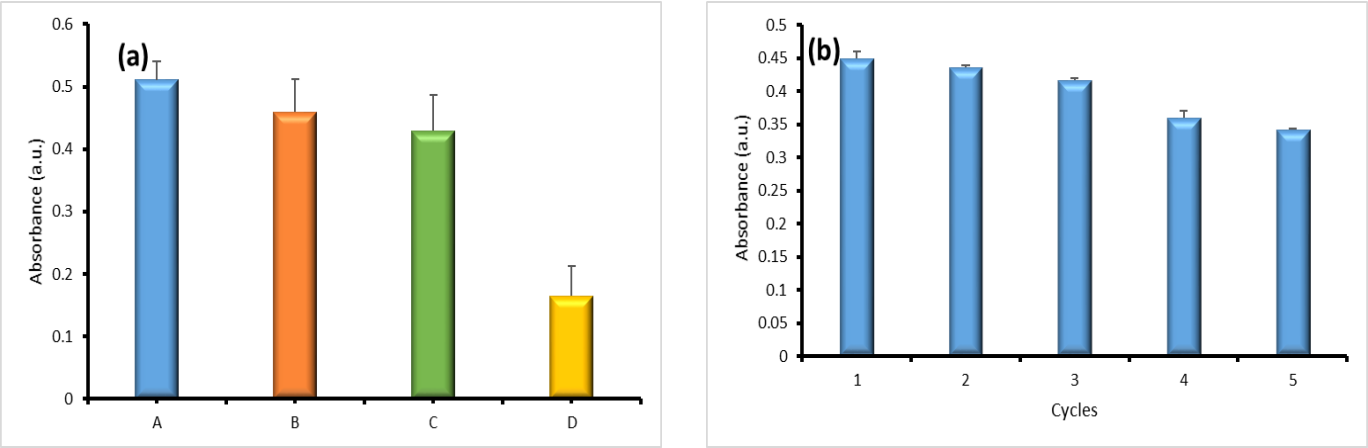

Fig. S9 (a) Effect of various quenchers on PPO mimic activity of Mn_3O_4 NPs (A: control without any scavenger; B: in the presence of sodium azide; C: in the presence of isopropyl alcohol; D: in the presence of ascorbic acid) (b) Recycling experiment

Fig. S10 Change in color with different concentrations of resorcinol 0.1mM (1) TMB+H_2O_2+ Resorcinol (2,3and 4) TMB+ H_2O_2+ NPs+no resorcinol, 0.2mM Resorcinol, 0.8mM Resorcinol respectively.

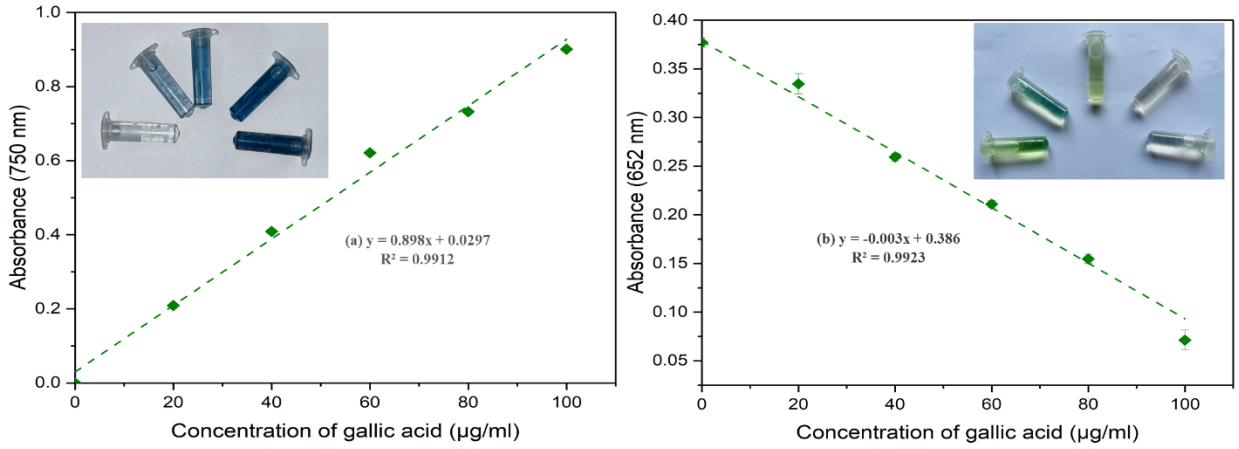

Fig. S11 Standard calibration curves using gallic acid for detection of polyphenols using a) FC-method b) Nanoparticles

S.8 Statistical analysis

A linear alignment of points along the reference line in the normal probability plot indicates that the residuals are approximately normally distributed, validating the assumption of normality in the least-squares regression model. Because the residuals have a normal distribution pattern, the points are located on a straight line (Fig. S6 (a)). Fig. S6 (b) displays random scatter plot for actual and predicted response. All of these findings suggest proposed model's strong correlation in predicting PPO mimic activity of NPs towards catechol.

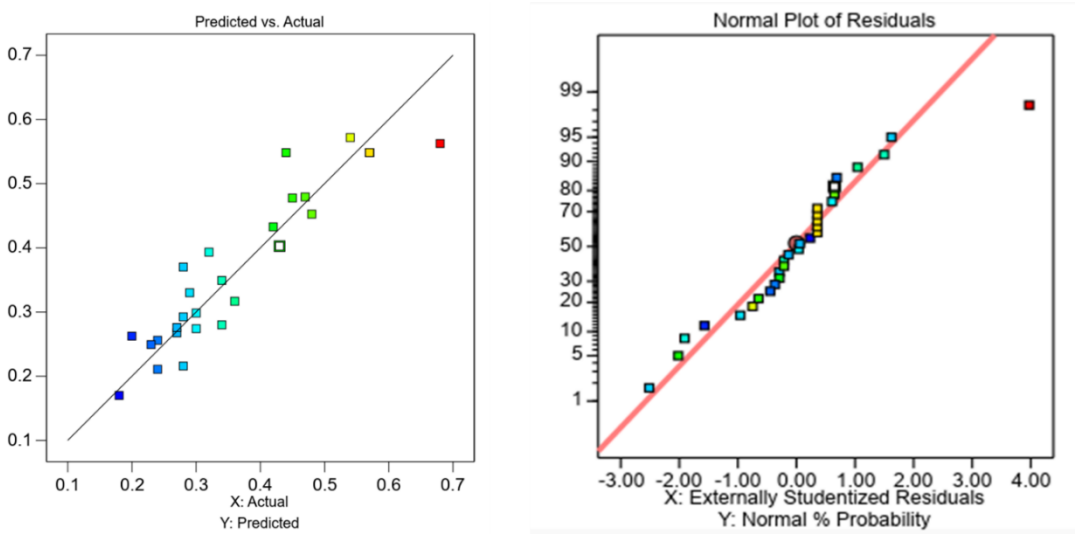

Fig. S12 a) Correlation with predicted values of enzyme activity (using catechol), (b) Normal probability plot of residuals obtained by ANOVA for the enzyme activity of Mn_3O_4 NPs

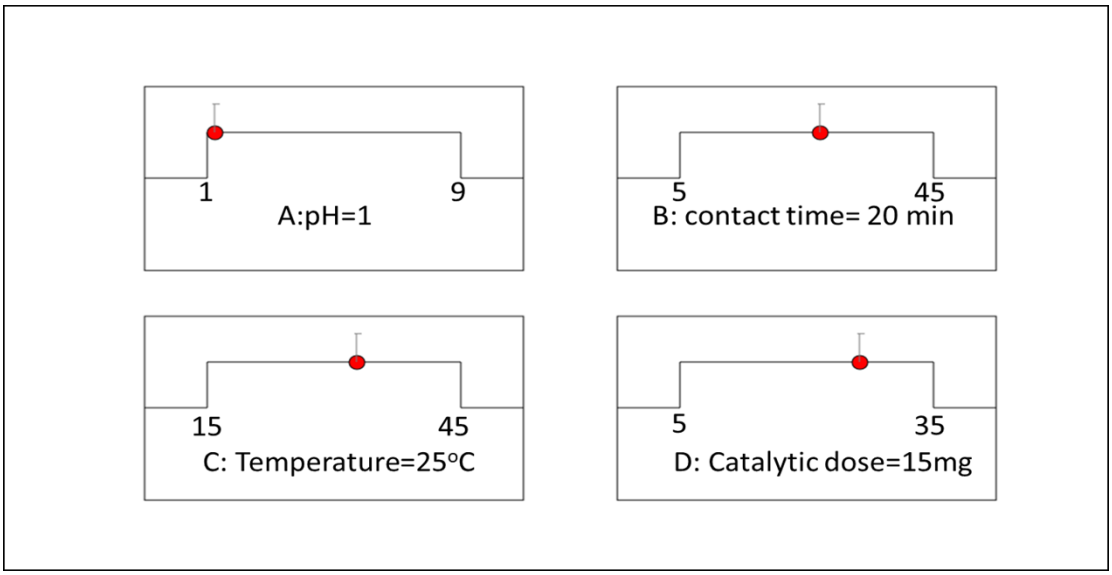

Fig. S13 Optimized parameters for enzyme mimic activity of Mn_3O_4 NPs

The enzyme mimic activity can be represented using the equation-

$$\text{Absorbance} = -0.403519+0.0007129A+0.012685B+0.019604D+0.000125AB+0.000500AD+0.001510A^2-0.000463B^2-0.000696C^2-0.000323D^2$$

Table S2: Randomized design matrix and noticed responses

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1
Run	**A:pH**	**B:catalytic dose**	**C:Temperature**	**D:contact time**	**Absorbance**
		mg	˚C	Minutes	
1	5	20	30	25	0.44
2	9	20	15	25	0.28
3	5	20	15	5	0.24
4	1	20	45	25	0.42
5	9	20	30	5	0.3
6	5	20	45	45	0.3
7	5	35	45	25	0.28
8	5	5	45	25	0.18
9	5	35	15	25	0.29
10	1	20	30	5	0.32
11	9	20	45	25	0.27
12	5	20	30	25	0.57
13	5	5	30	5	0.24
14	1	20	15	25	0.45
15	1	20	30	45	0.54
16	9	35	30	25	0.43
17	5	5	30	45	0.23
18	5	20	30	25	0.57
19	5	20	30	25	0.57
20	1	5	30	25	0.48
21	5	35	30	5	0.27
22	5	20	30	25	0.57
23	5	20	30	25	0.57
24	5	5	15	25	0.34
25	5	35	30	45	0.47
26	5	20	45	5	0.28
27	5	20	15	45	0.34
28	9	5	30	25	0.2

29	1	35	30	25	0.68
30	9	20	30	45	0.36

Table S3: ANOVA for enzyme mimic activity of Mn_3O_4 NPs

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.4805	14	0.0343	8.24	0.0001* (significant)
A-pH	0.0919	1	0.0919	22.05	0.0003*
B-catalytic dose	0.0469	1	0.0469	11.25	0.0043*
C-Temperature	0.0037	1	0.0037	0.8820	0.3625
D-contact time	0.0290	1	0.0290	6.96	0.0186*
AB	0.0002	1	0.0002	0.0540	0.0194*
AC	0.0001	1	0.0001	0.0240	0.059
AD	0.0064	1	0.0064	1.54	0.0343*
BC	0.0056	1	0.0056	1.35	0.324
BD	0.0110	1	0.0110	2.65	0.0646
CD	0.0016	1	0.0016	0.3840	0.5448
A^2	0.0040	1	0.0040	0.9611	0.3424
B^2	0.0744	1	0.0744	17.86	0.0007*
C^2	0.1683	1	0.1683	40.39	< 0.0001*
D^2	0.1144	1	0.1144	27.46	< 0.0001*
Residual	0.0625	15	0.0042		
Lack of Fit	0.0484	10	0.0048	1.72	0.2859 (non-significant)
Pure Error	0.0141	5	0.0028		
Cor Total	0.5430	29			
R^2 = 0.8849					

*= significant values

References

1 J. Uhrovčík, *Talanta*, 2014, **119**, 178–180.

2 G. J. Molole, A. Gure and N. Abdissa, *BMC Chemistry*, 2022, **16**, 48.