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1. Introduction

Hydrogen peroxide exists widely in biological systems, food production, and pharmaceutical, industrial and environmental applications.¹⁻³ It is a natural product in oxidative metabolic processes in biology, being harmful to organisms when the concentration of H_2O_2 reaches 0.5 mmol L^{-1} .⁴ It is also a source of toxic oxygen that produces ·OH radicals,5 which can lead to disease and senescence in a body. Therefore, the trace determination of H₂O₂ is very important in environmental analysis, biochemical analysis and clinical diagnostics. In recent years, various methods for the determination of H₂O₂ have been developed, such as fluorometry,⁶ spectrophotometry,^{7,8} chemiluminescence,9 liquid chromatography10 and electrochemistry.^{11,12} Among these methods, the horseradish peroxidase (HRP)-catalyzed reaction is the most widely used enzymatic reaction.13 The characteristics of the enzyme have been systematically studied with H2O2 as an oxidizing agent and in

Fluorometric enhancement of the detection of H₂O₂ using different organic substrates and a peroxidase-mimicking polyoxometalate†

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Simple, sensitive and stable fluorometric sensors based on the polyoxotungstate intrinsic peroxidase (Na₁₀[α -SiW₉O₃₄]) induced fluorescent enhancement of benzoic acid (BA), thiamine (TH) and 3-(4-hydroxyphenyl)propionic acid (HPPA) for the detection of hydrogen peroxide (H₂O₂) are developed for the first time. In three assays, the three non-fluorescent substrates BA, TH and HPPA were oxidized with the ·OH radicals decomposed from H₂O₂ under the catalysis of Na₁₀[α -SiW₉O₃₄] under basic pH conditions. The optimal conditions for the detection of H₂O₂ were evaluated and possible mechanisms are also discussed. The fluorescence intensity increases were linearly related to the concentration of H₂O₂ in the ranges 1 × 10⁻⁸ to 1.6 × 10⁻⁶, 1.6 × 10⁻⁶ to 1 × 10⁻⁴, and 1 × 10⁻⁵ to 2.5 × 10⁻⁴ M with BA, TH, and HPPA as substrates, respectively. Detection limits for the three systems were found to be 6.7 × 10⁻⁹, 2.2 × 10⁻⁷ and 9.6 × 10⁻⁶ M (3 σ), respectively. The RSD values ranged from 2.57% to 4.66%, 0.82% to 4.06%, and 1.08% to 2.75%, respectively. The rates of recoveries were between 99.73% and 113.06%, 95.20% and 104.22%, and 95.28% and 128.76%, respectively. Moreover, the effects of interference were studied. The proposed work was successfully applied to the determination of H₂O₂ in water and a sensitive, rapid and easy to operate assay was built, which has great potential applications in environmental science.

the presence of various substances as fluorogenic substrates.⁴ Although HRP has high specificity and sensitivity, its instability and high cost restrict its application.¹⁴ Compared to HRP, artificial enzyme mimics are more economic due to their high thermal stability, high tolerance of practical conditions, good adaptability to abiotic/biotic reactions and low cost of preparation and purification.

The development of artificial enzyme mimics as well as their expanding applications in various fields, such as pharmaceuticals, fine-chemical syntheses, chemical and biological sensing, and proteome analyses, have been actively pursued for decades.¹⁵ To date, a large number of artificial enzymes have been explored to mimic the structures and functions of naturally occurring enzymes including cyclodextrins, metal complexes, porphyrins, polymers, dendrimers, biomolecules and nanomaterials.¹⁶⁻²⁸ Among these, polyoxometalates have aroused special attention due to their mimicking enzyme activity towards peroxidase substrates. Polyoxometalates (POMs) are a large family of inorganic metal-oxide cluster compounds with many remarkable physical and chemical properties and biological activities.29,30 In particular, POMs undergo a fast and reversible multi-electron redox process without any significant structural changes, that makes them useful as redox sensors. Based on the outstanding properties of POMs, POMs and their hybrids have been utilized in

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a colorimetric assay of HepG2 cells, H_2O_2 and glucose.³¹⁻³⁸ However, by far the majority of POMs with intrinsic peroxidaselike activity are stable under acid aqueous conditions. While the complex practical conditions for H_2O_2 detection require high temperature and alkalinity (20–98 °C, pH \geq 7),^{7,10} which hamper the applications of POMs. In a recent paper, we first reported the fluorescent quenching of CdTe quantum dots for the measurement of H_2O_2 with catalysis by $Na_{10}[\alpha$ -SiW₉O₃₄] under basic pH aqueous conditions.³⁹ Considering the HPR enzymemimicking properties of $Na_{10}[\alpha$ -SiW₉O₃₄], new fluorometric methods based on it for the detection of H_2O_2 and several fluorogenic substrates under alkaline conditions can subsequently be developed. Additionally, more research is needed into the effects of the POM enzyme-mimicking reaction for biological and chemical analysis.

From a combination of the above reasons, herein we design for the first time new fluorometric enhancement methods that explore the catalytic effect of $Na_{10}[\alpha$ -SiW₉O₃₄] to convert weakly fluorescent substrates into strongly fluorescent substrates in the presence of H₂O₂. Benzoic acid (BA),¹³ thiamine (TH)⁴⁰ and 3-(4-hydroxyphenyl)propionic acid (HPPA)⁴¹ have been employed as fluorogenic substrates for hydrogen peroxide detection via the formation of fluorescent products. In this fluorometric assay, different analytical parameters, such as concentration of substrates and Na₁₀[α-SiW₉O₃₄], buffer pH, and reaction time were optimized. The catalysis activities and kinetic mechanics of Na₁₀[α-SiW₉O₃₄] were investigated upon the reaction of hydrogen peroxide with its oxidized substrates, BA, TH and HPPA. Under the optimal reaction conditions, the detection system of BA-Na₁₀[α -SiW₉O₃₄]-H₂O₂ shows the most sensitive response to H_2O_2 over the other two systems. The present assays have been successfully applied to the determination of H₂O₂ in water.

2. Experimental section

2.1. Reagents and chemicals

All the chemicals used were of analytical grade without further purification. Benzoic acid (BA), thiamine (TH), 3-(4-hydroxyphenyl)propionic acid (HPPA) and Na₂WO₄·2H₂O were obtained from the Guoyao Chemical Research Institute (Shenyang, China). Na₂SiO₃ was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Na₃PO₄, NaH₂PO₄, NaCl, HCl, Na₂CO₃ and hydrogen peroxide (H₂O₂, 30%) were purchased from Beijing Chemical Works (Beijing, China). The water used in the experiments was purified. The ρ of the water was 18 M Ω cm.

2.2. Instrumentation

The fluorescence measurements were performed on a Shimadzu RF-5301 PC fluorescence spectrofluorophotometer (Kyoto, Japan). A 1 cm path length quartz cuvette was used in the experiment. The widths of the excitation and emission slits were set to 5.0 and 5.0 nm, respectively. The Fourier Transform Infrared (FTIR) spectrum was recorded in the range 400– 4000 cm⁻¹ on KBr (FTIR IRAffinity-1s, Shimadzu, Japan). The pH measurements were performed by a PHS-25 pH meter (Shanghai INESA Scientific Instrument Co. Ltd, China). The purified water was obtained from a SMART-N Heal Force Water Purification System (Shanghai Canrex Analytic Instrument Co., Ltd, Pudong Shanghai China).

2.3. Synthesis of $Na_{10}[\alpha$ -SiW₉O₃₄]

Na₁₀[α -SiW₉O₃₄] was prepared according to the literature method.⁴² Briefly, 0.133 mol of Na₂WO₄·2H₂O and 12.5 mmol of Na₂SiO₃ were dissolved in 200 mL of distilled hot water, and then HCl (6 M, 32.5 mL) was slowly added to the above solution with vigorous stirring for 30 min. The mixture was boiled to a volume of 75 mL and then the filtrate was collected. Then, 3.2 mol of Na₂CO₃ solution was slowly added into the filtrate under stirring. The precipitate was collected after 1 h. The solid was stirred with 250 mL of 4 M NaCl and dried under vacuum. The characteristic peaks of Na₁₀[α -SiW₉O₃₄] (KBr pellet, cm⁻¹) were 987, 936, 869, 836, 705, 655 (Fig. S1†), which are nearly consistent with the values reported in the literature.

2.4. H₂O₂ detection

100 µL of different substrates (the concentrations of BA, TH, and HPPA were 5, 20, and 80 mmol L⁻¹, respectively), 100 µL of 8 mmol L⁻¹ Na₁₀[α -SiW₉O₃₄], 750 µL of 20 mmol L⁻¹ phosphate buffer solution (pH = 11.0) were mixed at room temperature. 50 µL of various concentrations of H₂O₂ were added into the mixture, sequentially. After the addition of H₂O₂, the fluorescence values of the above solutions were measured by a fluorescence spectrophotometer (BA: $\lambda_{ex} = 295$ nm, $\lambda_{em} = 405$ nm. TH: $\lambda_{ex} = 375$ nm, $\lambda_{em} = 440$ nm. HPPA: $\lambda_{ex} = 330$ nm, $\lambda_{em} = 416$ nm). The process of H₂O₂ addition to BA, TH and HPPA with Na₁₀[α -SiW₉O₃₄] as catalyst is shown in Scheme 1.

3. Results and discussion

3.1. The peroxidase-like activity of the $Na_{10}[\alpha$ -SiW₉O₃₄]

The peroxidase-like activity of $Na_{10}[\alpha$ -SiW₉O₃₄] was verified by fluorometric experiments using BA, TH and HPPA as the substrates. $Na_{10}[\alpha$ -SiW₉O₃₄] catalyzed the conversion of weakly fluorescent BA to produce fluorescent adduct hydroxylated benzoic acid (OHBA) in the presence of H₂O₂, which showed strong fluorescence at 405 nm when excited at 295 nm. As shown in Fig. 1A, when Na₁₀[α-SiW₉O₃₄] was added into the BA– H₂O₂ solution, a strong fluorescence peak was obtained. However, there were no strong fluorescence peaks when the solution did not contain H2O2 and/or Na10[a-SiW9O34], which confirmed that the reaction between BA and H₂O₂ could be catalyzed by $Na_{10}[\alpha$ -SiW₉O₃₄]. Thiamine is a non-fluorescent substrate, but thiochrome, the oxidation product of thiamine with H₂O₂, is strongly fluorescent, showing an excitation maximum at 375 nm and a fluorescence emission maximum at 440 nm. The fluorescence spectrum of the TH-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system is shown in Fig. 1B. In comparison, no fluorescence peaks were observed in the TH, TH-Na₁₀[α -SiW₉O₃₄] or TH-H₂O₂ systems, which confirmed that the reaction between TH and H_2O_2 could also be catalyzed by $Na_{10}[\alpha$ -SiW₉O₃₄]. HPPA

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Scheme 1 A schematic illustration of the processes of H_2O_2 addition to BA, TH and HPPA with $Na_{10}[\alpha-SiW_9O_{34}]$ as catalyst.

is one of the most efficient substrates for evaluating peroxidase activity.⁴³ More specifically, non-fluorescent HPPA can be oxidized by hydrogen peroxide to yield a strong fluorescent dimer *via* peroxidase catalysis. Following the addition of Na₁₀[α -SiW₉O₃₄] to the HPPA-H₂O₂ system, a strong fluorescence peak was observed, as shown in Fig. 1C. Like the BA and TH systems, no fluorescence peaks were observed in the HPPA, HPPA-Na₁₀[α -SiW₉O₃₄] and HPPA-H₂O₂ systems, which indicated that the reaction between HPPA and H₂O₂ could be catalyzed by Na₁₀[α -SiW₉O₃₄].

3.2. Condition optimization

In order to optimize the possible analytical methods for H_2O_2 determination, the effects of experimental conditions, including reaction time, solution pH, the concentration of substrates, and the concentration of $Na_{10}[\alpha$ -SiW₉O₃₄] were investigated.

3.2.1. Effect of reaction time. As shown in Fig. 2A, the fluorescence intensity of the generated OHBA rapidly increases with reaction time initially, and then tends towards saturation after 600 s, which indicates that the added H₂O₂ is completely consumed by oxidation of the substrate BA. Thus, 10 min was chosen as the optimal reaction time. Fig. 2B shows that the fluorescence intensity of the generated thiochrome changed from 0 to 600 seconds, and then tended towards to a constant after 600 s, indicating that the TH-Na₁₀[α -SiW₉O₃₄]-H₂O₂ solution was stable during this period. Fig. 2C shows the effects of reaction time (0-900 seconds) on the fluorescence intensity of HPPA-Na₁₀[α -SiW₉O₃₄] in the presence of H₂O₂. When H₂O₂ was added into an HPPA-Na₁₀[α-SiW₉O₃₄] aqueous solution, the fluorescence intensity rapidly increased from 0 to 60 seconds, and then tended towards saturation after 600 s, which indicated that the added H₂O₂ was completely consumed by oxidation of the substrate HPPA. Thus, 10 min was chosen as the optimal reaction time in the three different substrate systems.



Fig. 1 Fluorescence spectra of the (A) $BA-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system, (B) $TH-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system and (C) $HPPA-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system. Reaction conditions: 8 mmol $L^{-1} Na_{10}[\alpha-SiW_9O_{34}]$; 100 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 20 mmol $L^{-1} TH$; 80 mmol $L^{-1} H_2O_2$; 5 mmol $L^{-1} BA$; 80 mmol $L^{-1} H_2O_2$; 7 mmol $L^{-1} H_2O_2$; 8 mmol $L^{-1} H_2O_2$; 9 mmol $L^{-1} H_2O_2$; 9



Fig. 2 The effects of reaction time on the fluorescence intensities of the (A) BA-Na₁₀[α-SiW₉O₃₄] system, (B) TH-Na₁₀[α-SiW₉O₃₄] system and (C) HPPA-Na₁₀[α -SiW₉O₃₄] system in the presence of H₂O₂. Reaction conditions: 8 mmol L⁻¹Na₁₀[α -SiW₉O₃₄]; 100 mmol L⁻¹H₂O₂; 5 mmol L⁻¹ BA; 80 mmol L⁻¹ HPPA; pH, 11.0; temperature, 25 °C.

3.2.2. Effect of pH. The solution pH is an important factor affecting catalytic efficiency. To investigate the influence of pH on the different substrate-Na₁₀[α -SiW₉O₃₄]-H₂O₂ systems, the fluorescence intensities were examined in buffer solutions with various pH values (5.0-11.0). As shown in Fig. 3, the fluorescence intensities of different substrate-Na₁₀[\alpha-SiW₉O₃₄]-H₂O₂ systems were much faster in basic solutions than in neutral or acidic solutions. Moreover, the BA-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system, TH-Na₁₀[\alpha-SiW₉O₃₄]-H₂O₂ system and HPPA-Na₁₀[\alpha-SiW₉O₃₄]-H₂O₂ system reached their maximum fluorescence intensities when the pH value was 11.0. Therefore, pH 11.0 was selected to be the optimal pH in the different substrate-Na₁₀[α -SiW₉O₃₄]-H₂O₂ systems.

3.2.3. Effect of different substrate concentrations. To maximize the activity of $Na_{10}[\alpha$ -SiW₉O₃₄], the effects of varying concentrations of the substrates were also investigated. Fig. 4A and D show the influence of BA concentrations on the

fluorescence intensity of the reaction solution. For a given concentration of H_2O_2 (100 mmol L⁻¹), the fluorescence intensity was enhanced with an increase in the BA concentration up to 5 mM, and then slowly increased beyond 5 mM of BA. Hence, 5 mM BA was selected as the optimal concentration. As shown in Fig. 4B and E, the relationship between the fluorescence intensity of the product and the concentration of TH was investigated between 0.3125 and 50 mM. When the concentration of TH reached 20 mM, the fluorescence intensity of the TH- $Na_{10}[\alpha$ -SiW₉O₃₄]-H₂O₂ system reached its maximum. Thus, 20 mM TH was selected as the optimal concentration. As shown in Fig. 4C and F, the fluorescence intensity of the reaction system was enhanced as the concentration of HPPA increased to 80 mM, after which it decreased. Thus, 80 mM HPPA was selected as the optimal concentration.

3.2.4. Effect of $Na_{10}[\alpha$ -SiW₉O₃₄] concentration. In order to achieve the best performance, we investigated the effect of



Fig. 3 The effects of pH on the fluorescence intensities of the (A and D) $BA-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system, (B and E) $TH-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system and (C and F) $HPPA-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system. Reaction conditions: 8 mmol L^{-1} $Na_{10}[\alpha-SiW_9O_{34}]$; 100 mmol L^{-1} H_2O_2 ; 5 mmol L⁻¹ BA; 20 mmol L⁻¹ TH; 80 mmol L⁻¹ HPPA; temperature, 25 °C.

BY-NC



Fig. 4 The effects of different substrate concentrations on the fluorescence intensities of the (A and D) BA-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system, (B and E) TH-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system, and (C and F) HPPA-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system. Reaction conditions: 8 mmol L⁻¹ Na₁₀[α -SiW₉O₃₄]; 100 mmol L⁻¹ H₂O₂; pH, 11.0; temperature, 25 °C.

 $Na_{10}[\alpha$ -SiW₉O₃₄] concentrations (0.5–12 mM) on the fluorescence intensities of BA, TH and HPPA in the presence of H₂O₂. As shown in Fig. 5A and B, the fluorescence intensities all increased quickly with an increase in $Na_{10}[\alpha$ -SiW₉O₃₄] concentrations up to 8 mM, and then moderately slowly increased beyond 8 mM of $Na_{10}[\alpha$ -SiW₉O₃₄] in the BA and TH systems. Whereas, in the HPPA-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system, the fluorescence intensity first increased and then decreased, as shown in Fig. 5C. As shown in Fig. 5, when the concentration of Na₁₀[α -SiW₉O₃₄] reached 8 mM, the effect of Na₁₀[α -SiW₉O₃₄] on the fluorescence intensity in different substrate-Na₁₀[α -SiW₉O₃₄]-H₂O₂ systems achieved the best activity. Thus, aiming to obtain



Fig. 5 The effects of $Na_{10}[\alpha-SiW_9O_{34}]$ concentrations on the fluorescence intensities of the (A and D) BA $-Na_{10}[\alpha-SiW_9O_{34}] - H_2O_2$ system, (B and E) TH $-Na_{10}[\alpha-SiW_9O_{34}] - H_2O_2$ system and (C and F) HPPA $-Na_{10}[\alpha-SiW_9O_{34}] - H_2O_2$ system. Reaction conditions: 100 mmol L⁻¹ H₂O₂; 5 mmol L⁻¹ BA; 20 mmol L⁻¹ TH; 80 mmol L⁻¹ HPPA; pH, 11.0; temperature, 25 °C.

the best catalytic activity of $Na_{10}[\alpha$ -SiW₉O₃₄] and low economic cost, 8 mM $Na_{10}[\alpha$ -SiW₉O₃₄] was selected as the optimal concentration.

3.3. Steady-state kinetic assay

For a further understanding of the influence of different substrates on the catalytic mechanism of $Na_{10}[\alpha$ -SiW₉O₃₄] in the presence of H₂O₂, steady-state kinetic assays for different substrates were determined in detail. As shown in Fig. 6, the typical Michaelis-Menten curve was obtained for $Na_{10}[\alpha$ - SiW_9O_{34}] in the presence of H_2O_2 . Michaelis-Menten constant $(K_{\rm M})$ and maximum initial velocity $(V_{\rm max})$ were counted from the Michaelis-Menten curve using a Lineweaver-Burk plot. The kinetic parameters of BA, TH, and HPPA are given in Table 1. The $K_{\rm M}$ of Na₁₀[α -SiW₉O₃₄] with BA, TH and HPPA as substrates were 6.03 \times 10⁻⁴, 7.95 \times 10⁻³ and 2.12 \times 10⁻² M, respectively. The V_{max} of $Na_{10}[\alpha$ -SiW₉O₃₄] with BA, TH, and HPPA as substrates were 4.06 \times 10 $^{-4}$, 3.68 \times 10 $^{-5}$ and 5.75 \times 10 $^{-5}$ M s $^{-1}$, respectively. The $K_{\rm M}$ of BA, TH, and HPPA using Na₁₀[α - SiW_9O_{34}] as a catalyst with H_2O_2 as the substrate were 3.75 \times 10^{-7} , 8.31×10^{-5} and 2.51×10^{-2} M, respectively. The V_{max} of BA, TH, and HPPA using $Na_{10}[\alpha$ -SiW₉O₃₄] as a catalyst with H₂O₂ as the substrate were 5.82 \times $10^{-4},$ 3.02 \times 10^{-5} and 3.85 \times 10^{-5} M s⁻¹, respectively. The $K_{\rm M}$ value of Na₁₀[α -SiW₉O₃₄] with BA as the substrate was apparently lower than those values in the TH and HPPA systems. This indicated that BA has a higher affinity to $Na_{10}[\alpha$ -SiW₉O₃₄] in comparison with TH and HPPA. The apparent $K_{\rm M}$ value of BA with H_2O_2 as the substrate was apparently lower than those values in TH and HPPA. It was clear that BA has a higher affinity for H₂O₂ compared with those of TH and HPPA.

Table 1 Comparison of the K_{M} and V_{max} values of BA, TH and HPPA using Na₁₀[α -SiW₉O₃₄] as a catalyst

Enzyme	Substrate	K_{M} (M)	$V_{\rm max} \left({ m M} \ { m S}^{-1} ight)$
Na ₁₀ [α-SiW ₉ O ₃₄]	BA	$6.03 imes10^{-4}$	$4.06 imes 10^{-4}$
$Na_{10}[\alpha$ -SiW ₉ O ₃₄]	H_2O_2	$3.75 imes10^{-7}$	$5.82 imes10^{-4}$
Na ₁₀ [α-SiW ₉ O ₃₄]	TH	7.95×10^{-3}	3.68×10^{-5}
$Na_{10}[\alpha$ -SiW ₉ O ₃₄]	H_2O_2	8.31×10^{-5}	$3.02 imes10^{-5}$
$Na_{10}[\alpha$ -SiW ₉ O ₃₄]	HPPA	$2.12 imes10^{-2}$	$5.75 imes10^{-5}$
$Na_{10}[\alpha$ -SiW ₉ O ₃₄]	H_2O_2	2.51×10^{-2}	3.85×10^{-5}

3.4. Calibration curve for H₂O₂ detection

Under the optimized reaction conditions, the relationships between the fluorescence intensities and H2O2 concentrations in the BA-Na₁₀[α -SiW₉O₃₄]-H₂O₂, TH-Na₁₀[α -SiW₉O₃₄]-H₂O₂ and HPPA-Na₁₀[α-SiW₉O₃₄]-H₂O₂ systems were investigated. As shown in Fig. 7D, the fluorescence intensity of BA-Na₁₀[α-SiW₉O₃₄]-H₂O₂ increased linearly with increasing concentrations of H₂O₂. The linear regression equation was as follows: Y = 4.45 + 5.22 \times 10⁷ \times C_{H,O₂} with a correlation coefficient of 0.97403; the linear range was from 1×10^{-8} to 1.6×10^{-6} M. The lower limit of detection (3 σ , LOD) of Na₁₀[α -SiW₉O₃₄] for ${
m H_2O_2}$ was found to be 6.7 imes 10⁻⁹ M. As shown in Fig. 7E, the fluorescence intensity of TH-Na10[a-SiW9O34]-H2O2 increased linearly with increasing concentrations of H2O2. The linear regression equation was as follows: $Y = 8.41 + 4.35 \times 10^6 \times$ C_{H,O_2} with a correlation coefficient of 0.99049; the linear range was from 1.6 \times 10⁻⁶ to 1 \times 10⁻⁴ M. The LOD of the Na₁₀[α -SiW₉O₃₄] for H₂O₂ was found to be 2.2×10^{-7} M. Moreover, as shown in Fig. 7F, the fluorescence intensity of HPPA-Na₁₀[α- SiW_9O_{34}]-H₂O₂ increased linearly with increasing



Fig. 6 The steady-state kinetic assays and catalytic mechanisms of $Na_{10}[\alpha-SiW_9O_{34}]$ as a catalyst in different substrates: (A and D) BA $-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system, (B and E) TH $-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system and (C and F) HPPA $-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system. Reaction conditions: 8 mmol L⁻¹ $Na_{10}[\alpha-SiW_9O_{34}]$; pH, 11.0; temperature, 25 °C; incubation time, 10 min.



Fig. 7 Linear calibration plots for H_2O_2 : (A and D) $BA-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system, (B and E) $TH-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system, and (C and F) $HPPA-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$ system. Reaction conditions: 8 mmol L^{-1} $Na_{10}[\alpha-SiW_9O_{34}]$; 5 mmol L^{-1} BA; 20 mmol L^{-1} TH; 80 mmol L^{-1} HPPA; pH, 11.0; temperature, 25 °C; incubation time, 10 min.

concentration of H₂O₂. The linear regression equation was as follows: $Y = 237.55 + 4.60 \times 10^5 \times C_{H_2O_2}$ with a correlation coefficient of 0.98489; the linear range was from 1×10^{-5} to 2.5 $\times 10^{-4}$ M. The LOD of Na₁₀[α -SiW₉O₃₄] for H₂O₂ was found to be 9.6 $\times 10^{-6}$ M. Comparing these systems for the determination of H₂O₂, as shown in Table 2, the proposed method is superior in its low detection limit.

3.5. Interference study

For further evaluating the detection selectivity of BA–Na₁₀[α -SiW₉O₃₄], TH–Na₁₀[α -SiW₉O₃₄] and HPPA–Na₁₀[α -SiW₉O₃₄] systems for H₂O₂ determination, investigations were carried out at H₂O₂ concentrations of 5 × 10⁻⁷, 5 × 10⁻⁵ and 5 × 10⁻⁴ mol L⁻¹ with various coexisting substrates added. The results for BA–Na₁₀[α -SiW₉O₃₄]–H₂O₂ are shown in Table 3. And the results for TH–Na₁₀[α -SiW₉O₃₄]–H₂O₂ and HPPA–Na₁₀[α -SiW₉O₃₄]–H₂O₂ and S1 and S2.† It can

be observed that none of the substances shows any obvious interference. Thus, these proposed fluorometric methods display a high selectivity for the determination of H_2O_2 .

3.6. Determination of H₂O₂ in water samples

In order to evaluate the feasibility of the proposed methods, we tested the content of H_2O_2 in water samples under the optimum conditions. Each sample was analyzed at the same time with all the methods in order to avoid any possible differences caused by degradation of the analyte in the sample. The results of the BA–Na₁₀[α -SiW₉O₃₄]–H₂O₂ system are presented in Table 4, where it can be seen that the data for recoveries was between 99.73% and 113.06%. The RSD of detection ranged from 2.57% to 4.66%. The results of the TH–Na₁₀[α -SiW₉O₃₄]–H₂O₂ system are shown in Table S3.† For each sample, three parallel experiments were conducted, and the RSD of detection ranged from 0.82% to 4.06%. The recoveries of the samples were between

Table 2 Comparison of different fluorescent systems for the detection of H_2O_2					
System	Linear range ^{<i>a</i>}	Detection limit ^a	Reference		
BA–BFO MNPs system	$2.0 imes10^{-8}$ to $2.0 imes10^{-5}$	4.5×10^{-9}	13		
BA-·OH	$1.1 imes 10^{-5}$ to $1.1 imes 10^{-3}$	1.0×10^{-6}	44		
CuO NPs/HPPA	$5.0 imes10^{-5}$ to $4.0 imes10^{-4}$	$8.1 imes10^{-7}$	41		
Hb-H ₂ O ₂ -thiamine	$1.0 imes10^{-7}$ to $8.0 imes10^{-5}$	2.6×10^{-8}	40		
HRP-Cy.7.Cl	$1.8 imes10^{-7}$ to $7.2 imes10^{-6}$	5.6×10^{-8}	4		
HBcAb-HRP-CdTe QDs	$1.0 imes10^{-7}$ to $1.5 imes10^{-4}$	$6.9 imes10^{-8}$	45		
$CdTe-Na_{10}[\alpha-SiW_9O_{34}]-H_2O_2$	7.8 $ imes$ 10 ⁻⁹ to 2.5 $ imes$ 10 ⁻⁷	3.8×10^{-9}	39		
BA-Na ₁₀ [α -SiW ₉ O ₃₄]-H ₂ O ₂	$1.0 imes10^{-8}$ to $1.6 imes10^{-6}$	$6.7 imes10^{-9}$	This work		
TH-Na ₁₀ [α -SiW ₉ O ₃₄]-H ₂ O ₂	$1.6 imes10^{-6}$ to $1.0 imes10^{-4}$	$2.2 imes10^{-7}$	This work		
HPPA-Na ₁₀ [α-SiW ₀ O ₂₄]-H ₂ O ₂	1.0×10^{-5} to 2.5×10^{-4}	9.6×10^{-6}	This work		

^{*a*} mol L^{-1} .

Table 3 The interference study for the determination of H_2O_2 (5 \times 10^{-7} mol $L^{-1})$ by the proposed method

Coexisting substance	Content (mol L^{-1})	$\Delta I^{a}/I$ (%)
Na ⁺	$5 imes 10^{-3}$	1.25
K ⁺	5×10^{-3}	-1.96
NH^{4+}	$5 imes 10^{-3}$	-1.26
SO_4^{2-}	$2 imes 10^{-3}$	-2.80
Mn ²⁺	$2 imes 10^{-3}$	2.90
Cl^-	$2 imes 10^{-3}$	2.38
CO_{3}^{2-}	$2 imes 10^{-3}$	-7.50
Glucose	$2 imes 10^{-3}$	-2.47
Citric acid	$2 imes 10^{-3}$	1.77

 $^{a}\Delta I = I_0 - I$, where I_0 and I are the fluorescence intensities of the BA-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system in the absence and presence of interfering species.

Table 4 Results of the analyses of H_2O_2 in water samples, when the substrate was BA

Sample	Added (µM)	Detected (µM)	Recovery (%)	$\begin{array}{l} \text{RSD} (n \\ = 3, \%) \end{array}$
1	0.4	0.455 ± 0.0212	113.06	4.66
2	0.8	0.878 ± 0.0239	109.46	2.73
3	1.6	1.599 ± 0.0411	99.73	2.57

95.20% and 104.22%. The results for the HPPA-Na₁₀[α -SiW₉O₃₄]-H₂O₂ system are listed in Table S4,† From Table S4,† the recoveries of H₂O₂ were found to range from 95.28% to 128.76%, and the RSD of detection ranges from 1.08% to 2.75%. The results demonstrated that these proposed methods for H₂O₂ determination were acceptable and suitable.

4. Conclusions

In summary, we established for the first time new fluorometric enhancement methods for the detection of $Na_{10}[\alpha-SiW_9O_{34}]$ in alkaline H_2O_2 bleaching systems. H_2O_2 can be decomposed into ·OH radicals in the presence of $Na_{10}[\alpha-SiW_9O_{34}]$, which could turn non-fluorescent substrates into a strongly fluorescent product. Under the optimal reaction conditions, linear correlation was established between fluorescence intensity and the concentration of H_2O_2 . These methods will help in monitoring H_2O_2 doses and its related products, such as hydroxyl radicals, under complex practical conditions, but also in the further detection of the meaningful fluorogenic substrates, BA, TH and HPPA.

Conflicts of interest

There are no conflicts to declare.

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References

- 1 Y. Tao, E. G. Ju, J. S. Ren and X. G. Qu, *Chem. Commun.*, 2014, **50**, 3030–3032.
- 2 H. Q. Chen, H. P. Yu, Y. Y. Zhou and L. Wang, *Spectrochim. Acta, Part A*, 2007, **67**, 683–686.
- 3 X. S. Chai, Q. X. Hou, Q. Luo and J. Y. Zhu, *Anal. Chim. Acta*, 2004, **507**, 281–284.
- 4 B. Tang, L. Zhang and K. H. Xu, Talanta, 2006, 68, 876-882.
- 5 B. Tang, L. Zhang and Y. Geng, *Talanta*, 2005, 65, 769–775.
- 6 G. C. Van de Bittner, E. A. Dubikovskaya, C. R. Bertozzi and C. J. Chang, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 21316–21321.
- 7 P. A. Tanner and A. Y. S. Wong, *Anal. Chim. Acta*, 1998, **370**, 279–287.
- 8 B. Tang, Y. Wang, Y. Sun and H. X. Shen, *Spectrochim. Acta, Part A*, 2002, **58**, 141–148.
- 9 J. M. Lin and M. Yamada, Anal. Chem., 1999, 71, 1760-1766.
- 10 H. F. Yue, X. Bu, M. H. Huang, J. Young and T. Raglione, *Int. J. Pharm.*, 2009, **375**, 33–40.
- 11 L. S. Jahnke, Anal. Biochem., 1999, 269, 273-277.
- 12 K. Hensley, K. S. Williamson, M. L. Maidt, S. P. Gabbita, P. Grammas and R. A. Floyd, *HRC J. High Resolut. Chromatogr.*, 1999, 22, 429–437.
- 13 W. Luo, Y. S. Li, J. Yuan, L. H. Zhu, Z. D. Liu, H. Q. Tang and S. S. Liu, *Talanta*, 2010, **81**, 901–907.
- 14 X. Chen, D. Li, H. Yang, Q. Zhu, H. Zheng and J. Xu, *Anal. Chim. Acta*, 2001, **434**, 51–58.
- 15 E. Kuah, S. Toh, J. Yee, Q. Ma and Z. Q. Gao, *Chem.–Eur. J.*, 2016, 22, 8404–8430.
- 16 R. Breslow and L. E. Overman, *J. Am. Chem. Soc.*, 1970, **92**, 1075–1077.
- 17 Y. Aiba, J. Sumaoka and M. Komiyama, *Chem. Soc. Rev.*, 2011, **40**, 5657–5668.
- 18 R. P. Bonarlaw and J. K. M. Sanders, J. Am. Chem. Soc., 1995, 117, 259–271.
- 19 G. P. Royer and I. M. Klotz, *J. Am. Chem. Soc.*, 1969, **91**, 5885–5886.
- 20 X. Zhang, H. P. Xu, Z. Y. Dong, Y. P. Wang, J. Q. Liu and J. C. Shen, *J. Am. Chem. Soc.*, 2004, **126**, 10556–10557.
- 21 D. J. Cram and J. M. Cram, Science, 1974, 183, 803-809.
- 22 J. M. Lehn and C. Sirlin, J. Chem. Soc., Chem. Commun., 1978, 949–951.
- 23 Z. Y. Dong, Y. G. Wang, Y. Z. Yin and J. Q. Liu, *Curr. Opin.* Colloid Interface Sci., 2011, 16, 451–458.
- 24 G. Wulff and A. Sarhan, *Angew. Chem., Int. Ed. Engl.*, 1972, **11**, 341–342.
- 25 T. Takagish and I. M. Klotz, Biopolymers, 1972, 11, 483-491.
- 26 T. Pan and O. C. Uhlenbeck, *Biochemistry*, 1992, **31**, 3887–3895.
- 27 R. R. Breaker and G. F. Joyce, Chem. Biol., 1994, 1, 223-229.
- 28 S. J. Pollack, J. W. Jacobs and P. G. Schultz, *Science*, 1986, 234, 1570–1573.
- 29 D. L. Long, R. Tsunashima and L. Cronin, *Angew. Chem., Int. Ed.*, 2010, **49**, 1736–1758.
- 30 M. T. Pope and A. Müller, Angew. Chem., Int. Ed., 1991, 30, 34-48.

- 31 J. J. Wang, X. G. Mi, H. Y. Guan, X. H. Wang and Y. Wu, *Chem. Commun.*, 2011, 47, 2940–2942.
- 32 S. Liu, J. Q. Tian, L. Wang, Y. W. Zhang, Y. L. Luo, H. Y. Li, A. M. Asiri, A. O. Al-Youbi and X. P. Sun, *Chempluschem*, 2012, 77, 541–544.
- 33 J. J. Wang, D. X. Han, X. H. Wang, B. Qi and M. S. Zhao, *Biosens. Bioelectron.*, 2012, **36**, 18–21.
- 34 D. Li, H. Y. Han, Y. H. Wang, X. Wang, Y. G. Li and E. B. Wang, *Eur. J. Inorg. Chem.*, 2013, 1926–1934.
- 35 C. L. Sun, X. L. Chen, J. Xu, M. J. Wei, J. J. Wang, X. G. Mi, X. H. Wang, Y. Wu and Y. Liu, *J. Mater. Chem. A.*, 2013, 1, 4699–4705.
- 36 Z. Sun, H. Z. Bie, M. J. Wei, J. J. Wang, X. G. Mi, X. H. Wang and Y. Wu, *Chin. Chem. Lett.*, 2013, **24**, 76–78.
- 37 Y. Ji, J. Xu, X. L. Chen, L. Han, X. H. Wang, F. Chai and M. S. Zhao, *Sens. Actuators*, B, 2015, 208, 497–504.

- 38 Z. Ma, Y. F. Qiu, H. H. Yang, Y. M. Huang, J. J. Liu, Y. Lu, C. Zhang and P. A. Hu, ACS Appl. Mater. Interfaces, 2015, 7, 22036–22045.
- 39 R. Tian, B. Y. Zhang, M. M. Zhao, Q. Ma and Y. F. Qi, *Talanta*, 2018, **188**, 332–338.
- 40 C. L. Xu and Z. J. Zhang, Anal. Sci., 2001, 17, 1449-1451.
- 41 H. H. Deng, X. Q. Zheng, Y. Y. Wu, X. Q. Shi, X. L. Lin, X. H. Xia, H. P. Peng, W. Chen and G. L. Hong, *Analyst*, 2017, 142, 3986–3992.
- 42 A. Téazéa, G. Hervéa, R. G. Finke and D. K. Lyon, *Inorg. Synth.*, 1990, 27, 85–96.
- 43 K. Zaitsu and Y. Ohkura, Anal. Biochem., 1980, 109, 109-113.
- 44 F. Si, X. Zhang and K. L. Yan, RSC Adv., 2014, 4, 5860-5866.
- 45 T. T. Gong, J. F. Liu, Y. W. Wu, Y. Xiao, X. H. Wang and S. Q. Yuan, *Biosens. Bioelectron.*, 2017, **92**, 16–20.