Supporting information for

Dihydro-benzo[4,5]imidazo[1,2-c]quinazoline-based probe with aggregation-induced ratiometric emission for the ratiometric fluorescent detection of peroxynitrite in living cells and zebrafish

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S1 Experimental section

S1.1. Materials and instrumentation

Solvents and starting materials for syntheses were purchased commercially and used as received. Elemental analyses were carried out on an Elemental Vario EL analyzer. ^{1}H NMR spectra were recorded on a Bruker AV 400MHz spectrometer in DMSO-d_6 solution. The UV spectra were recorded on a Purkinje General TU-1800 spectrophotometer. Fluorescence spectra were determined on a Varian CARY Eclipse spectrophotometer, in the measurements of emission and excitation spectra the pass width is 5 nm and the voltage of the photomultiplier tube is 650 V. ESI-MS spectra were obtained on a Bruker Daltonics Esquire 6000 mass spectrometer. The cytotoxic effect exerted by **1** on cultured HeLa cells was ascertained by a standard MTT assay according to the literature method [1]. Fluorescent images were taken on Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope.

S1.2. General UV–vis and fluorescence spectra measurements

The spectral analyses were performed in water (H_2O)–dimethyl sulfoxide (DMSO) mixtures at room temperature. The concentration of the sensor **1** for UV-vis and fluorescence measurement was 10 μM. Anions and reactive oxygen species were prepared with sodium or potassium salt solution of water.

UV-vis and fluorescence spectrophotometric titrations were conducted directly in 2 mL cuvette by successive addition of corresponding chemical reagent using a microliter syringe. Upon addition of every aliquot, the solution was well mixed then the spectrum was measured.

*S1.3. Synthesis of the probe **1***

To a solution of 3-(benzothiazol-2-yl)-2-hydroxy-5-methyl-benzaldehyde (0.538 g, 2.0 mmol) in ethanol (30 mL) was added 2-(2-aminophenyl)benzimidazole (0.272 g, 2.0 mmol). The mixture was refluxed with stirring for 2 h after which the color of the resulting solution turned pale-yellow. The precipitate was formed after the solution was cooled to room temperature, which was filtered to produce probe **1** (0.718 g, yield: 78%). Elemental analysis for **1** ($C_{28}H_{20}N_4OS$) (%): Calcd: C, 73.02; H, 4.38; N, 12.17; S, 6.96. Found: C, 72.80; H, 4.58; N, 11.95; S, 6.79. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.89 (s, 1H, OH), 8.21-8.23 (d, 1H, ArH), 8.14-8.16 (d, 1H, ArH), 7.98-7.99 (d, 1H, ArH), 7.67-7.69 (q, 2H, ArH), 7.60-7.64 (m, 1H, ArH), 7.52-7.56 (m, 1H, ArH), 7.43 (s, 1H, CH), 7.42 (s, 1H, NH), 7.07-7.26 (m, 4H, ArH), 6.88-6.90 (d, 1H, ArH), 6.81-6.85 (t, 1H, ArH), 6.758-6.762 (d, 1H, ArH), 2.13 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.06, 147.71, 143.60, 132.86, 132.09, 129.76, 128.29, 125.10, 122.94, 122.74, 122.47, 119.15, 118.44, 117.12, 115.20, 111.77, 110.60, 63.57, 40.65, 40.44, 40.23, 40.02, 39.81, 39.60, 39.39, 20.50. ESI-MS (m/z): ($C_{34}H_{27}N_2O_3S^+$), Calcd: 461.1391. Found: 461.1437.

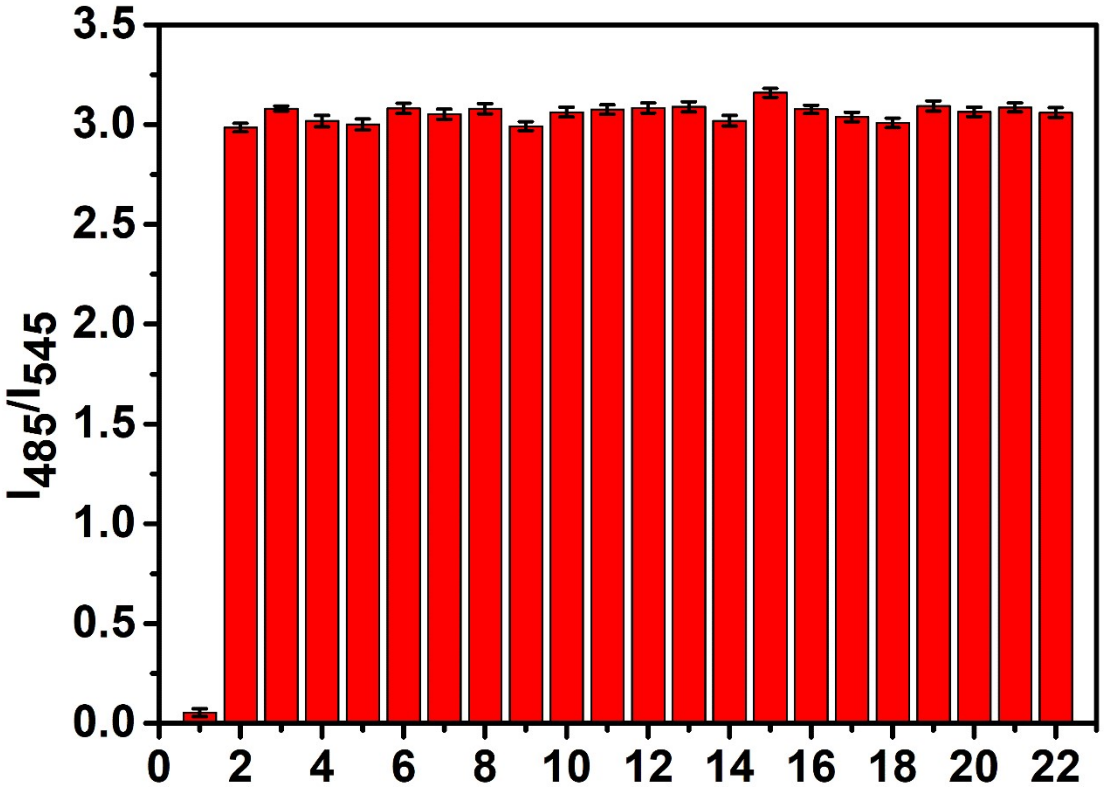


Fig. S1 Fluorescent intensity ratio (I_{485}/I_{545}) of **1** (10 μM) and **1**+$ONOO^-$ (8.5 μM) with various interfering analytes (100 μM) in PBS buffer (20 mM, pH 7.4, 0.5% DMSO). 1. Probe; 2-21, **1**+$ONOO^-$ with analytes: 2. AcO^-, 3. Br^-, 4. Cl^-, 5. ClO_4^-, 6. SO_4^{2-}, 7. HSO_4^-, 8. F^-, 9. $H_2PO_4^-$, 10. HPO_4^-, 11. I^-, 12. PO_4^{3-}, 13. S^{2-}, 14. HSO_3^-, 15. SO_3^{2-}, 16. ClO^-, 17. H_2O_2, 18. $^1O_2$, 19. ·OH, 20. NO, 21. ROO^-; 22. **1**+$ONOO^-$. The excitation wavelength was 360 nm.

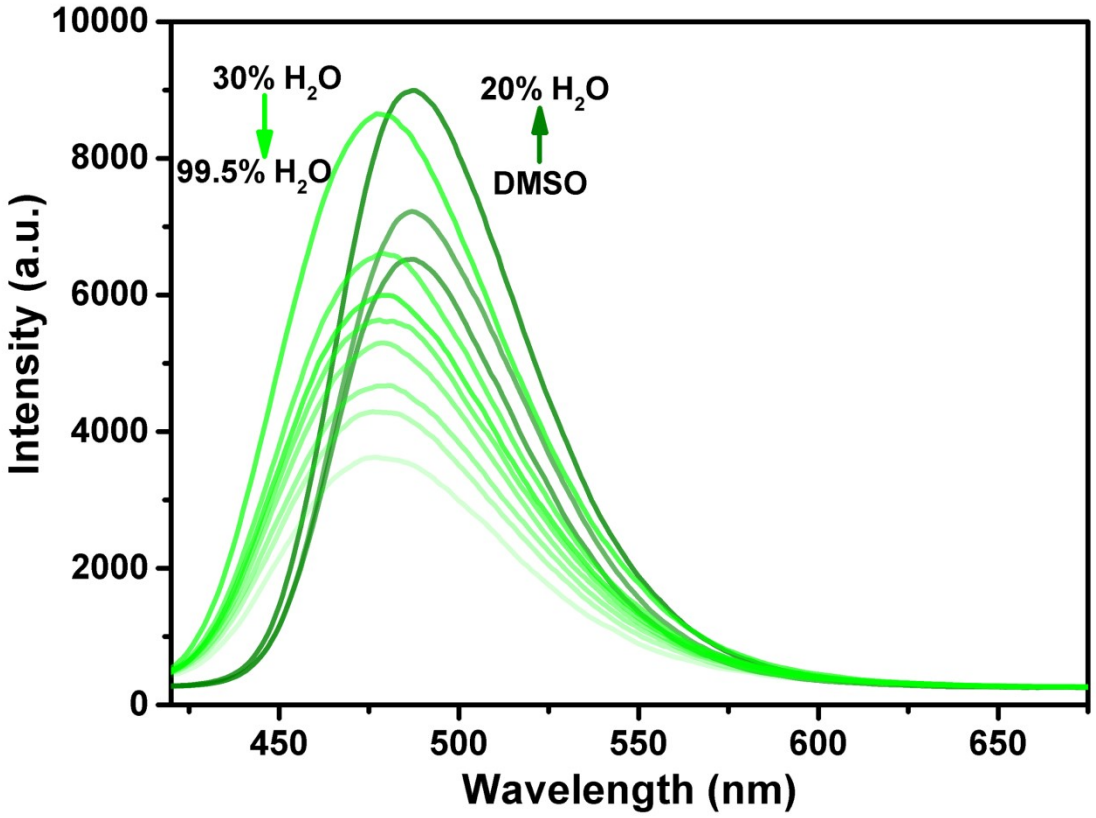


Fig. S2 Fluorescence spectra of **1** (10 μM)+$ONOO^-$ (8.5 μM) in H_2O/DMSO mixtures with different water fractions. Excitation wavelength was 360 nm.

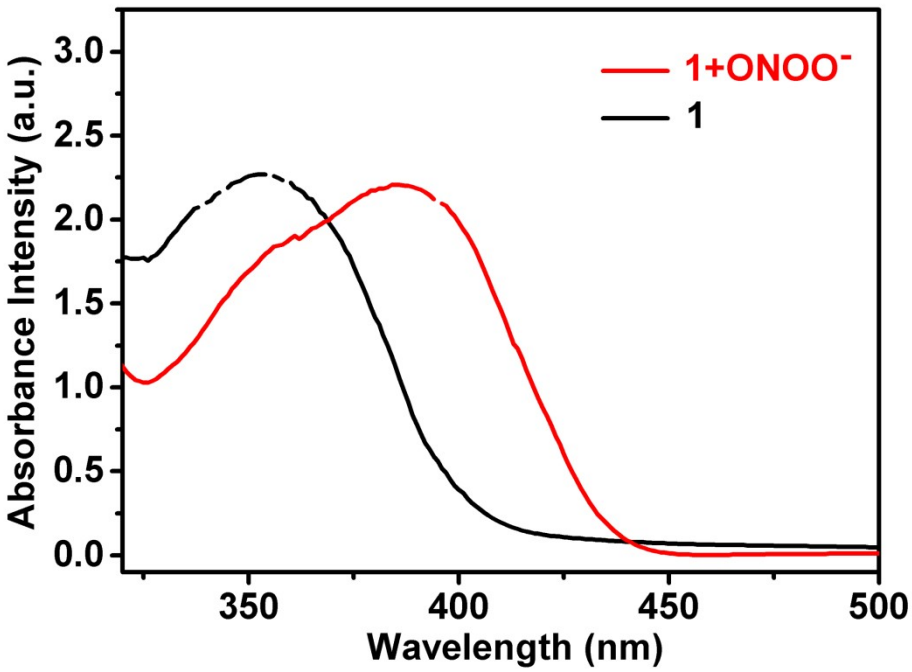


Fig. S3 UV–vis spectra of **1** (10 μM) and **1**+$ONOO^-$ (8.5 μM) in PBS buffer (20 mM, pH 7.4, 0.5% DMSO).

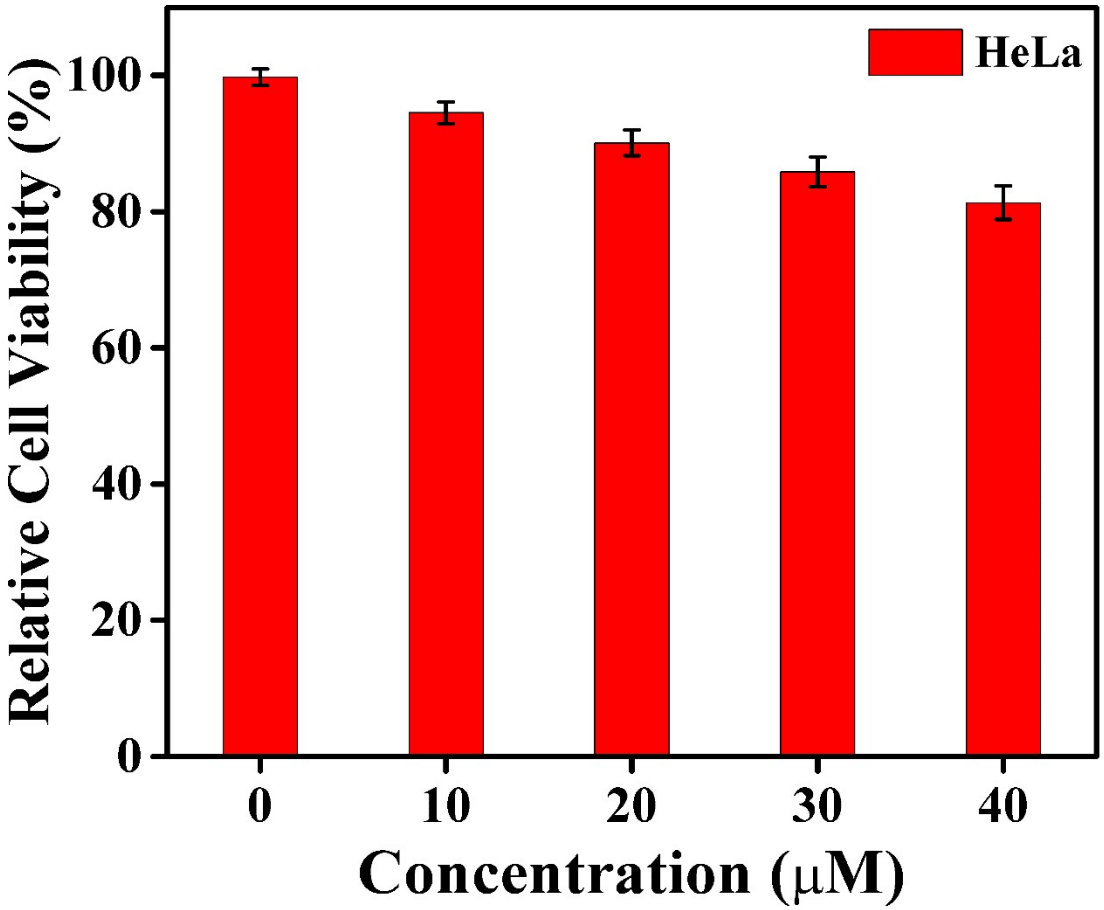


Fig. S4 The viability assay of cell with different concentration of **1** (0-40 μM) on HeLa cells using the MTT assay for 24 h. All samples were done in triplicate.

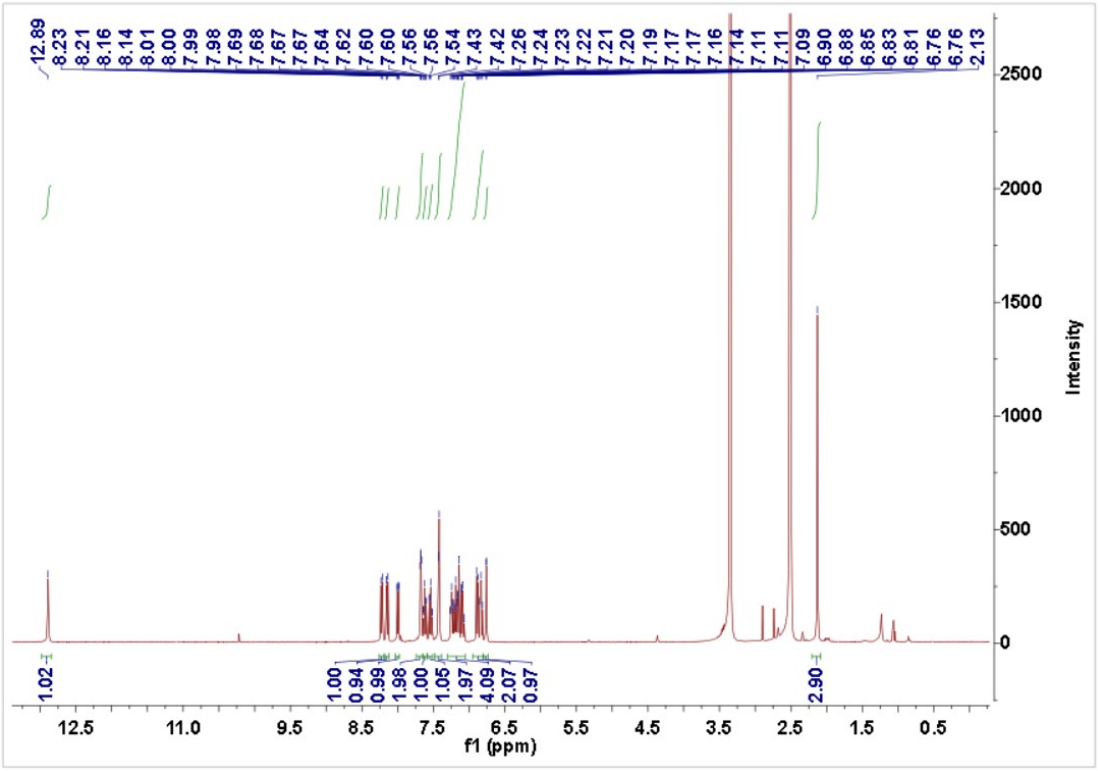


Fig. S5 ^{1}H NMR spectrum of **1** in DMSO-d_6.

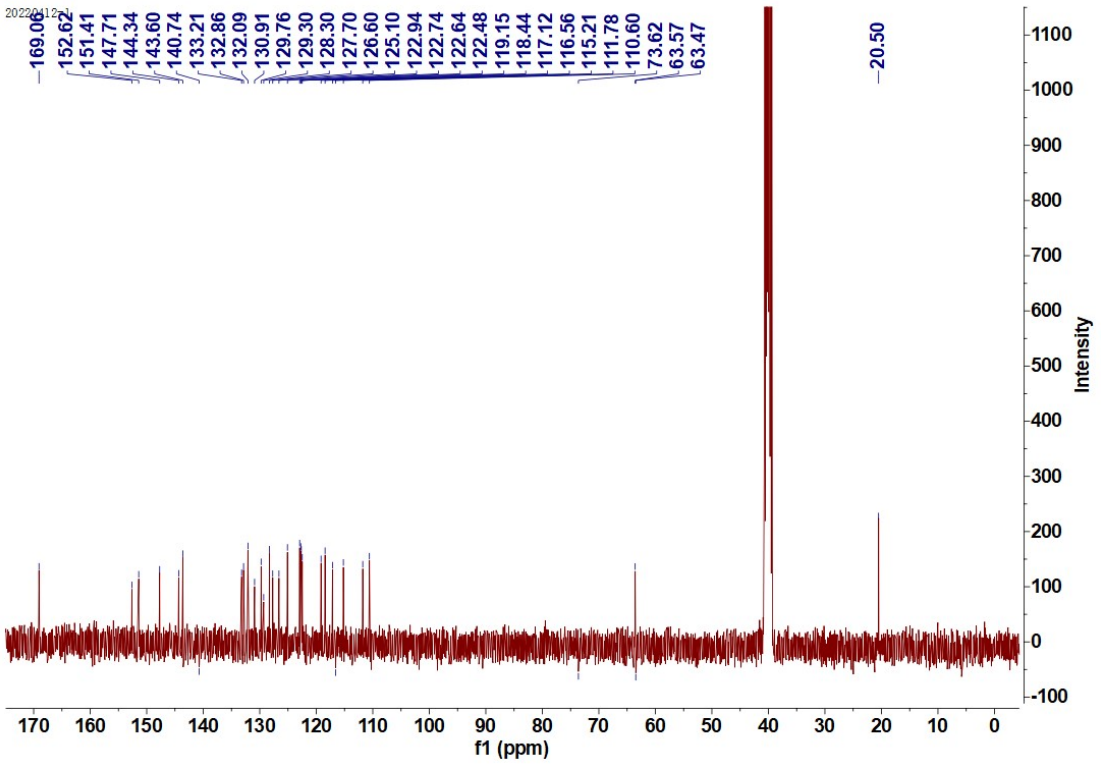
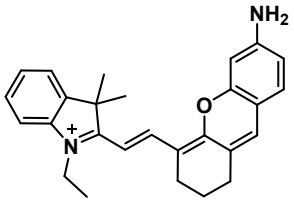
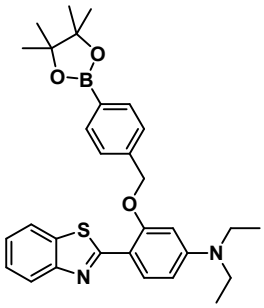
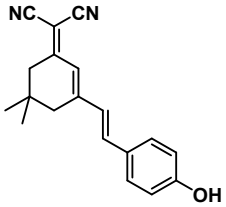
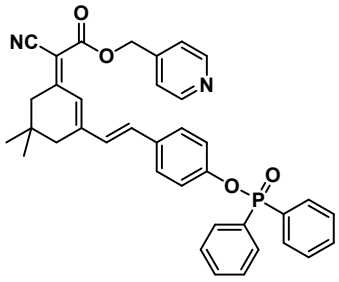
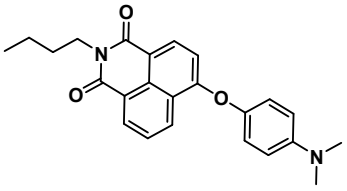
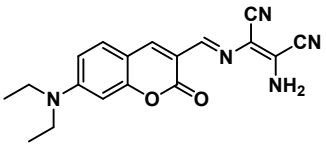
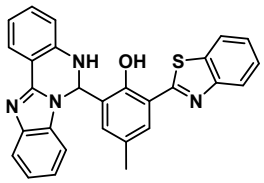


Fig. S6 ^{13}C NMR spectrum of **1** in DMSO-d_6.

Table S1 Comparison of probe **1** with some reported $ONOO^-$ probes.

Ref.	Probe	Media	LOD (nM)	pH	Response Time
[2]		PBS	33	7.0-12.0	120 s
[3]		1% DMSO	75	7.0-12.0	60 s
[4]		10% DMSO	78.7	6.5-8.5	1 h
[5]		30% DMSO	53	3.0-10.0	20 min
[6]		10% DMF	69	-	100 s
[7]		10% DMF	135	4.0-9.0	5 min
This work		0.5%DMSO	17.6	2.0-13.0	30 s

References

[1] J. Zhang, J. F. Kan, Y. Y. Sun, M. Won, J. H. Kim, W. F. Zhang, J. Zhou, Z. S. Qian, J. S. Kim,ACS. Appl. Bio. Mater. 4 (2021) 2080-2088.

[2] D. Y. Zhou, J. Ou-Yang, Y. F. Li, W. L. Jiang, Y. Tian, Z. M. Yi, C. Y. Li, Dyes. Pigm. 161 (2018) 288-295.

[3] M. L. Li, H. Han, S. M. Song, S. M. Shuang, C. Dong, Spectrochim. Acta. A. 261 (2021) 120044.

[4] Y. B. Zhang, D. G. Ma, Spectrochim. Acta. A. 244 (2021) 118890.

[5] B. Gu, C. F. Liu, Y. Wu, C. X. Zhang, Y. M. Shen, M. Q. Liu, ACS Omega. 5 (2020) 27530-27535.

[6] W. L. Sheng, K. Wang, N. Gao, L. Z. Wang, R. C. Wang, X, M, Zhang, X. Q. Chen, Y. Zhang, B. C. Zhu, K. C. Liu, Analyst. 146 (2021) 5264-5270.

[7] C. Wang, W. Shu, Q. Q. Chen, C. L. Yang, S. Su, M. X. Gao, R. B. Zhang, J. Jing, X. L. Zhang, Spectrochim. Acta. A. 260 (2021) 119990.