

## SUPPLEMENTARY MATERIAL

### Manganese Oxides (MnO<sub>x</sub>)–Based Colorimetric Nanosensor for Indirect Measurement of Lipophilic and Hydrophilic Antioxidant Capacity

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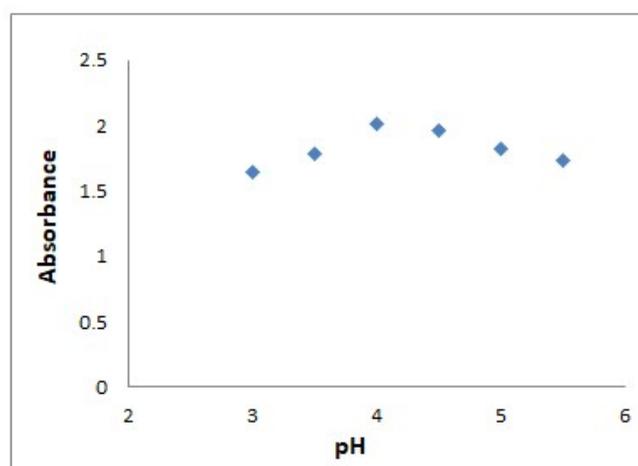
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#### Optimization conditions for the proposed sensor

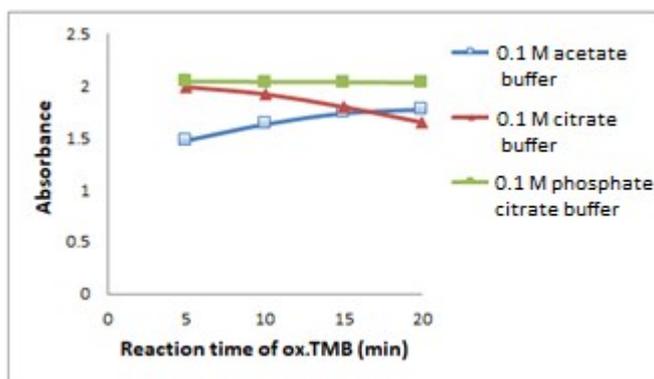
The catalytic properties of MnO<sub>x</sub> NPs depended on the pH of the medium. As shown in Fig. S1, the absorbance of the reaction system was maximal at pH 4.0, because lower pH might enhance the dissolution of catalyst while higher pH could support hydrolysis stabilizing a single valency of Mn, obstructing the catalytic activity of multi-valent MnO<sub>x</sub>.



**Figure S1.** The effect of pH on TMB–MnO<sub>x</sub> NPs reaction system. Working conditions: 0.1 mL of suspended MnO<sub>x</sub> NPs solution + 4.0 mL of buffer solution (with a pH ranging from 3.0 to 5.5) + 0.15 mL of  $1.0 \times 10^{-2}$  M of TMB + 0.75 mL of water (waited for 5 min).

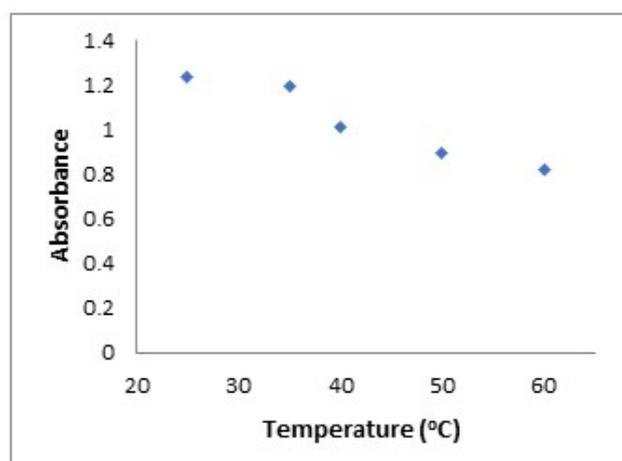
In the buffer selection stage of the method, we studied with acetate buffer (0.1 M acetic acid, 0.1 M sodium acetate), citrate buffer (0.1 M citric acid, 0.1 M sodium citrate) and phosphate-citrate buffer (0.1 M citric acid, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O) solutions to achieve optimal pH. As shown in Fig. S2,

when working with acetate and citrate buffer solutions, the reaction time between MnO<sub>x</sub> NPs and TMB lasted 30 minutes. However, when working with phosphate-citrate buffer solution, the reaction was equilibrated within 5 minutes. Consequently, we selected pH 4.0 phosphate-citrate buffer as the ideal buffer solution to enable rapid equilibration.



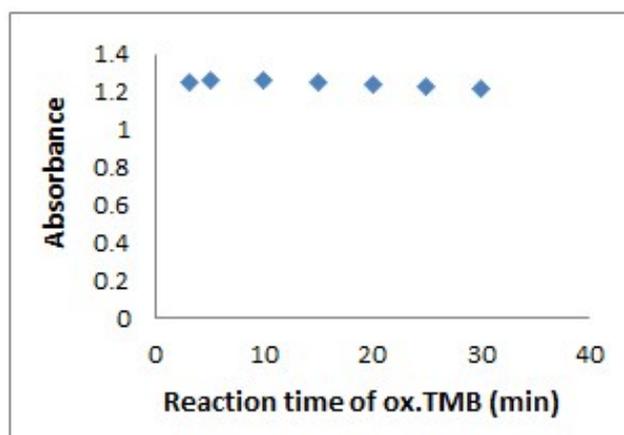
**Fig S2.** Optimization conditions for buffer selection. Working conditions: 0.1 mL of suspended MnO<sub>x</sub> NPs solution + 4.0 mL of buffer solution (*i.e.*, 0.1 M acetate buffer, 0.1 M citrate buffer, 0.1 M phosphate-citrate buffer adjusted to pH 4.0) + 0.15 mL  $1.0 \times 10^{-2}$  M of TMB + 0.75 mL of water (waited for 5 min).

The color development of TMB in MnO<sub>x</sub> nanoparticles suspension was dependent on reaction temperature. As shown in Fig. S3, the absorbance decreased as the temperature was increased from 25 to 60 °C, resulting in the selection of room temperature for the sensing procedure.



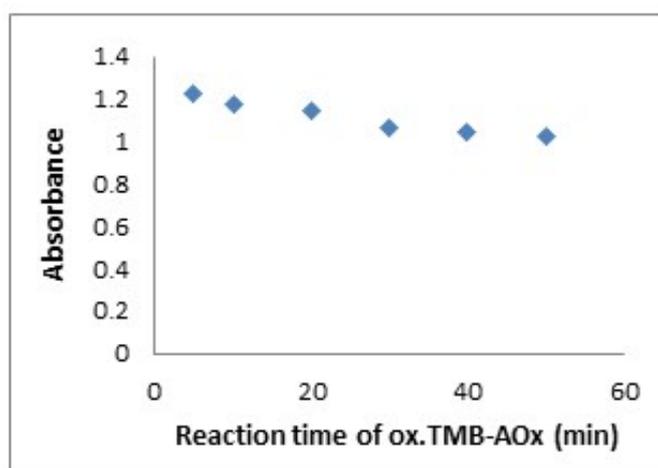
**Figure S3.** Effect of temperature on the color development of TMB in MnO<sub>x</sub> nanoparticles suspension. Working conditions: 0.1 mL of suspended MnO<sub>x</sub> NPs solution + 4.0 mL of 1.0 M phosphate-citrate buffer (pH 4.0) + 0.15 mL of  $1.0 \times 10^{-2}$  M of TMB + 0.75 mL of water, followed by incubation for 5 min at different temperatures from 25 °C to 60 °C.

To choose the optimal time period for color development of TMB oxidation product, the redox reaction of TMB (*i.e.*, conversion to blue-colored oxTMB) rapidly reached completion in 5 min, owing to the catalytic activity of MnO<sub>x</sub> nanoparticles (Fig. S4).



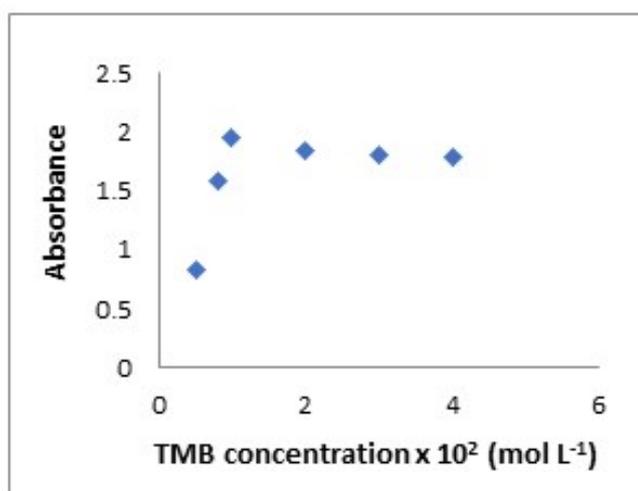
**Figure S4.** Optimization of reaction time for redox reaction of TMB. Working conditions: 0.1 mL of suspended MnO<sub>x</sub> NPs solution + 4.0 mL of 1.0 M phosphate-citrate buffer (pH 4.0) + 0.15 mL of  $1.0 \times 10^{-2}$  M of TMB + 0.75 mL of water, followed by waiting for the TMB redox reaction to reach completion at incubation times from 3 to 30 min.

To choose the optimal time for color bleaching of TMB oxidation product in the presence of antioxidant compounds, the incubation time of the reaction between oxidized species on nano-MnO<sub>x</sub> surfaces, oxTMB, and antioxidants giving rise to TMB color bleaching was determined as 30 min (Fig. S5).



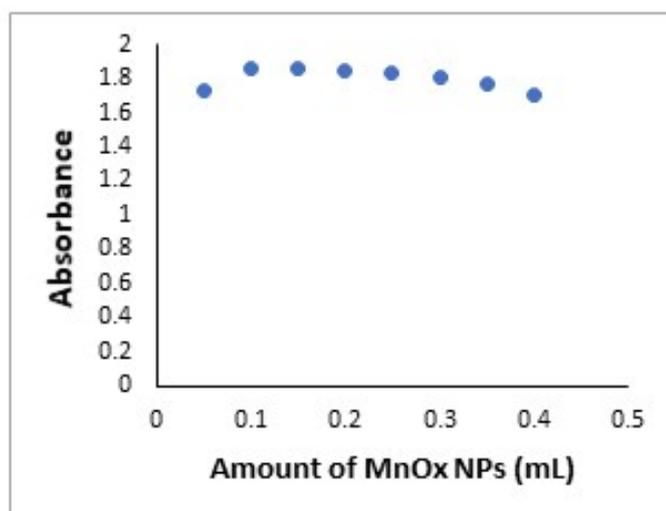
**Figure S5.** Optimization of the time of reaction between oxTMB and antioxidants. Working conditions: 0.1 mL of suspended  $\text{MnO}_x$  NPs solution + 4.0 mL of 1.0 M phosphate-citrate buffer (pH 4.0) + 0.15 mL of  $1.0 \times 10^{-2}$  M of TMB + 0.75 mL of water (waited for 5 min) + x mL of antioxidant + (1-x) mL of water, and then incubated for a period of 5-50 min.

Then, the chromophoric probe (TMB) amount was optimized. As shown in Fig. S6, TMB concentration was chosen as  $1.0 \times 10^{-2}$  M in final solution.



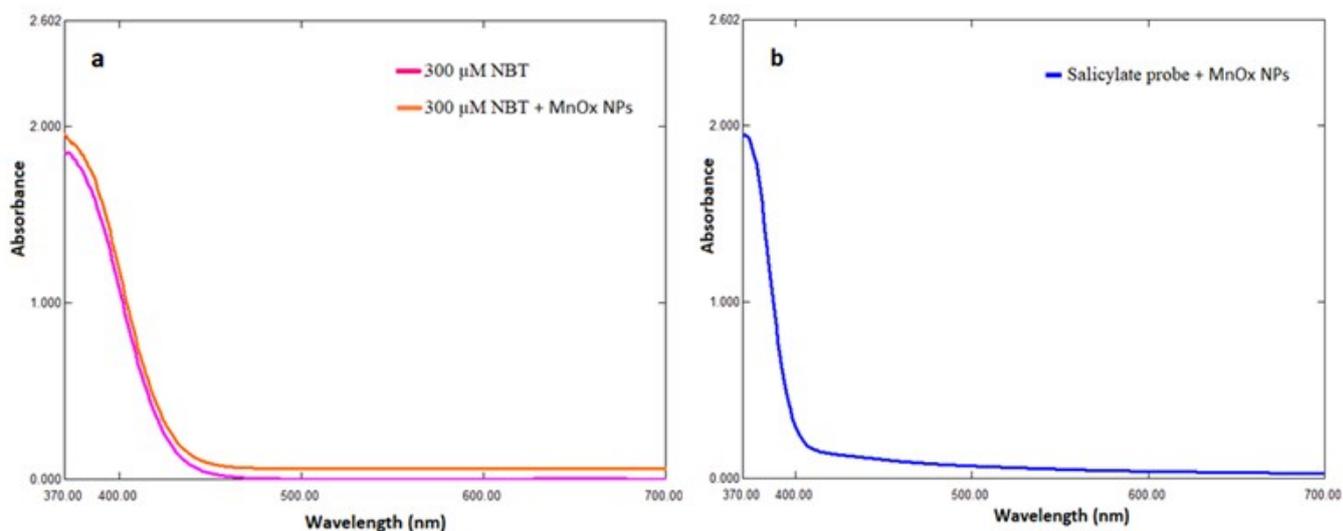
**Figure S6.** Effect of TMB concentration on absorbance at 650 nm. Working conditions: 0.1 mL of suspended  $\text{MnO}_x$  NPs solution + 4.0 mL of 1.0 M phosphate-citrate buffer (pH 4.0) + 0.15 mL of TMB at different initial concentrations + 0.75 mL of water (waited for 5 min).

For optimizing the volume of manganese oxide nanoparticles solution affecting color development, the optimal amount of  $\text{MnO}_x$  NPs was determined as 0.1 mL in 0.8 mg/mL suspended  $\text{MnO}_x$  NPs solution (Fig. S7).



**Figure S7.** Optimization of the amount of MnO<sub>x</sub> NPs. Working conditions: amount of suspended MnO<sub>x</sub> NPs solution ranging from 0.05 to 0.4 mL + 4.0 mL of 1.0 M phosphate citrate buffer (pH 4.0) + 0.15 mL of  $1.0 \times 10^{-2}$  M of TMB + 0.75 mL of water (waited for 5 min).

Studies for testing the probable presence of reactive oxygen species (ROS) are shown in Fig. S8. NBT test for superoxide anion radicals and salicylate probe for hydroxyl radicals showed the absence of both ROS (Fig. S8-a,b).



**Figure S8:** UV-vis absorption spectra for detecting the probable presence of reactive oxygen species in the system: (a) O<sub>2</sub><sup>•-</sup> radical testing with colorimetric assay using nitroblue tetrazolium (NBT); (b) <sup>•</sup>OH radical testing with colorimetric assay using salicylate probe.

Data in Table S1 demonstrate the higher sensitivity of the proposed nanosensor over other similar nanosensors.

**Table S1:** Comparison of the analytical performance of nanoparticle-based spectroscopic sensors for the detection of trolox.

Analytical sensor	Linear range ( $\mu\text{M}$ )	LOD ( $\mu\text{M}$ )	Ref.
Au NPs	3.07–90.5	0.2	[1]
Ag NPs	0.128 –112	0.23	[2]
Iron oxide NPs	150-1500	47.0	[3]
MnOx NPs	0.5–12.5	0.047	This work

Data in Table S2 show precise and accurate recoveries of antioxidants from real sample solutions using the proposed nanosensor.

**Table S2:** Analytical results for the standard addition experiments of the proposed method applied to real samples (n=3).

	Added ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Recovery (%)	RSD (%)
<b>CAT addition to green tea extract</b>	5.00	4.86	97	5.65
<b>TR addition to green tea extract</b>	5.00	5.41	108	4.35
<b>AA addition to orange juice extract</b>	6.67	6.85	103	2.84

## References

- [1] M.Bener, F. Şen, B. And R. Apak, , *Talanta*, 2018,**187**, 148-155.
- [2] M. Özyürek, N. Güngör, S. Baki, K. Güçlü and R. Apak, *Analytical Chemistry*, 2012, **84**, 8052-8059.
- [3] A. Szydłowska-Czerniak, A. Łaszewska, and A. Tułodziecka, *Analytical Methods*, 2015, **7**, 4650-4660.