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8, 5554Multi-emitting fluorescence sensor of MnO₂–
OPD–QD for the multiplex and visual detection of
ascorbic acid and alkaline phosphatase†Qian Yang,^{ab} Chuyao Li,^{ab} Jinhua Li,^b Maryam Arabi,^b Xiaoyan Wang,^{bc}
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A blue-, yellow- and red-emitting fluorescence sensor was constructed via the synergy of manganese dioxide (MnO₂) nanosheets, *o*-phenylenediamine (OPD) and red-fluorescent quantum dots (r-QDs), namely MnO₂–OPD–QD, for visualization of ascorbic acid (AA) and alkaline phosphatase (ALP). Initially, by virtue of the oxidase-like activity of MnO₂ nanosheets, OPD was oxidized into yellow-fluorescent OPDox (emission: 569 nm), which quenched the r-QDs (emission: 659 nm). After interaction with AA, one reaction product of ALP and 2-phospho-L-ascorbic acid (AA2P), the MnO₂ nanosheets were reduced and lost the oxidase-like activity, so that the generation of OPDox was lessened and the fluorescence of the r-QDs was thereupon recovered. Further increasing AA or ALP, the MnO₂ nanosheets were fully decomposed so that OPD was not oxidized but was reduced into blue-fluorescent OPDred (emission: 441 nm), while the r-QDs were not affected. Overall, AA or ALP triggered emission intensity changes at 441, 569 and 659 nm and led to profuse color variation over the yellow–orange–red–purple range, realizing multiplex and visual determination of AA and ALP in the ranges of 0.5–70 μM and 0.1–100 mU mL^{−1}, respectively. The color variation range of the ternary-emission mode was greatly enlarged, especially compared with that of the single-/dual-emission mode. Accordingly, the MnO₂–OPD–QD sensor provided detection limits for AA and ALP as low as 0.18 μM and 0.06 mU mL^{−1}, respectively. Furthermore, the highly selective/sensitive measurement and accurate readout of AA and ALP were accomplished in complicated food and serum samples. The multi-emitting fluorescence sensor held great potential for onsite visualization of various targets in more fields.

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Introduction

Nowadays, fluorescence-based sensors with a visualization effect enjoy great popularity for analyte identifying and measuring, owing to the extraordinary sensitivity and distinct fluorescence emission within the visible range.^{1,2} As is well known, dual-emission fluorescence sensing technology has aroused great interest since coming into use.^{3–6} After analyte

detection by dual-emission fluorescence sensors, the intensities of two emission peaks would change out of sync, for instance, one peak is enhanced but the other remains at a constant intensity.^{7,8} Thereupon, the test results are not compromised by various analyte-independent factors like background signal, fluorescent material concentration, instrumental and operational fluctuation, *etc.*, which often easily cause interference in traditional single-emission fluorescence sensors.^{9,10} This is exactly the attractive self-calibration function of ratiometric fluorescence detection. More importantly, the hard-to-observe fluorescence brightness change due to the quenching/enhancing of a solo emission peak could be upgraded into fluorescence color variation in the “dual-emission” mode, which is highly desirable for observers.

However, the color variation provided by dual-emission sensors is still limited within a certain range. No matter what two fluorescence colors are present, the widest available color variation is located within their transition color. For instance, when a green- and blue-emission fluorescence sensor interacts with the target of diniconazole, the green fluorescence peak is

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quenched while the blue one remains unchanged, and consequently the sensor presents continuous color changes from green to blue (Fig. S1a, ESI†).¹¹ Likewise, a yellow- and blue-emission fluorescence sensor can show fluorescence color evolution within the limited yellow–blue window (Fig. S1b, ESI†). In order to significantly increase the color variation, Cai *et al.*¹² and Yang *et al.*¹³ have developed excellent tricolor-emission fluorescence sensors by mixing three different fluorescence providers of red, green and blue simultaneously. Three emission peaks, *i.e.*, three emission colors, are affected by the target recognition behavior and change at different rates and trends. Under this combined action, the color variation is significantly broadened and becomes more profuse (Fig. S1c, ESI†), following a red–yellow–green–blue¹² or green–yellow–red–blue¹³ route. Meanwhile, the detection sensitivities, reflected by the detection limits and ratiometric intensity change rates, have been remarkably improved accordingly.

Generally, for a visual sensor, selectivity and sensitivity are vital indexes. Various ligands (natural, engineered and random library based) have been grafted on sensors to recognize targets through specific interactions.^{14–16} However, a number of them often suffer from drawbacks *viz.* high price, physical/chemical instability, and preparation complexity. For overcoming the limitations, eco-friendly nanomaterials with specific properties are adopted, like manganese dioxide (MnO₂) nanosheets, which have a specific interaction with antioxidant substances. MnO₂ nanosheets are a typical sheet-like transition-metal dioxide, and are characterized by wide UV-vis absorption and oxidase-like activity. After interaction with antioxidants, MnO₂ nanosheets could be decomposed and reduced into Mn²⁺ ions and therefore lost their UV-vis absorption and oxidase-like activity. Based on this mechanism, MnO₂ nanosheets are now shining in fluorescence sensor construction for detection of various antioxidants such as glutathione (GSH)^{17,18} and ascorbic acid (AA).^{19,20} By cooperating with a fluorescent material, MnO₂ nanosheets could quench its fluorescence by fluorescence resonance energy transfer (FRET) or inner filter effect (IFE) depending on their wide absorption band; after decomposition by the antioxidants, the color of the MnO₂-based sensor would be changed from black brown to clear and transparent with reduced UV-vis absorption, so that FRET/IFE would be restrained and the fluorescence of the fluorescent material would be recovered (named an “off-on” fluorescence sensor).^{18,21} Alternatively, by mixing with a non-fluorescent substance like *o*-phenylenediamine (OPD) and dopamine, MnO₂ nanosheets could cause their oxidation to provide a specific fluorescence signal; after being reduced by the antioxidants, the produced Mn²⁺ ions have no oxidase-like capacity, and therefore the generation of a fluorescent oxide and fluorescence signal would be limited (defined as an “on-off” fluorescence sensor).²² However, as far as we are aware, among all the reported MnO₂-based fluorescence sensors, only three studies are of the “dual-emission” mode,^{23–25} and no study of the “ternary-emission” mode has been conducted.

Therefore, herein, we propose a blue, yellow and red ternary-emission fluorescence sensor through the cooperation of MnO₂

nanosheets, OPD and red-fluorescent quantum dots (r-QDs), *viz.* MnO₂–OPD–QD, for visual detection of AA and alkaline phosphatase (ALP) as the model targets, since fluorescence intensity changes of the three emission peaks resulted in profuse color evolution (Fig. S1c, ESI†). AA plays an important role in human daily life as an enzyme cofactor, reducing agent and nutritional factor,^{20,26} and ALP, an essential hydrolase existing in living organisms, can catalyze dephosphorylation of 2-phospho-L-ascorbic acid (AA2P) to produce AA.²⁷ The as-constructed MnO₂–OPD–QD sensor was fully characterized and explored for the possible detection principle, and several variables were optimized systematically and excellent analytical performances were demonstrated. Furthermore, the sensor was applied for the determination of AA in food and ALP in serum samples with satisfactory results, indicating promise for onsite visual detection of trace targets in complicated matrices.

Experimental section

Reagents and materials

KMnO₄ was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). OPD and Cys were provided by Solarbio Science & Technology Co. Ltd (Beijing, China). 4-Morpholine-ethanesulfonic acid (MES), AA, *N*-ethylmaleimide (NEM), GSH, oxalic acid, pepsin, trypsin and ribonuclease A were obtained from Aladdin (Shanghai, China). ALP, AA2P, lysozyme, lipase and acetylcholinesterase (AChE) were offered by Sigma-Aldrich (Shanghai, China). Citric acid was obtained from XiLong Chemical (Guangdong, China). Malic acid, tartaric acid and glucose were purchased from Damao Chemical Reagent Factory (Tianjin, China). All used reagents were at least of analytical grade. Red-fluorescent CdTe/ZnS QDs (r-QDs) were synthesized as reported^{28,29} with necessary modification (with synthesis details given in the ESI†). Phosphate buffer (0.1 M, pH 8.0) was used in the AA and ALP detection procedures. Ultrapure water of 18.2 MΩ specific resistance (Millipore, USA) was used in all experiments.

Apparatus

Fluorescence emission and UV-vis absorption spectra were recorded using a fluorescence spectrophotometer (F-7000, HITACHI) and a UV-vis spectrophotometer (Tu-1900, PERSEE), respectively. Fluorescence images were observed under a 365 nm UV lamp (WFH-203C, SHANGHAI JINKE). The shape, morphology, and dimensions of the MnO₂ nanosheets were investigated using a transmission electron microscope (TEM, JEOL 2100F) and an atomic force microscope (AFM, Dimension Edge, Bruker). Elemental and structural analyses were performed by energy dispersive spectroscopy (EDS, X-Max, Oxford), Raman spectroscopy (LabRAM HR Evolution, HORIBA) and X-ray photoelectron spectroscopy (XPS, ESCALAB 250xi, Thermo Scientific).

Synthesis of MnO₂ nanosheets

MnO₂ nanosheets were synthesized by reducing KMnO₄ under ultrasound.¹⁷ Briefly, 9 mL of KMnO₄ (0.01 M) was mixed with 12 mL of MES (0.1 M, pH 6.0) and sonicated for 5 min.

Then, the obtained black brown product, *i.e.*, MnO₂ nanosheets, was purified with ultrapure water five times and dispersed in ultrapure water for future use.

Procedure for ternary-emission fluorescence detection of AA and ALP

For AA detection, 750 μL of AA at different concentrations was firstly mixed with 100 μL of MnO₂ nanosheets (0.8 mg mL^{-1}) for 10 min. Then, 100 μL of phosphate buffer (0.1 M, pH 8.0) and 50 μL of OPD (0.3 M) were added to the mixtures successively, followed by incubating in a water bath at 40 $^{\circ}\text{C}$ for 60 min. Finally, 20 μL of r-QDs were added for recording fluorescence spectra (excitation wavelength: 365 nm; slit widths: 10/10 nm).

For ALP detection, 750 μL of ALP at different activities was firstly reacted with 100 μL of AA2P (10 mM, in phosphate buffer) at 37 $^{\circ}\text{C}$ for 60 min. The following procedure of adding MnO₂ nanosheets, OPD and r-QDs, and recording fluorescence spectra was the same as that of AA detection above, but changing the volume of r-QDs to 10 μL .

The MnO₂-OPD-QD sensor's selectivity toward AA and ALP was investigated by replacing AA or ALP with other antioxidant substances and enzymes at a fixed concentration of 30 μM and activity of 30 mU mL^{-1} , respectively, to participate in the analysis procedure mentioned above.

Application in real samples

Several fresh food samples of yellow pepper, kiwi fruit and tomato were firstly squeezed by a juicer. Then, the obtained juices and another two commercial fruit juices (orange juice, and apple and banana juice) were centrifuged at 8000 rpm for 20 min and filtered through a 0.22 μm filter membrane to obtain the aqueous samples. After being neutralized and diluted to a detectable concentration range, these samples were analyzed for AA determination. Additionally, human, pig, goat and duck sera were diluted to a detectable concentration range and 0.3 mM NEM³⁰ was added for ALP activity determination using the developed MnO₂-OPD-QD sensor. The test results of AA concentrations and ALP activities were compared with those measured by commercial AA and ALP assay kits (Nanjing Jiancheng Bioengineering Institute, China), respectively.

Results and discussion

Construction, characterization and possible detection principle of the MnO₂-OPD-QD sensor

Fig. 1 schematically illustrates the construction of the ternary-emission MnO₂-OPD-QD fluorescence sensor and its possible sensing principle toward AA and ALP. MnO₂ nanosheets were firstly synthesized, and then OPD and r-QDs were successively added. In the presence of AA (or coexistence of ALP and AA2P), which could reduce MnO₂ nanosheets and remove their oxidase-like activity, the oxidation of OPD into yellow-fluorescent OPDox (emission: 569 nm) was lessened, the fluorescence intensity of the r-QDs (emission: 659 nm) was recovered, and the reducing behavior of OPD into blue-fluorescent OPDred (emission: 441 nm) was triggered.

Fig. 1 Illustration of the construction of the ternary-emission MnO₂-OPD-QD sensor and its possible sensing principle toward AA and ALP. Left dotted box: Illustration of how ALP catalyzes the dephosphorylation of AA2P to generate AA.

Fig. 2 (A) TEM image, (B) AFM image, (C) elemental mapping, (D) XPS spectrum and (E) Raman spectrum of MnO₂ nanosheets. Insets of (D): XPS spectra of Mn 2p and Mn 3s.

As displayed in Fig. 2A and B, the synthesized MnO₂ nanosheets possessed a typical sheet-like structure with numerous wrinkles and folds, and the average lateral and longitudinal dimensions were about 100 nm and 2.5 nm, respectively. Expectedly, elements of Mn and O, as well as the characteristic functional group of Mn-O, were observed in the elemental mapping (Fig. 2C), XPS (Fig. 2D) and Raman spectra (Fig. 2E). The spin-orbit doublet in the Mn 2p region, namely Mn (2p_{1/2}) of 654.7 eV and Mn (2p_{3/2}) of 642.8 eV (inset of Fig. 2D), could be assigned to a mixed-valence Mn system, most likely Mn⁴⁺ and Mn³⁺.³¹ The average oxidation state (AOS) of Mn was estimated to be 3.64, according to the equation of $\text{AOS} = 8.95 - 1.13 \Delta E \text{ (eV)}$,³² where ΔE means the peak energy separation of the Mn 3s multiplet splitting (inset of Fig. 2D). Subsequently, MnO₂ nanosheets were mixed with OPD and r-QDs, where r-QDs are especially suitable for multi-emitting sensor construction owing to their wide excitation spectrum but narrow symmetrical emission peak, large Stokes shift, and low toxicity from the ZnS shell's protection.^{8,13} As can be seen, the non-fluorescent OPD (black line of Fig. S2, ESI†) was oxidized into yellow-fluorescent OPDox emitting at 569 nm

(red line of Fig. S2, ESI†) with a wide absorption band over 325–525 nm to overlap the excitation band of the r-QDs and therefore quench them (blue line of Fig. S2, ESI†) via the IFE mechanism^{33,34} rather than the FRET process because no spectral overlap occurred between the OPDox absorption and r-QD emission (Fig. S3, ESI†). Thereupon, the MnO₂-OPD-QD sensor initially presented yellow fluorescence under a 365 nm UV lamp (Fig. S2a, ESI†).

In the presence of a small amount of AA, MnO₂ nanosheets were partially decomposed into Mn²⁺ ions and lost their oxidase-like activity, leading to lower generation of OPDox and fluorescence recovery of the r-QDs (green line of Fig. S2, ESI†). Therefore, the sensor's fluorescence was converted into orange and further red (Fig. S2b, ESI†). With increasing AA concentration, the MnO₂ nanosheets were completely consumed so that no OPDox was produced and the red fluorescence was fully recovered. Besides, in alkaline media, the AA residue reduced OPD into OPDred with blue fluorescence at 441 nm (purple line of Fig. S2, ESI†). Consequently, the sensor's fluorescence further evolved into red-purple or even blue-purple (Fig. S2c, ESI†). To sum up, with the out-of-sync change of three kinds of emission peaks, the MnO₂-OPD-QD sensor displayed AA-dependent profuse fluorescence color evolution over the yellow–orange–red–purple window for its visualization. On the other hand, ALP could catalyze the dephosphorylation of AA2P to produce AA. Hence, it was well founded to detect ALP by the proposed MnO₂-OPD-QD sensor based on the aforementioned principle.

Condition optimization for MnO₂-OPD-QD sensor construction

In the tricolor-emission MnO₂-OPD-QD sensor, OPD was utilized to be oxidized by MnO₂ nanosheets in order to produce OPDox and provide yellow fluorescence. The concentration of OPD and the temperature and time of the reaction between OPD and MnO₂ nanosheets were optimized. As can be seen in Fig. S4 (ESI†), the fluorescence intensity of the MnO₂-OPD mixture was enhanced with OPD increasing from 0.1 to 0.3 M and remained stable after that, so OPD of 0.3 M was chosen. Besides, it was found that the fluorescence intensity of the MnO₂-OPD mixture in a sealed centrifuge tube gradually increased with the reaction time, no matter what the reaction temperature was (Fig. S5, ESI†). However, at 30 °C and 40 °C, the fluorescence intensity reached a plateau within 120 and 60 min, respectively (Fig. S5A and B, ESI†), while, at higher temperatures of 50 °C and 60 °C, the fluorescence intensity of the MnO₂-OPD mixture kept increasing, and the intensity of the OPD solution itself also rose (Fig. S5C and D, ESI†). This fact indicated that the reaction temperature determined the self-oxidization rate of OPD, which had a significant effect and could not be neglected at high temperature. To make the fluorescence intensity of OPDox closely link to the MnO₂ residue (depending on the analyte concentration), the self-oxidization of OPD should be avoided, and therefore a reaction temperature of 40 °C and reaction time of 60 min were adopted.

Additionally, the red fluorescence was provided by r-QDs, which should be added in an appropriate amount to ensure the

best visualization effect. Excessive amounts of r-QDs went against enlarging the fluorescence evolution range because (i) OPDox at a certain amount had limited absorbance and could only quench some r-QDs, so a sensor with an overdose of r-QDs would display initial orange fluorescence rather than yellow; and (ii) for a mixture of blue-emission OPDred and too many r-QDs it was hard to attain the final blue-purple fluorescence but it presented red-blue. Alternatively, too few r-QDs provided weak intensity and had an adverse effect on displaying profuse fluorescence variation, because the solo existence of red fluorescence was hard to catch by the naked eye, and for color mixing with yellow/blue it was difficult to produce orange or purple fluorescence. It looked like only a change from yellow to blue. For AA determination, as shown in Fig. S6 (ESI†), the MnO₂-OPD-QD sensor with r-QDs higher than 20 μL presented an initial fluorescence color of orange and a final color of red-purple. However, when AA consumed all MnO₂ nanosheets, the recovered fluorescence of r-QDs less than 20 μL was hard to observe with the naked eye. The r-QDs at 20 μL exhibited an initial color of yellow, middle color of bright red, and a final color of blue-purple. So, 20 μL of QDs was used in the AA detection procedure. In addition, the addition of AA2P would consume some MnO₂ nanosheets and lower the fluorescence intensity of OPDox,²⁵ as shown in Fig. S7 (ESI†), so the optimal amount of r-QDs was reduced to 10 μL for the ALP activity assay.

Analytical performance of the MnO₂-OPD-QD sensor for AA

Under the optimized conditions, the tricolor-emission MnO₂-OPD-QD sensor was used for detecting AA at different concentrations. As shown in Fig. 3A and the inset, in the absence of AA, OPD was oxidized by MnO₂ nanosheets into yellow-fluorescent OPDox emitting at 569 nm, with a wide absorption band to quench the r-QDs' fluorescence. A yellow fluorescence image was presented under the 365 nm UV lamp (upper of Fig. 3A). With AA increasing from 0 to 30 μM, MnO₂ nanosheets were gradually decomposed and finally disappeared, and therefore the generation of OPDox was gradually lessened. The decreased OPDox led to weakened yellow fluorescence and UV-vis absorbance, and the fluorescence of the r-QDs was therefore recovered. Consequently, the fluorescence color of the MnO₂-OPD-QD sensor turned from yellow to orange to red (upper of Fig. 3A). It should be pointed out that the seemingly constant fluorescence intensity of the r-QDs was a result of spectral overlap with the wide emission peak of OPDox. After eliminating the overlap effect, the fluorescence intensity of the r-QDs obviously increased with increasing AA (Fig. S8, ESI†). Then, with further increasing AA up to 100 μM, OPD was not oxidized but reduced into OPDred by the excess AA in the alkaline conditions, with accompanying emitted blue fluorescence at 441 nm. The increasing blue fluorescence cooperated with the intensity-stable red fluorescence to evolve the sensor's color from red to red-purple to blue-purple, with the overall intensity growing (upper of Fig. 3A). The profuse fluorescence color variation was in favor of AA visualization by the naked eye. Additionally, it was observed that the fluorescence intensity decrease $((F_0 - F)/F_0)$ of OPDox at 569 nm was linearly related

Fig. 3 (A) Fluorescence spectra and images of the ternary-emission $\text{MnO}_2\text{-OPD-QD}$ sensor toward 0–100 μM AA, and (B) the corresponding fitting curves for the fluorescence intensity decrease at 569 nm (red curve) and increase at 441 nm (blue curve), respectively. (C) Fluorescence spectra and images of the dual-emission $\text{MnO}_2\text{-OPD}$ sensor toward 0–100 μM AA, and (D) the corresponding fitting curves for the fluorescence intensity decrease at 569 nm (red curve) and increase at 441 nm (blue curve), respectively. Insets of (A and C): Magnified fluorescence spectra of the $\text{MnO}_2\text{-OPD-QD}$ sensor and $\text{MnO}_2\text{-OPD}$ sensor toward 0–30 μM AA, respectively.

to AA within 0.5–15 μM , and the fluorescence intensity increase $((F - F_0)/F_0)$ of OPDred at 441 nm was linearly related to AA from 25 to 70 μM , with high correlation coefficients (r^2) of 0.9959 and 0.9891, respectively (Fig. 3B). An ultralow detection limit of 0.18 μM was calculated based on the 3σ IUPAC criteria.

For comparison, the sensing performance of the dual-emission $\text{MnO}_2\text{-OPD}$ sensor was also investigated. Likewise, the increase of AA over 0–30 μM caused less OPDox generation and reduced yellow fluorescence at 569 nm, and a further increase of AA produced more OPDred and enhanced blue fluorescence at 441 nm (Fig. 3C). The linear intensity decrease/increase followed the same behavior as mentioned above (Fig. 3D). However, the dual-emission sensor only displayed firstly weakened yellow and subsequently strengthened blue fluorescence images (upper of Fig. 3C), lacking the color evolution over the orange–red–purple window compared with that of the ternary-emission sensor (upper of Fig. 3A). It was not satisfactory for AA visualization. Additionally, the $\text{MnO}_2\text{-OPD-QD}$ sensor possessed a self-correction function owing to the almost unchanged red fluorescence peak. An abnormal red-emission peak could indicate something wrong in the detection procedure. Therefore, the superiorities of the tricolor-emission $\text{MnO}_2\text{-OPD-QD}$ sensor were corroborated reasonably and fully, especially the visualization effect and self-correction function.

Analytical performance of the $\text{MnO}_2\text{-OPD-QD}$ sensor for ALP

ALP can catalyze AA2P to produce AA, so that its activity could be detected by the developed $\text{MnO}_2\text{-OPD-QD}$ sensor. For the

ALP activity assay, the zymolyte of AA2P was additionally added to the sensor. The dosage of AA2P determined the concentration of AA, and was optimized firstly. As shown in Fig. S9 (ESI[†]), at the same activity of ALP, the quenching effect of OPDox was gradually strengthened with increasing AA2P, by virtue of the higher output of AA. Meanwhile, a higher sensitivity but a narrower linear range was presented because higher concentrations of AA could fully reduce the MnO_2 nanosheets sooner. Besides, as mentioned above, AA2P containing trace AA wasted partial MnO_2 nanosheets and therefore decreased the original yellow fluorescence of OPDox (Fig. S7, ESI[†]). The weak fluorescence is not conducive to visual detection. Thus, after comprehensive consideration of the sensitivity, linear range and initial fluorescence intensity, AA2P at a concentration of 10 mM was added for ALP determination.

Fig. 4A exhibits the ALP activity test results using the ternary-emission $\text{MnO}_2\text{-OPD-QD}$ sensor. Likewise, yellow-fluorescent OPDox was less and less generated within 0–40 mU mL^{-1} ALP because of the decomposition of MnO_2 nanosheets, while the blue fluorescence of OPDred was regularly enhanced. Different from that in the AA determination, the blue-emission of OPDred appeared even when the MnO_2 nanosheets were not completely reduced, that is, the yellow fluorescence didn't completely disappear. It was reasonably deduced that although the oxidation product of AA was fully oxidized when it reacted with MnO_2 nanosheets, ALP and AA2P continued to produce AA in the procedure of OPD oxidization by the MnO_2 residue, and the newly-produced AA competed with MnO_2 nanosheets to reduce OPD. Hence, with more ALP to produce more AA, fewer MnO_2 nanosheets were left and yellow fluorescent OPDox was less generated. On the other hand, more later-generated AA interacted with more OPD residue to give rise to higher intensity blue fluorescence. In brief, the yellow fluorescence was decreased and the blue fluorescence was enhanced in the meanwhile. Then, with ALP higher than 40 mU mL^{-1} , all MnO_2 nanosheets were decomposed so that no yellow emission would appear, but OPDred continued to be generated so the blue fluorescence kept rising. The ratiometric fluorescence intensity change of the blue- and yellow-emission peaks, $(I_{441}/I_{569})/(I_{441}/I_{569})_0$, obeyed

Fig. 4 Fluorescence spectra of the (A) ternary-emission $\text{MnO}_2\text{-OPD-QD}$ sensor and (B) dual-emission $\text{MnO}_2\text{-OPD}$ sensor toward 0–100 mU mL^{-1} ALP. Uppers: The corresponding fluorescence images under a 365 nm UV lamp. Insets: Fitting curves for the corresponding ratiometric intensity changes of $(I_{441}/I_{569})/(I_{441}/I_{569})_0$, with the related parameter values given in Table S1 (ESI[†]).

the logistic function (inset of Fig. 4A), and an ultralow LOD of 0.06 mU mL^{-1} was obtained based on the 3σ IUPAC rule. The simultaneous quenching of yellow fluorescence and enhancement of blue fluorescence, as well as the firstly increasing and then unchanging red fluorescence, gave rise to the profuse fluorescence variation involving yellow, orange, red, red-purple, blue-purple, and their transitional colors (upper of Fig. 4A).

Similarly, diminishing yellow fluorescence and increasing blue fluorescence were also observed in the dual-emission MnO_2 -OPD sensor (Fig. 4B). However, without the contribution of red fluorescence, the fluorescence change presented just over the yellow-blue range. The preeminence of the ternary-emission MnO_2 -OPD-QD sensor was proved again.

Selectivity and practical application of the MnO_2 -OPD-QD sensor

The MnO_2 -OPD-QD sensor realized AA and ALP determination by virtue of the oxidase-like activity and recognizer role toward reducing substances of MnO_2 nanosheets, as well as the specific reduction reaction of OPD by AA. Hence, the selectivity of the sensor was demonstrated by comparing the fluorescence intensity changes of the yellow- and blue-emission peaks, and the fluorescence images, against other reductive substances and enzymes (at the same concentration or activity). As shown in Fig. 5, GSH and Cys reduced the fluorescence intensity at

Fig. 5 Fluorescence intensity decrease at 569 nm and increase at 441 nm of the tricolor-emission MnO_2 -OPD-QD sensor toward various reducing substances and enzymes (AA and other reducing substances: $30 \mu\text{M}$; ALP and other enzymes: activity of 30 mU mL^{-1} ; NEM: $100 \mu\text{M}$). Upper: The corresponding fluorescence images under a 365 nm UV lamp.

569 nm, owing to their considerable reducing capacity that could decompose MnO_2 nanosheets, while no reaction between them and OPD was observed so that no emission at 441 nm appeared. Accordingly, red and orange fluorescence images were observed under a 356 nm UV lamp, respectively (upper of Fig. 5). After the masking effect of NEM, which could specifically react with the thiol group of GSH and Cys,³⁰ the interference effects were blocked (Fig. 5 and the upper). Other reductive substances (glucose, oxalic acid, tartaric acid, citric acid and malic acid) and enzymes (lipase, pepsin, trypsin, ribonuclease A, lysozyme and AChE) had no obvious effect on the fluorescence intensities and images (Fig. 5 and the upper). In contrast, AA and ALP caused a significant fluorescence intensity decrease and increase at 569 and 441 nm, respectively, turning the fluorescence color into red-purple and red, respectively (Fig. 5 and the upper). Hence, it was proved that the MnO_2 -OPD-QD sensor possessed outstanding selectivity toward AA and ALP.

With the certified high selectivity, the practical application of the MnO_2 -OPD-QD sensor was investigated. As listed in Table 1, AA concentrations in several fruit and vegetable juices were tested at 132.34 – $241.53 \mu\text{M}$ by the sensor, with relative standard deviations (RSDs) lower than 3.5%. The AA concentrations were also measured by a commercial AA assay kit. The inconspicuous relative errors of -5.63 to 11.00% between the two kinds of results confirmed the reliability of the developed MnO_2 -OPD-QD sensor. Also, ALP activities in four serum samples were detected by both the MnO_2 -OPD-QD sensor and the commercial ALP assay kit. As can be seen, the ALP activities in human, pig and goat serum samples were detected at 18.22 – $207.24 \text{ mU mL}^{-1}$ and were in accordance with those measured by the ALP assay kit. The abnormal value of 18.22 mU mL^{-1} in human serum (normal value: 30 – 120 mU mL^{-1} for healthy adults³⁵) may indicate certain specific diseases like AA deficiency, which should be further confirmed by combining with other clinical test results. Specifically, the ALP activity in duck serum was found at 6.13 mU mL^{-1} by the designed sensor, but could not be detected by the commercial ALP assay kit with a LOD of 4 mU mL^{-1} . ALP activity near the detection limit is hard to measure. So, it was demonstrated that this MnO_2 -OPD-QD sensor could resist various interferences in real samples and provide reliable test results, even when the analyte was at an ultralow content. Moreover, this sensor could realize analysis visualization conveniently *via* providing specific

Table 1 AA concentrations ($\mu\text{M} \pm \text{RSD} (\%)$) in fruit and vegetable samples and ALP activities ($\text{mU mL}^{-1} \pm \text{RSD} (\%)$) in serum samples measured by the developed ternary-emission MnO_2 -OPD-QD sensor and the commercial AA and ALP assay kits

Sample		Analyte	MnO ₂ –OPD–QD sensor	Commercial assay kit	Relative error (%)
Fruits and vegetables	Yellow pepper	AA	143.31 ± 2.65	151.86 ± 3.88	−5.63
	Kiwi fruit		132.34 ± 3.15	119.22 ± 2.81	11.00
	Tomato		150.89 ± 1.32	139.71 ± 3.92	8.00
	Orange juice		178.65 ± 2.53	168.11 ± 3.17	6.27
	Apple and banana juice		241.53 ± 3.41	231.01 ± 2.08	4.55
Serum	Human	ALP	18.22 ± 2.60	16.54 ± 3.97	10.16
	Pig		99.93 ± 3.18	93.74 ± 4.50	6.60
	Goat		207.24 ± 2.29	203.41 ± 4.82	1.88
	Duck		6.13 ± 3.58	Not detected	Not available

Table 2 Comparison of the construction strategy and sensing performance with those of reported MnO₂-based fluorescence sensors for detection of various antioxidants and their related substances

Sensor construction	Analyte	MnO ₂ characteristics	Emission	Assay time (min)	Linear range	LOD	Visualization effect	Ref.
MnO ₂ -dopamine	GSH	Oxidase-like activity	Single	35	0–350 μM	1.5 μM		22
MnO ₂ -graphene QDs	GSH	Wide absorption	Single	6	0.5–10 μM	0.15 μM		36
MnO ₂ -UCNP	GSH	Wide absorption	Single	3	0–10 mM	0.9 μM		17
MnO ₂ -carbon dot	AA	Wide absorption	Single	3	0.18–90 μM	42 nM		20
MnO ₂ -7-hydroxycoumarin	AA	Wide absorption	Single	3	0.5–40 μM	90 nM		19
MnO ₂ -polydopamine	ALP	Wide absorption	Single	42	1–80 mU mL ⁻¹	0.34 mU mL ⁻¹		37
MnO ₂ -perylene probe	AChE	Wide absorption	Single	63	5–100 mU mL ⁻¹	2.5 mU mL ⁻¹		38
MnO ₂ -carbon dot	Paraoxon	Wide absorption	Single	52	0.05–5 ng mL ⁻¹	0.015 ng mL ⁻¹		39
MnO ₂ -thiamine-Cu/Ag	GSH	Wide absorption, oxidase-like activity	Dual	25	0.01–70 μM	6.5 nM		24
MnO ₂ -OPD	AA	Oxidase-like activity	Dual	25	0.0–6.0 μM	10 nM		23
MnO ₂ -dopamine-QD	ALP	Oxidase-like activity	Dual	80	4–80 mU mL ⁻¹	Not provided		25
MnO ₂ -OPD-QD	AA, ALP	Oxidase-like activity	Ternary	130	0.5–70 μM AA 0.1–100 mU mL ⁻¹ ALP	0.18 μM AA 0.06 mU mL ⁻¹ ALP		This work

fluorescence images, not requiring professional technicians and expensive equipment, and allowing untrained people to readily perform analysis but without learning about the sophisticated sensing principle involved.

Method performance comparison

Recently, MnO₂ nanosheets have been widely used for fluorescence detection of antioxidants and their related substances, mainly including GSH,^{17,22,24,36} AA,^{19,20,23} ALP,^{25,37} AChE,³⁸ and paraoxon,³⁹ where the wide absorbance or oxidase-like activity of MnO₂ nanosheets is utilized. As listed in Table 2, most of the reported MnO₂-based fluorescence sensors possess just one emission peak, and display fluorescence intensity with a “turn-on” or “turn-off” behavior upon target recognition.^{17,19,20,22,36–39} Afterward, some improved dual-emission fluorescence sensors offered fluorescence variation involving an orange–blue, yellow–blue and green–red range for visually detecting AA,²³ GSH²⁴ and ALP,²⁵ respectively. Compared with a fluorescence brightness change, color changes are more desirable to read a more accurate and reliable result. Additionally, lowered detection limits make sensors promising to detect ultralow concentrations of targets.

In our developed tricolor-emission MnO₂-OPD-QD sensor, the MnO₂ nanosheets provide the oxidase-like activity and recognition reaction toward antioxidant substances; OPD acts as the precursor to evolve into yellow-fluorescent OPDox and blue-fluorescent OPDred with MnO₂ nanosheets and redundant AA, respectively; and r-QDs bring the red fluorescence window and self-correction function. Owing to the synergy of these multiple effects, the MnO₂-OPD-QD sensor exhibits wide linear ranges and low LODs, realizing accurate and reliable quantitative determination. The slightly lower LODs for AA in other studies are realized by reducing the usage of MnO₂ nanosheets^{19,20,23} at the expense of fluorescence brightness. However, excitingly, in our work for analyte visualization,

profuse fluorescence color change and sufficient brightness are both achieved: yellow to orange, red, red-purple, blue-purple, and their transitional colors with sufficient intensity.

Additionally, it is found that the entire assay time of these MnO₂-based sensors is determined by (i) the generation time of antioxidants (*e.g.*, AA),^{25,36–39} which is not required when the analytes themselves are antioxidants; (ii) the reaction time between the antioxidants and MnO₂ nanosheets; and (iii) the reaction time between the MnO₂ residue and (non-)fluorescent materials with wide absorption or oxidase-like activity. About (iii), the reaction time can be as short as a few minutes if wide absorption is utilized; otherwise, the time can be as long as tens of minutes because MnO₂ nanosheets take time to trigger the oxidation of non-fluorescent substances. So, as seen in Table 2, wide absorption based methods only take less than ten minutes to complete the detection of antioxidants (AA and GSH),^{17,19,20,36} while the assay time for non-antioxidant targets (ALP, AChE and paraoxon) based on the oxidase-like activity requires tens of minutes.^{23–25} However, the latter does not contain the long time (about several days) required to pre-synthesize fluorescent nanoparticles such as UCNPs, which are usually used in wide absorption based methods.^{17,20,36} The whole assay time of this work is relatively longer, especially for ALP; this is because ALP requires about 60 min to catalyze the production of AA, and the oxidation of OPD requires another 60 min. Even so, the method is acceptable for analyte detection and meets with the onsite visual detection requirements.

Conclusions

To sum up, a ternary-emission MnO₂-OPD-QD fluorescence sensor was successfully developed for visualization of AA and ALP in food and serum samples. Multifactor functions worked in the sensing performance, involving a recognizer role toward antioxidants and oxidase-like activity of MnO₂ nanosheets,

oxidation and reduction behaviors of OPD, and fluorescence recovery of r-QDs. The intensity changes of three emission peaks allowed the multiplex quantitative determination of AA and ALP, and induced profuse and wide-range color evolution over a yellow–orange–red–purple window, allowing the naked-eye readout of AA or ALP, providing extraordinary progress especially in comparison with traditional single-/dual-emission fluorescence sensors. Additionally, the sensor possessed satisfactory resistance to interference and reliable test results in complicated real samples, being consistent with those afforded by the commercial AA and ALP assay kits. As a successful proof of concept, we can foresee strong potential for multi-emitting fluorescence sensors in the routine monitoring of wider types of analytes with trace presence in various complicated samples.

Conflicts of interest

There are no conflicts to declare.

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