Electronic Supplementary Material (ESI) for RSC Advances.

Supplemental Information

Development of ultrasound-enhanced smartphone colorimetric biosensor for ultrasensitive hydrogen peroxide and its applications

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Preparation of solutions

50 mmol L^{-1} of phosphate buffer solution (PBS) was prepared by mixing disodium phosphate and monosodium phosphate were dissolved and adjusted with DI water. Then, solution was adjusted pH with 0.2 mol L^{-1} sodium hydroxide and/or 0.2 mol L^{-1} hydrochloric acid. For 2.0 mol L^{-1} of acetate buffer was prepared by mixing citric acid and sodium citrate and dissolved with DI water. Then, solution was adjusted pH with 0.2 mol L^{-1} sodium hydroxide and/or 0.2 mol L^{-1} hydrochloric acid. Stock standard solution of analyte and enzyme were prepared by dissolved in PBS pH 7.5. Stock solution of chromogenic agent at 150 mmol L^{-1} was prepared by TMB was dissolved and adjusted with ethanol. Additionally, stock solution of interference agent was prepared in PBS pH 7.5.

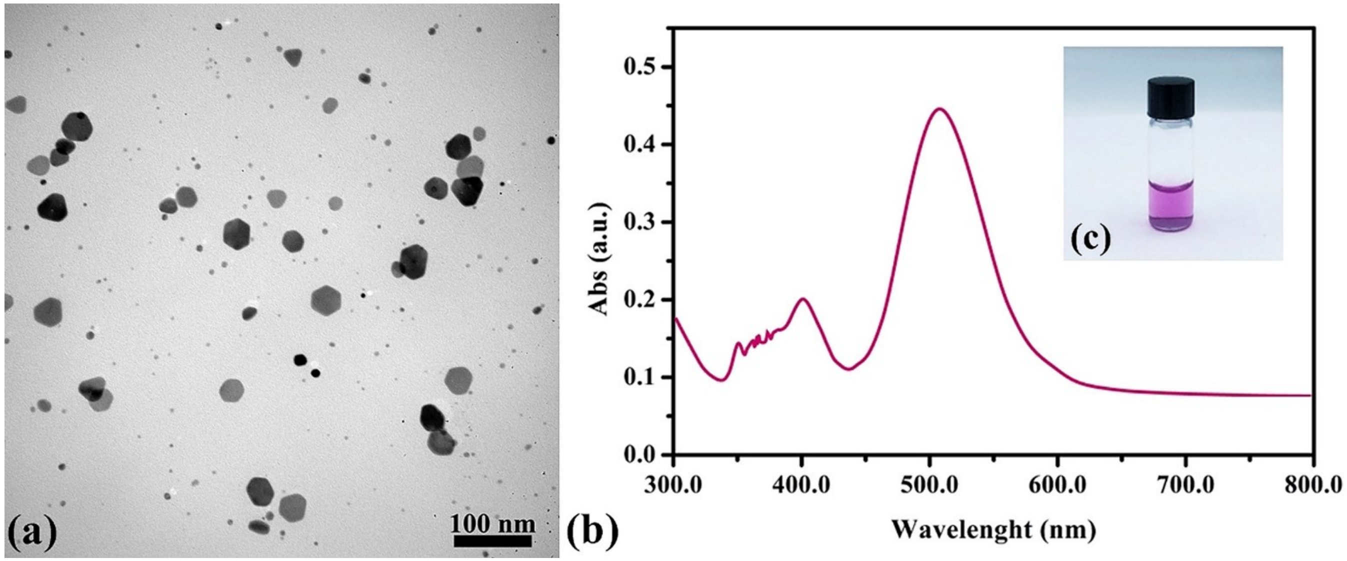


Figure. S1 Characteristic of silver nanoparticles; AgNPs (a) TEM image (b) absorbent spectrum (c) color solution of AgNPs.

Characteristic of AgNPs showed hexagonal shape with dimensional 45 ± 3 nm indicated TEM image in Figure. S1 (a). In addition, Evaluation for absorbent spectrum of them showed maximum absorbent at wavelength as 520 nm, which showed formation of hexagonal shape for AgNPs in Figure. S1 (b). Figure. S1 (c) showed solution of the synthesized AgNPs in proposed method. Thus, AgNPs was clearly studied and investigated to confirm a characteristic of them.

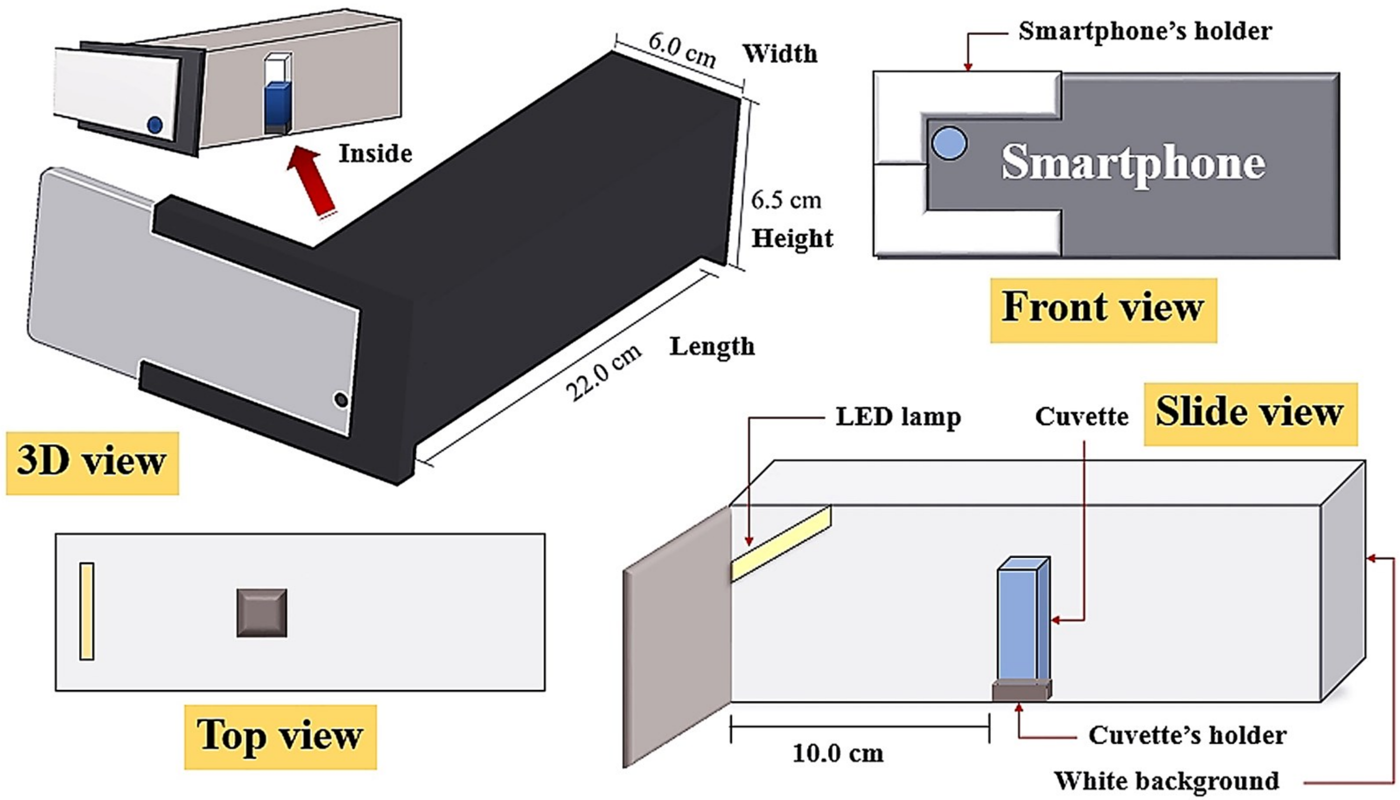


Figure. S2 Designed device for the smartphone colorimetric sensor.

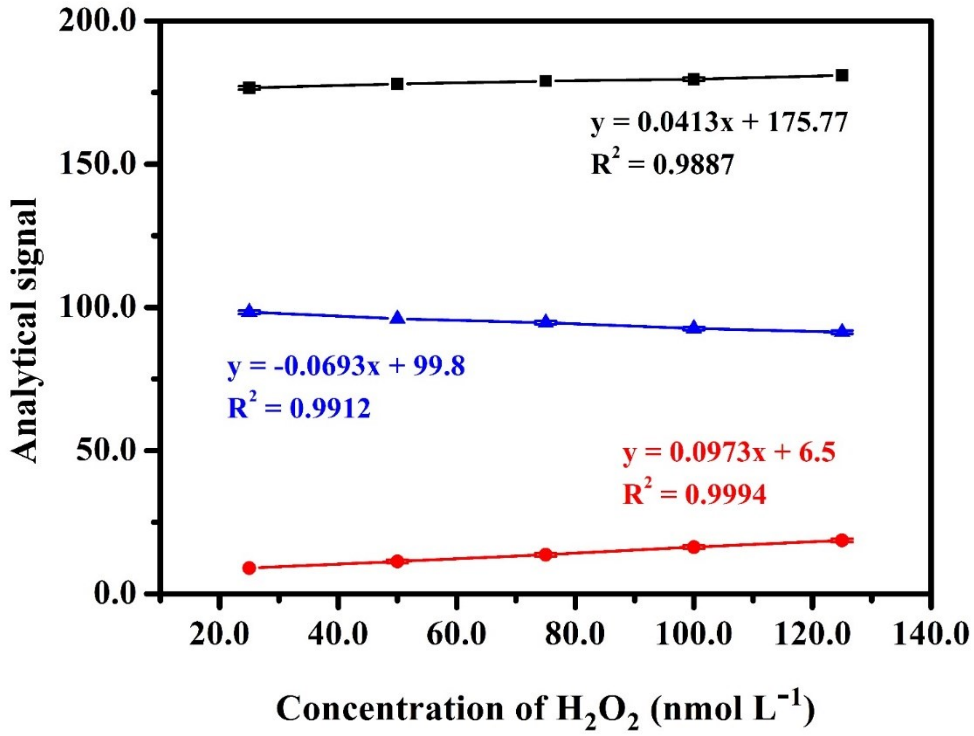


Figure. S3 Sensitivity of different channels obtained from HSV for the detection of H_2O_2 by proposed method consisting of H channel (back line), Saturation channel (red line) and V channel (blue line).

The final product color was measured with HSV channels to evaluate the suitable analytical signal for the proposed technique. Initially, AgNPs and TMB are pink-purple and colorless, respectively. After the oxidation-etching procedure and ultrasound, TMB changed to oxTMB which gave blue color as product color. In order to reduce discrepancies caused by the composition of the color ratio system and increase the measurement accuracy, the saturation channel was selected for the proposed method because this channel related to the direct color intensity of the product, which indicated most sensitivity and R^2.

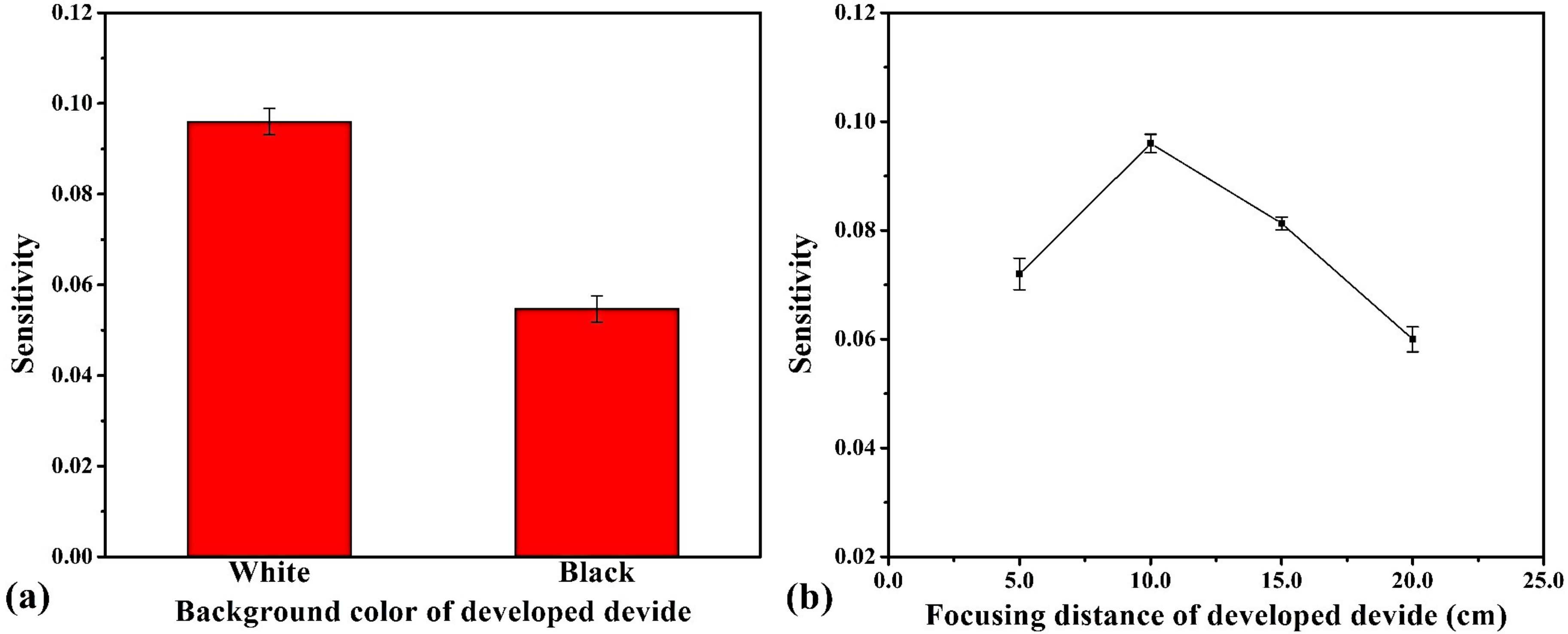
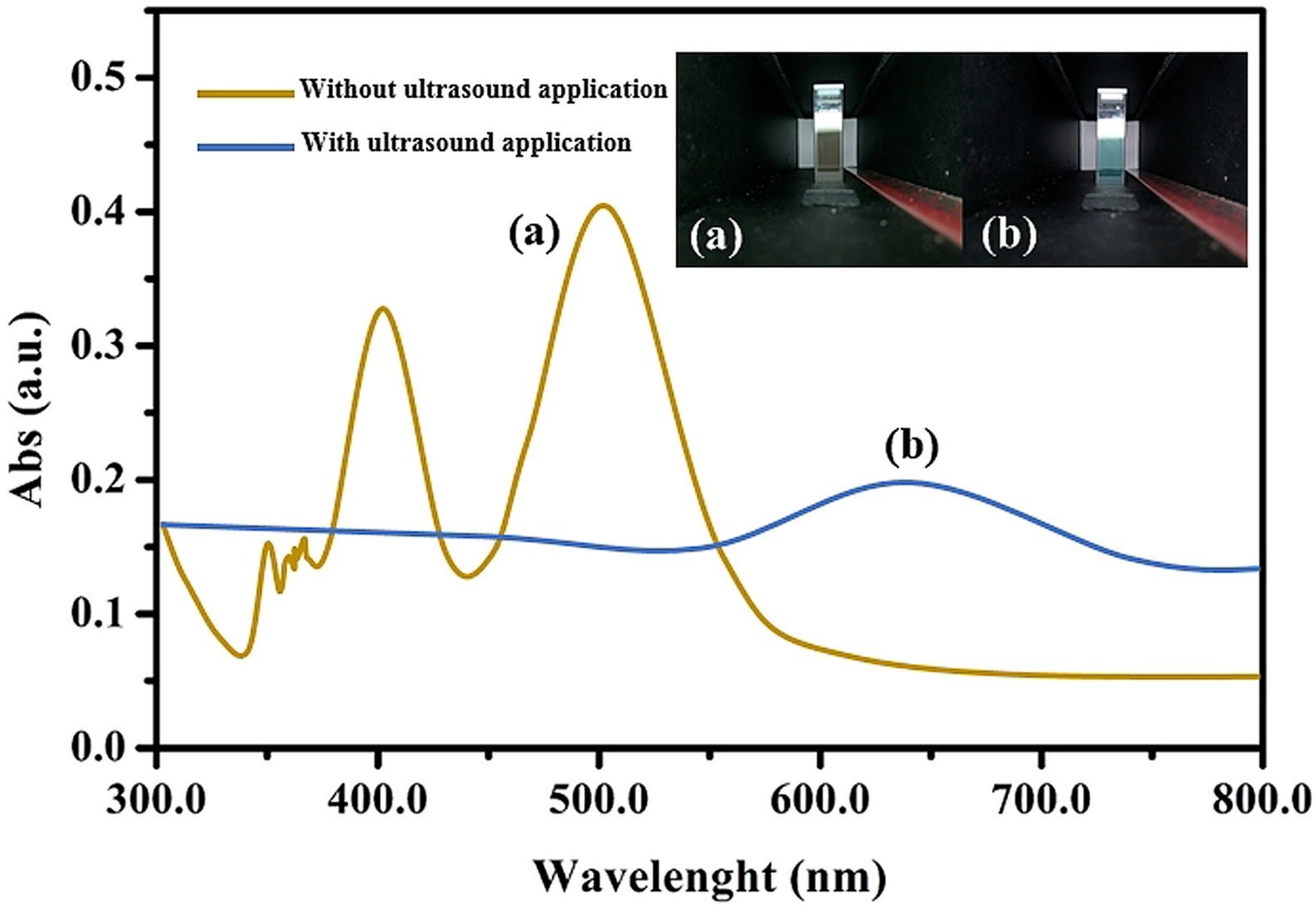


Figure. S4 (a) Sensitivity for difference background color and (b) Focusing distance of developed device in the proposed method.

We fabricated device with matte black acrylic plastic material to avoid interfering with the properties of the light. However, the internal background color of the device (black and white paper) which was put in the opposite to LED strips and focused distance were also studied. White background was chosen as suitable color of the internal background owing to good sensitivity whereas black color can absorb light affected to reduced intensity of the product (Fig. S4(a)). Besides, focusing distance between smartphone holder and cuvette was optimized because of affected to detectable intensity from resolution of smartphone's camera. The focusing distances were studied at 5.0, 10.0, 15.0, and 20.0 cm. As a result, we selected at 10.0 cm as appropriate focusing distance indicated in Figure. S4(b), which showed highest sensitivity. We proposed that the increasing of distance associated to reduce detectable camera performance of the smartphone.



Without ultrasound application
With ultrasound application
(a)
(b)
(a)
(b)
Abs (a.u.)
0.0
0.1
0.2
0.3
0.4
0.5
300.0
400.0
500.0
600.0
700.0
800.0
Wavelenght (nm)

Figure. S5 Absorbent spectrum of the H_2O_2 detection at 25.0 nmol L^{-1} by (a) without and (b) with ultrasound application in the proposed method.

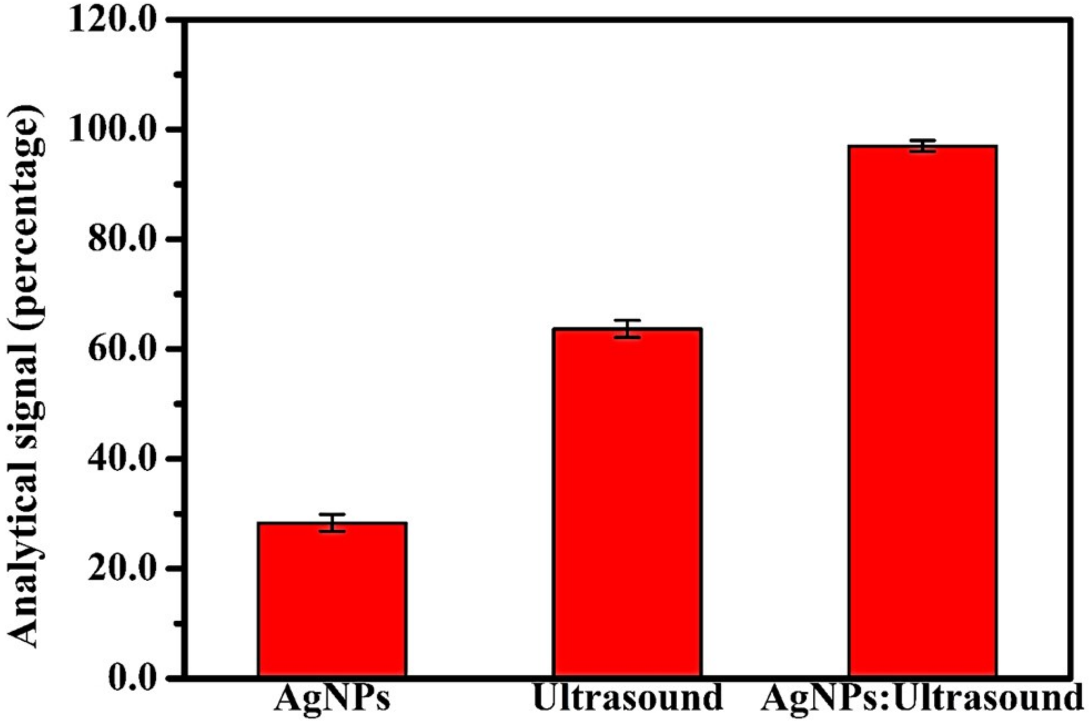


Figure. S6 Analytical signal for the H_2O_2 detection as 1000.0 nmol L^{-1} in the proposed method in the different conditions.

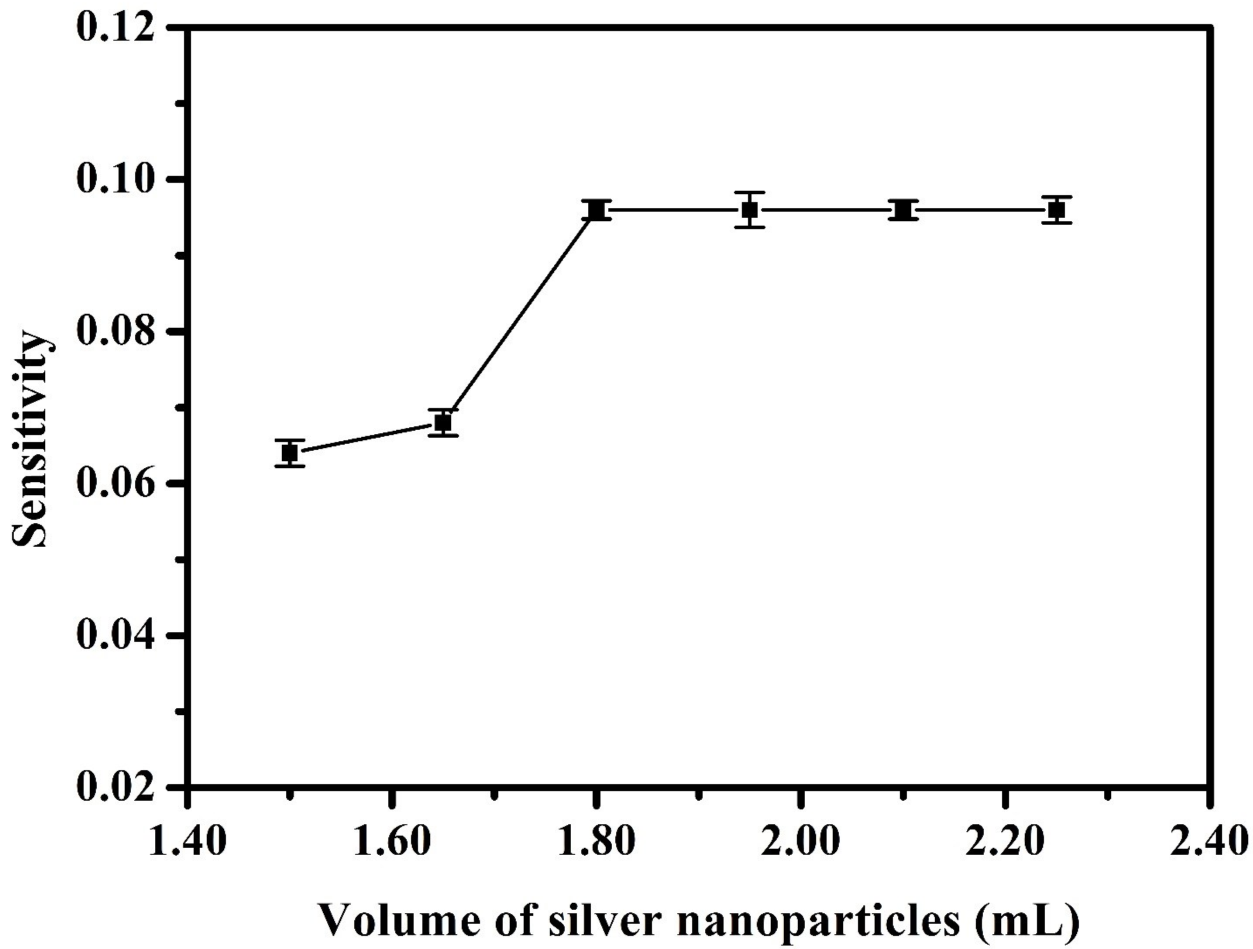


Figure. S7 Sensitivity of different volume of silver nanoparticles (AgNPs) for the H_2O_2 detection in the proposed method.

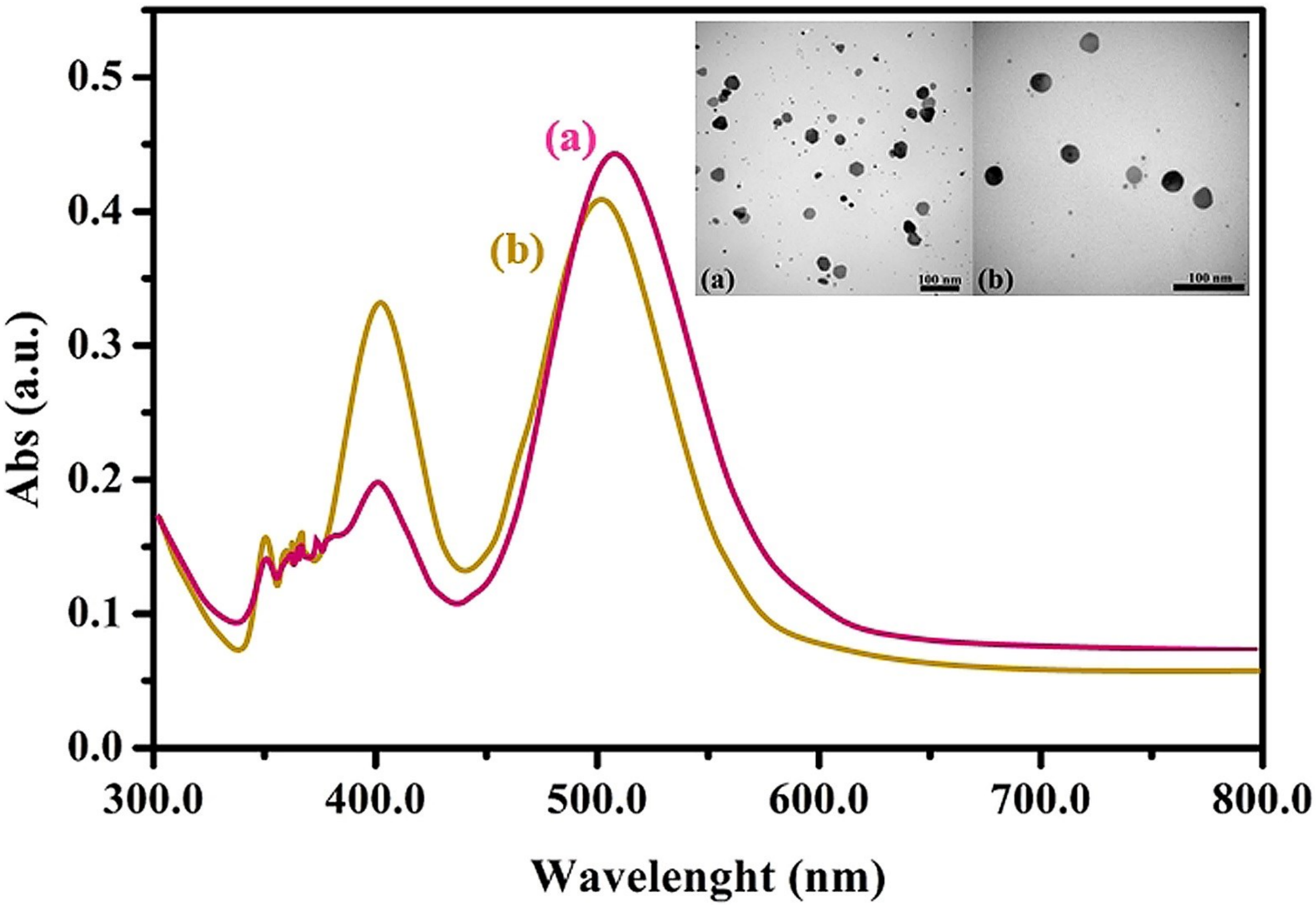


Figure. S8 Absorbent spectrum for locate surface plasmon resonance (LSPR) of AgNPs and TEM image between (a) pure AgNPs solution and (b) AgNPs solution containing acetate buffer pH 4.0.

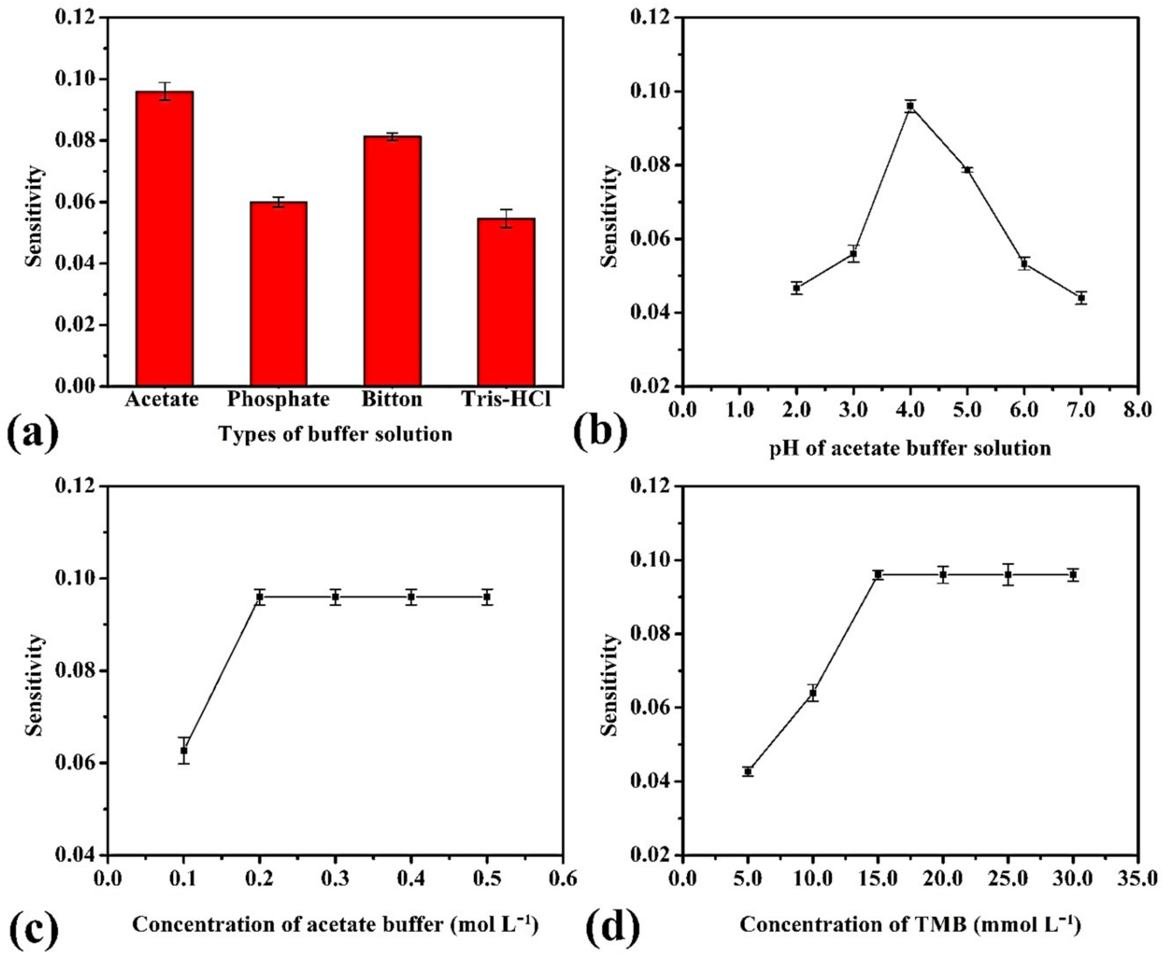


Figure. S9 Sensitivity of different types of (a) buffer solution (b) difference pH and (c) concentration of acetate buffer and (d) concentration of TMB for the H_2O_2 detection in the proposed method.

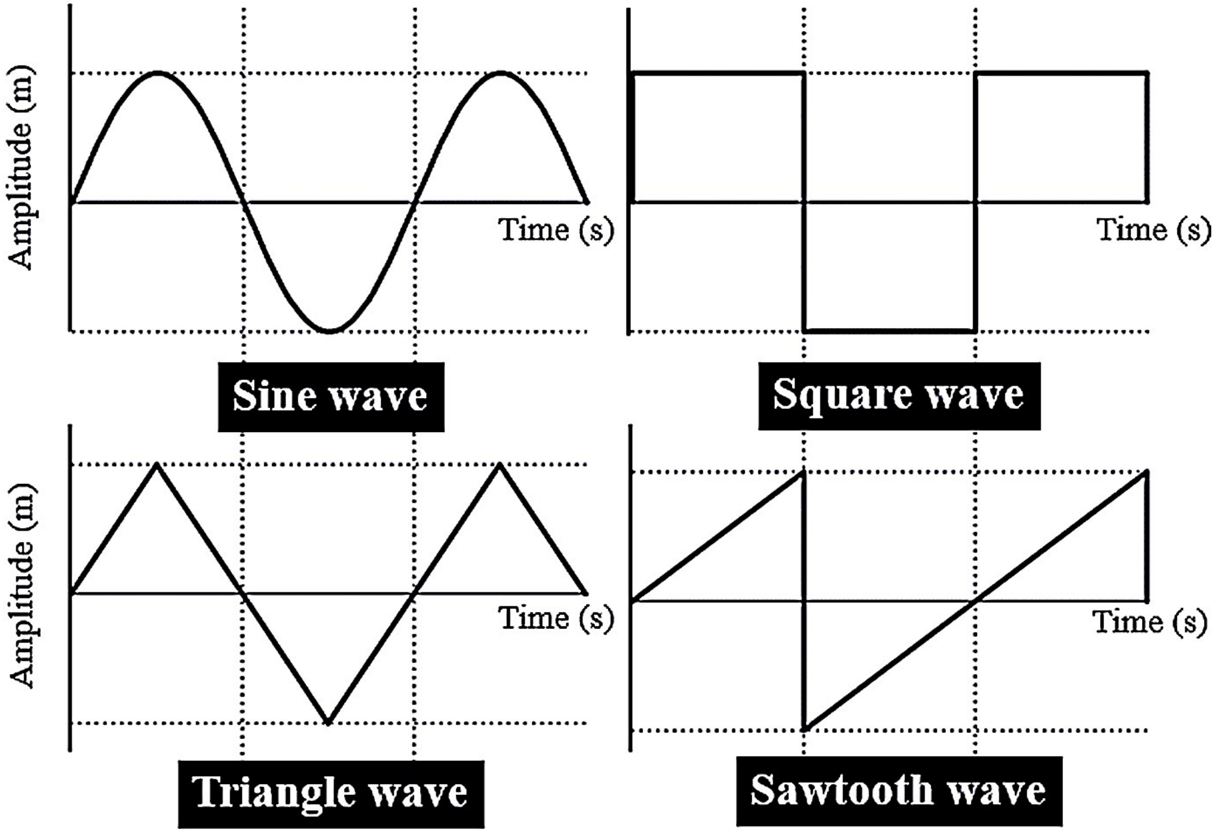


Figure. S10 The basically characteristic diagram of each sound waveform.

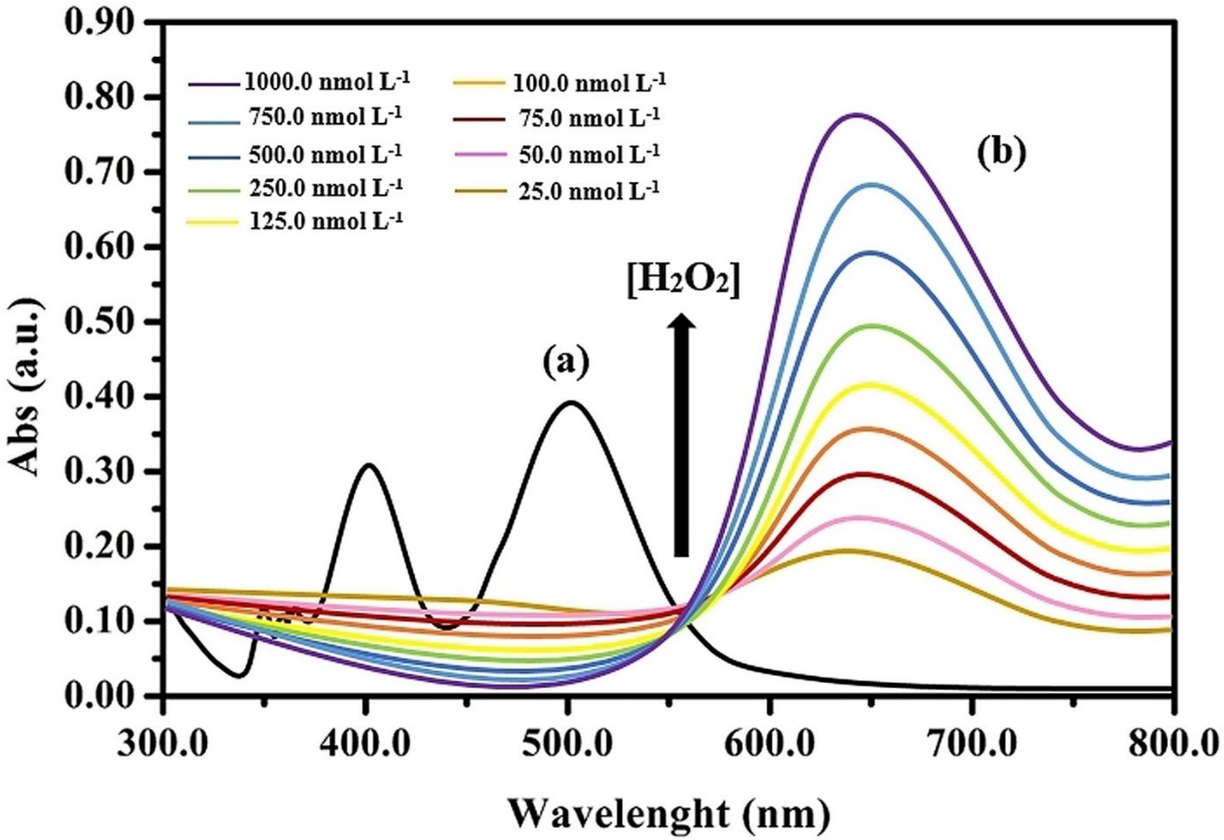


Figure. S11 Absorbent spectrum of (a) blank solution and (b) blue color product for the H_2O_2 detection in the proposed method.

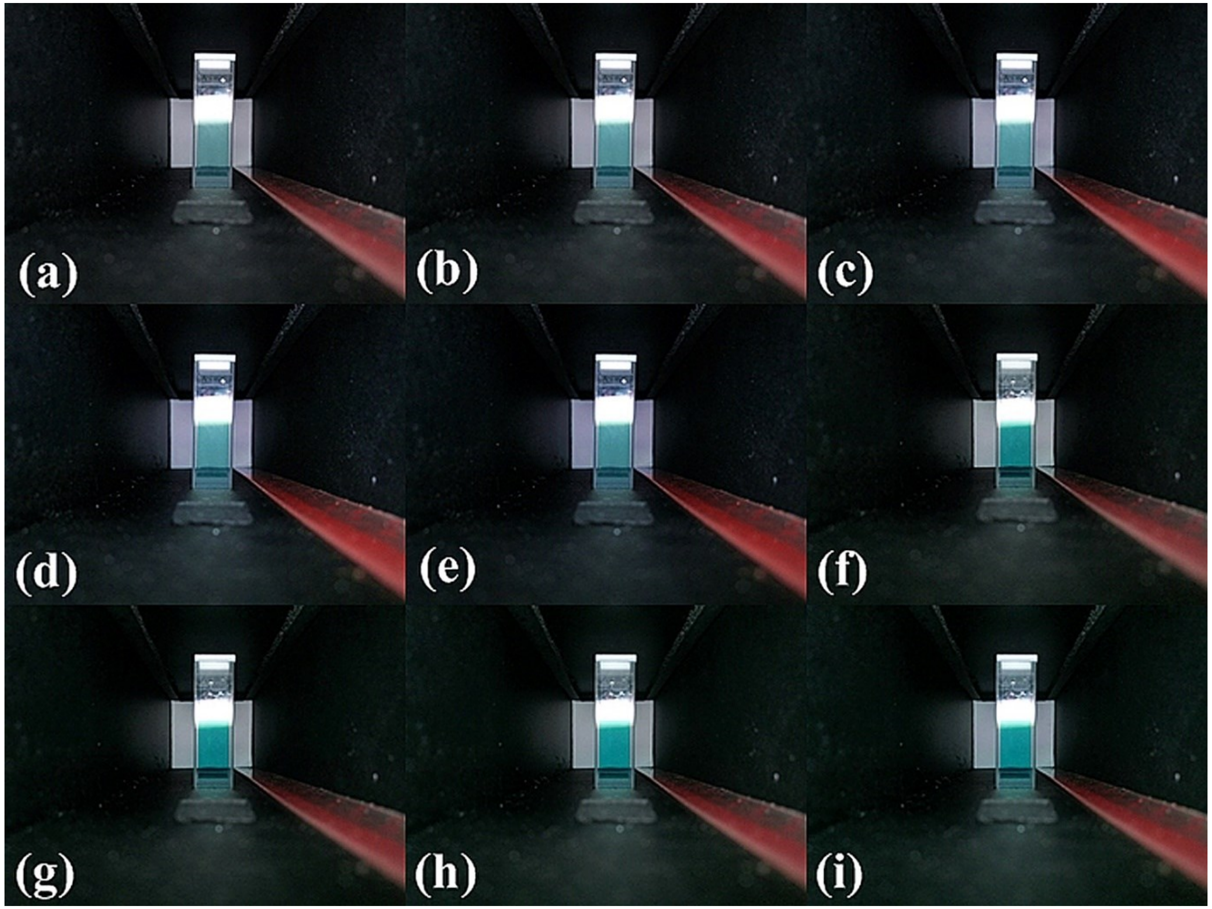


Figure. S12 The product solution in cuvette for H_2O_2 detection containing concentration linearity range for 25.0 to 1000.0 nmol L^{-1} (a-i) in developed device.

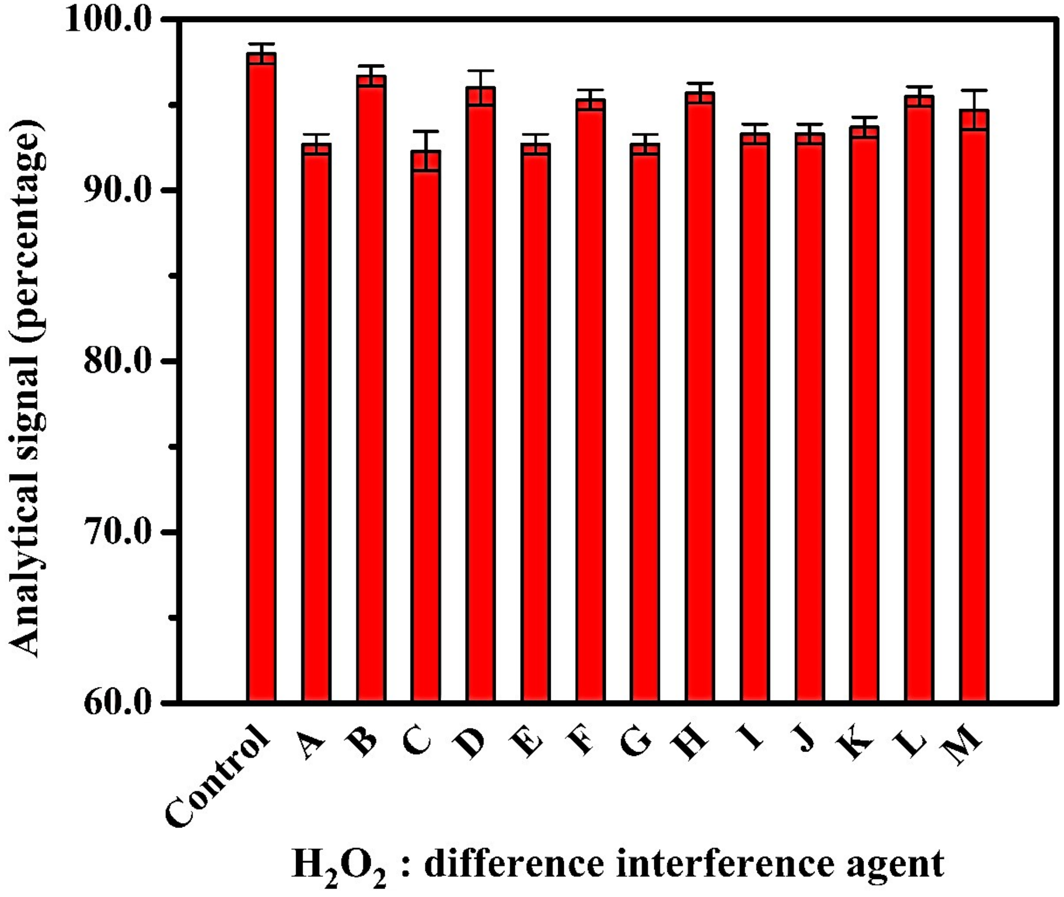


Figure. S13 Analytical signals for interference evaluation of the H_2O_2 (1.0 µmol L^{-1}) : interference agent (2.0 mmol L^{-1}), such as sodium chloride (NaCl); A, sodium nitrate ($NaNO_3$); B, potassium chloride (KCl); C, potassium dihydrogen phosphate (KH_2PO_4); D, magnesium chloride ($MgCl_2$); E, magnesium sulfate ($MgSO_4$); F, calcium chloride ($CaCl_2$); G, calcium carbonate ($CaCO_3$); H, salicylic acid; I, ascorbic acid; J, urea; K, creatinine; L and citric acid; M.

Table S1. Optimum condition studies for detection of H_2O_2 using proposed method (n=3)

Conditions	Optimum value
1. HSV channel	Saturation channel
2. Volume of silver nanoparticles (400 mg L^{-1})	1.80 mL
3. pH of acetate buffer solution	4.0
4. Concentration of acetate buffer solution	0.20 mol L^{-1}
5. Concentration of TMB	15.0 mmol L^{-1}
6. Frequency levels of the oscillation	22 kHz
7. Ultrasound timing	5.0 min
8. Type of waveform	Sawtooth waveform
9. Distance between generator probes and surface solution	1.0 cm
10. Intensity levels of smartphone	100%
11. Temperature	25 °C

Table S2. Types of the smartphone's brands

Smartphone' brand	Name of smartphone's brand	Smartphone's resolution
A	Huawei p9 plus	1080 x 1920 pixels, 16:9 ratio (~401 ppi density)
B	Vivo v9	1080 x 2280 pixels, 19:9 ratio (~400 ppi density)
C	Oppo a7	720 x 1520 pixels, 19:9 ratio (~271 ppi density)
D	Samsung j7	720 x 1280 pixels, 16:9 ratio (~267 ppi density)

Table S3. Types of the earphone's brands

Smartphone' brand	Sensitivity	F-test	T-test

Headphone' brand	Headphone's brand	Headphone's specification
A	Huawei (Type-C, AM116)	Impedance 32-ohm, Frequency Response 20 Hz -20 kHz, Sensitivity 109 dB/mW (at 1Khz)
B	Vivo HiFi (XE680)	Impedance 32-ohm, Frequency Response 20 Hz -20 kHz, Sensitivity 109 dB/mW (at 1Khz)
C	OPPO Headphone (MH320)	Impedance 32-ohm, Frequency Response 20 Hz -20 kHz, Sensitivity 106 dB/mW (at 1Khz)
D	Samsung (EO-EG920BBEGWW)	Impedance 32-ohm, Frequency Response 20 Hz -20 kHz, Sensitivity 98.5 dB/mW (at 1Khz)

Table S4. Ruggedness studies of proposed method by different smartphone's bands (n=3)

A	0.0913 ± 0.006	-	-
B	0.0904 ± 0.008	1.02	2.24
C	0.0904 ± 0.007	1.01	1.34
D	0.0897 ± 0.006	1.03	0.83

Table S5. Ruggedness studies of proposed method by different earphone's bands (n=3)

earphone' brand	Sensitivity	F-test	T-test
A	0.0915 ± 0.0027	-	-
B	0.0907 ± 0.0026	1.02	2.02
C	0.0903 ± 0.0028	1.03	1.84
D	0.0903 ± 0.0028	1.04	0.38