Electronic Supplementary Information

Highly sensitive quantification of ultratrace As(V) *via* iEESI-MS

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Content

Experimental Procedures

Materials

All chemicals used in this work were of analytical reagent grade and without further purification. Ultrapure water was used in all experiments. Cerium (III) nitrate (99.5%, $Ce(NO_3)_3 \cdot 6H_2O$) and cesium dihydrogen arsenate (99.99%, H_2AsCsO_4) were obtained from Beat Medicine (Shanghai, China). Sulfuric acid, ethylene glycol, hydrogen peroxide (30%, H_2O_2), anhydrous ethanol (≥ 99.5%, EtOH), sodium chloride (≥ 99.5%, NaCl), sodium hydroxide (≥ 96.0%, NaOH), nitric acid (36.0 − 38.0%, HNO_3), ferric chloride hexahydrate (≥ 99.0%, $FeCl_3 \cdot 6H_2O$), formic acid (≥ 99.5%, FA), ammonia (25.0% − 28.0%, $NH_3 \cdot H_2O$) and anhydrous sodium acetate (≥ 99.0%, NaAc) were purchased from Sinopharm Chemical Reagent. Co. Ltd. (Shanghai, China). Polyethylene glycol (PEG_{400}), other arsenate and arsenite were acquired by Macklin (Shanghai, China). Glycerol phosphorylcholine and ethanolamine phosphate were purchased from Invitrogen Molecular Probe (Carlsbad, CA), D-Glucose-6-phosphate disodium salt ($C_6H_{11}Na_2O_9P$), other arsenate and arsenite were acquired by Macklin (Shanghai, China). Different anion salts (i. e. carbonate, iodide, iodate, chloride, sulfate, fluoride, bromide, dihydrophosphate, arsenite, phosphate, phosphite, nitrate and nitrite) were purchased from Sinopharm Chemical Reagent. Co. Ltd. (Shanghai, China). 1.00 mL syringes were bought from Conley Medical devices (Hunan Province, China) and 0.22 μm filter membranes were obtained from Aboly (Tianjin, China).

Starch, agar powder, chitosan, gelatin, carrageenan were purchased from Solarbio (Beijing, China). Escherichia coli (E.coli) brought from Beina Chuanglian Institute of Biotechnology (BNCC17111513, Beijing, China). Five different water samples, Black Tiger Spring, Yellow River, Qushuiting Street, tap water were obtained from Jinan, Shandong Province.

Instrumentation

Q-Exactive Orbitrap (Thermo Scientific, San Jose, CA) in positive ion mode was used to record signals. The voltage of capillary and tube lens was set to +30 V. The temperature of the capillary was set as 320 °C, and sheath gas pressure was set to zero. iCAP Q (ICP-MS Thermo Scientific, San Jose, CA) in positive ion mode was used to record signals and Qtegra software was used to analyse data. The temperature of the interface was set as 35 °C, the nebulizer gas flow rate was set to 1.00 L/min and auxiliary gas flow rate was set at 0.800 L/min. Vacuum concentrator (ZLNS-1) was brought from Hunan Hengnuo Instrument and Equipment Co., Ltd. (Changsha, Hunan Province, China).

TEM was taken on a FEI TalosF200x (Super-X EDS was acquired in this experiment). XRD analysis was carried out on a D/Max 2500 V/PC X-ray diffractometer using Cu (40 kV, 30 mA) radiation. XPS spectra were performed with a Phobios 100 electron analyzer (SPECS GmbH) equipped with 5 channeltrons using an unmonochro-mated Mg Ka X-ray source (1253.6 eV). UV-VIS absorption was recorded on UV-2600 UV−visible spectrophotometer (Shimadzu, Japan). Zeta potential analysis and particle size distribution experiments were carried out by Malvern Zeta Sizer Nano (Malvern, England).

Synthesis of Fe_3O_4 magnetite microspheres

The magnetite microspheres were prepared according to the previous method.[1] Typically, $FeCl_3 \cdot 6H_2O$ (0.675 g), PEG_{400} (500 μL) and NaAc (1.80 g) were dissolved in ethylene glycol (40.0 mL) and stirred for 30 min. Then the obtained solution was transferred to a 50.0 mL Teflon-lined stainless steel autoclave and heated for 8 h at 200°C. Finally, the precipitate was collected by

centrifuge, followed by washed with EtOH for three times.

Synthesis of CeO_2 precursor

The ceria precursor was prepared by the solvothermal method.[2] 0.500 g $Ce(NO_3)_3 \cdot 6H_2O$ and 0.500 g NaOH were separately dissolved in EtOH (10.0 mL). Then the mixture was stirred vigorously for 24 h at 50 °C, followed by adding hydrogen peroxide (30% H_2O_2, 25.0 μL) and stirred for another 2 h. The obtained precipitate was washed and drying for 4 h under 60 °C. Then the precipitate (0.500 g) was dispersed in distilled water (10.0 mL) and the pH value was adjusted to 0.1 by HNO_3. The mixture was allowed to proceed for 2 h at 40 °C under continuous stirring. After cooled down to room temperature naturally, the yellow solution was obtained.

Synthesis of Fe_3O_4@CeO_2

The Fe_3O_4@CeO_2 microspheres were prepared in accordance with the following procedure:[3] 0.100 g Fe_3O_4 nanoparticles and 3.00 mL of ceria precursor were mixed with 30.0 mL distilled water for 15 min ultrasonic treatment. Then 0.500 M $NH_3 \cdot H_2O$ was added to the mixture for adjusting pH value to 6.80. The solution was agitated vigorously by stirring 4 h at 60 °C. After reaction completed, the synthesized nanoparticles were washed thoroughly with ethanol for three times and dried at 60 °C overnight.

Setup of iEESI-MS

Generally, the 1.00 mL syringe with a magnet under bottom was connect with a 0.22 μm filter membrane. A micro tee connection was used to connect the syringe and the metal spray needle. A high voltage was applied to the syringe needle and an external injection pump was used to control the flow rate of the syringe.

Quantification of As(V) by iEESI-MS

2.75 mg Fe_3O_4@CeO_2 was suspended in 500 μL ultrapure water with sonication. GPC (500 μL, 100 μM) was added and incubated for 90 min at 25 °C, 300 rpm. After centrifugation, the precipitate was washed by ultrapure water for three times. Then the precipitate was further mixed with IS (20.0 μM, 400 μL, V_{MeOH}: V_{H2O}=1:1, pH=6) solution and H_2AsCsO_4 samples with different final concentrations (50.0 pM, 100pM, 200 pM, 500 pM, 1.00 nM, 10.0 nM, 50.0 nM, 60.0 nM, 80.0 nM, 100 nM, V_{MeOH}: V_{H2O}=1:1). The mixture was incubated for 90 min at 25 °C, 300 rpm, and then transferred to the 1.00 mL syringe mentioned above. We used FA or $NH_3 \cdot H_2O$ to adjust the pH value of the solution. Then the final nanocomposites were removed by the magnet on bottom of the syringe and the supernatant was detected by UV-Vis and iEESI-MS.

Specificity of As(V) by iEESI-MS

2.75 mg Fe_3O_4@CeO_2 was suspended in 500 μL ultrapure water. After sonication, GPC (500 μL, 100 μM) was added and incubated for 90 min, followed by centrifugation. The obtained precipitate was washed by ultrapure water for three times and further mixed with IS (400 μL, 20.0 μM) solution. Then the mixture was incubated with H_2AsCsO_4 sample (1.00 nM) 90 min, or other different types of ionic salts (100 nM), including nitrate, nitrite, phosphate, phosphite, sulfate, sulfite, fluoride, bromide, dihydrophosphate. Then all the samples were detected by iEESI-MS.

Recovery Experiments

For the recovery test, three As(V) samples were spiked with solution with known concentrations (20.0 nM, 60.0 nM, 80.0 nM), respectively. The MS signal ratios of m/z 296/306 of the three mixtures were determined by iEESI-MS method for six times.

ICP-MS

Several standard samples with different concentrations of As (Ar: 74.9) were prepared, includes

66.67 nM, 133.3 nM, 267 nM, 667 nM and 1.33 μM. Rh (Ar: 103) was chosen as IS here. All of the food and water samples used for ICP-MS tests were prepared with same processing steps for iEESI-MS. Then the final test samples were spiked with 200 nM As(V) and concentrated to 500 μL. And all the samples were mixed with same volume of 2% HNO_3. Different from iEESI-MS test, arsenic concentrations were measured using ICP-MS of standard additions that inherently take into account any matrix interferences that could affect the sensitivity for arsenic.

Analysis of food and water samples treatment

Each of the food samples, including starch (1.00 g), agar powder (1.00 g), chitosan (1.00 g), gelatin (1.00 g), carrageenan (1.00 g), were mixed with 0.150 M HNO_3 (10.0 mL) separately for overnight. Afterwards, each of the solution was hot-extracted in a thermostat at 90 °C for 2.5 h, and shook 1 min every half hour. Then the samples were cooled to room temperature and centrifuged at 4000 rpm/min for 5 min. The supernatant (5.00 mL) was filtered and diluted with H_2O_2 to 10.0 mL, followed by heating at 90 °C for 30 min under shaking condition. After cooled, the solution was further centrifuged and filtered for iEESI-MS detection.

Five water samples (from Da Ming Lake, Black Tiger Spring, Yellow River, Qushuiting Street and Tap water) were diluted with 30% H_2O_2 (v/v=1:1). All the samples were shaking at 90 °C for 30 min. After cooling, the obtained solution was centrifuged and filtered, and further used for subsequent iEESI-MS test.

Cultivation of bacteria

The Luria-Bertani broth medium (LB) was prepared according to the ratio of 1.00 g trypsin, 0.500 g yeast extract, and 1.00 g NaCl per 100 mL of water. The LB medium was sterilized before use.[4] E.coli (BNCC17111513) were cultured overnight in LB at 37°C and harvested at the exponential growth phase. The concentration of E.coli was monitored photometrically by OD_{600} (optical density at a wavelength of 600 nm). Before performing bacterial colorimetric detection experiments, the OD_{600} values of bacterial stock solutions were adjusted to 0.5 which corresponded to the concentrations of 5×10^7 CFU/mL for E. coli, obtained based on the gold standard colony counting method.[4, 5] Then the LB bacterial solution was evenly divided into 5 culture flasks, which contained different concentrations of As(V) at 0 mg/L, 0.500 mg/L, 1.00 mg/L, 2.00 mg/L, 5.00 mg/L.45 After 24 h of cultivation, the bacterial cells were collected, and resuspended in PBS. OD_{600} of the 5 culture flasks were recorded. Then all of the cells were ultrasonication and acetonitrile was added. After centrifugation, the arsenic content in bacterial lysate was detected by iEESI-MS with same processes as mentioned above.

Reuse of iEESI-MS

For reuse the iEESI-MS device, the 1.00 mL disposable syringe need to be washed by solvent (V_{MeOH}: V_{H2O}=1:1, pH=6) and the filter should be changed a new one.

Results and Discussion

1. Particle size of magnetic nano-materials

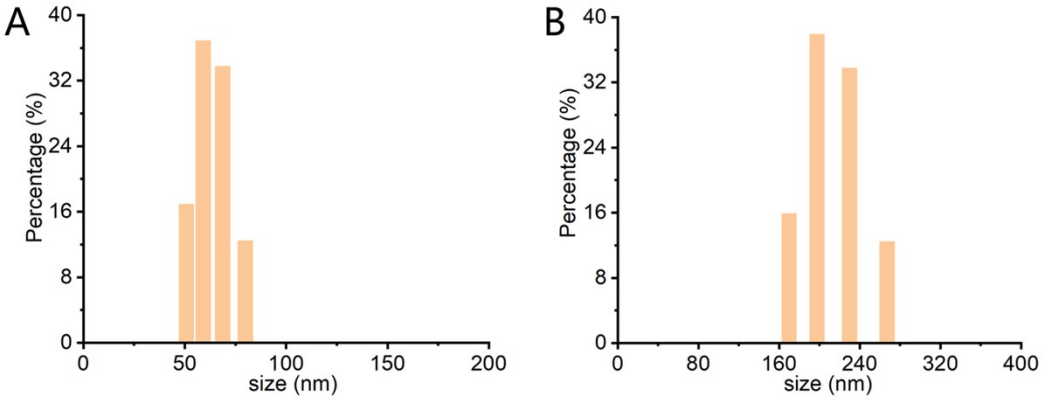

Fig. S1 Particle size distribution of (A) Fe_3O_4 and (B) Fe_3O_4@CeO_2.

2. EDS data of Fe_3O_4@CeO_2

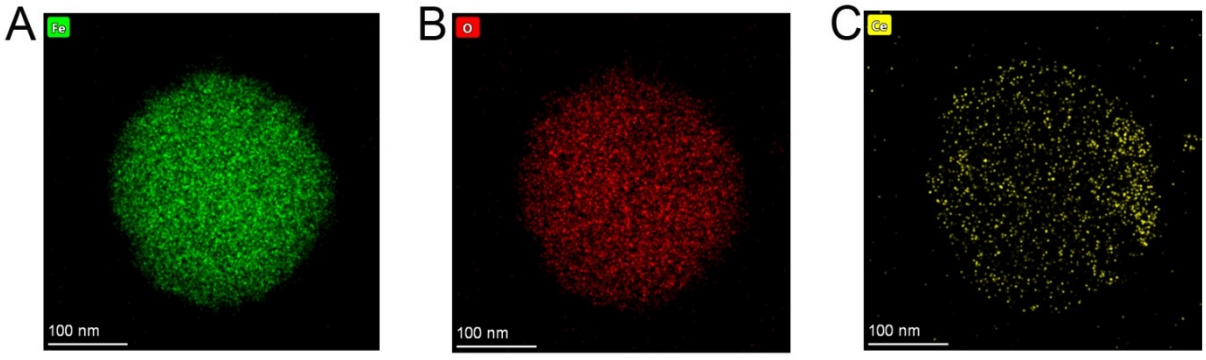

Fig. S2 EDS spectra of (A) Fe, (B) O and (C) Ce elements.

3. Characterization of nanocomposites

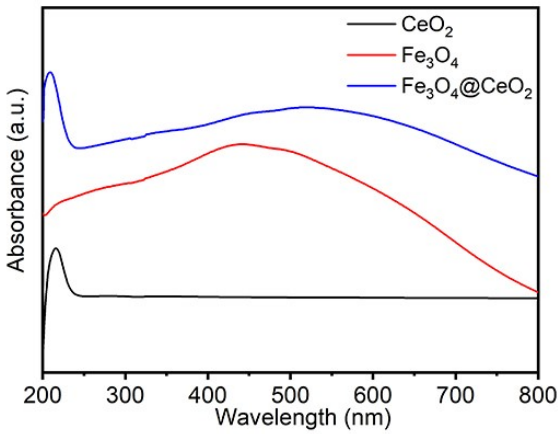

Fig. S3 UV-Vis spectra of CeO_2, Fe_3O_4 and Fe_3O_4@CeO_2.

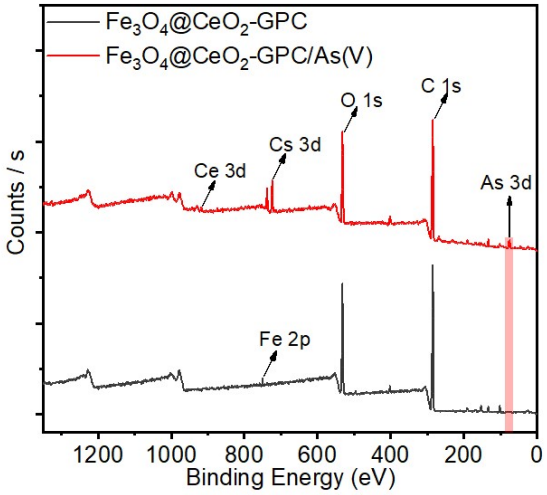

Fig. S4 XPS of Fe_3O_4@CeO_2-GPC and Fe_3O_4@CeO_2-GPC/As(V).

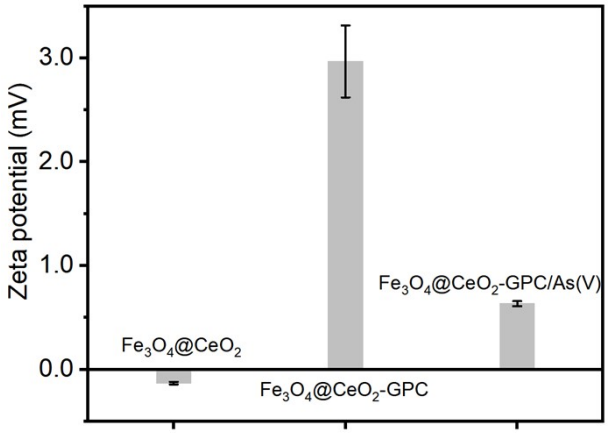

Fig. S5 Zeta potential of Fe_3O_4@CeO_2, Fe_3O_4@CeO_2-GPC and Fe_3O_4@CeO_2-GPC/As(V).

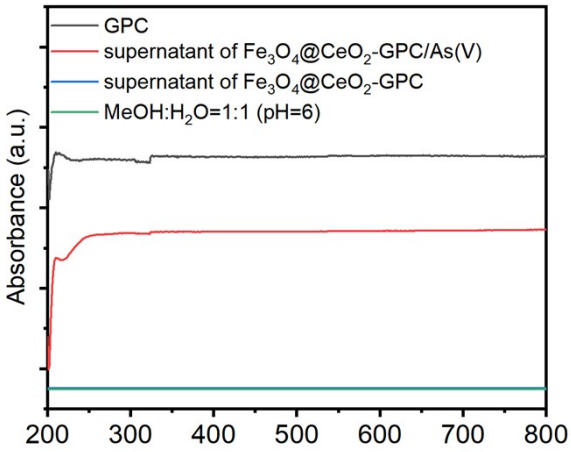

Fig. S6 UV-Vis spectra for GPC only, the supernatant of Fe_3O_4@CeO_2-GPC without As(V), the supernatant of Fe_3O_4@CeO_2-GPC with As(V) addition and solvent only.

4. Optimization of the experimental conditions

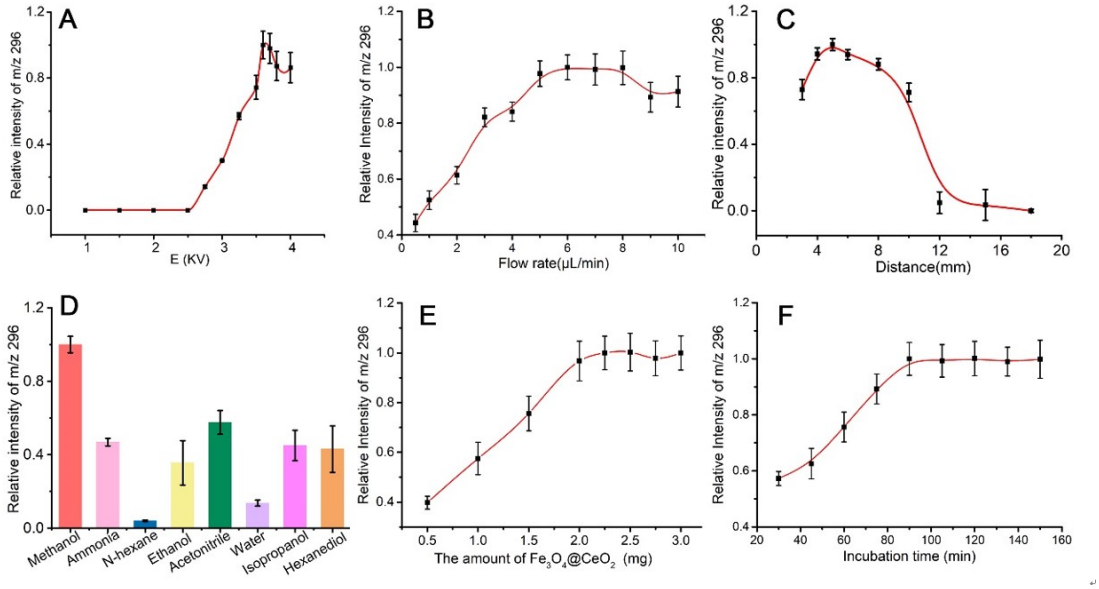

Fig. S7 The optimized experimental conditions of Fe_3O_4@CeO_2-GPC based iEESI-MS for As(V) detection.

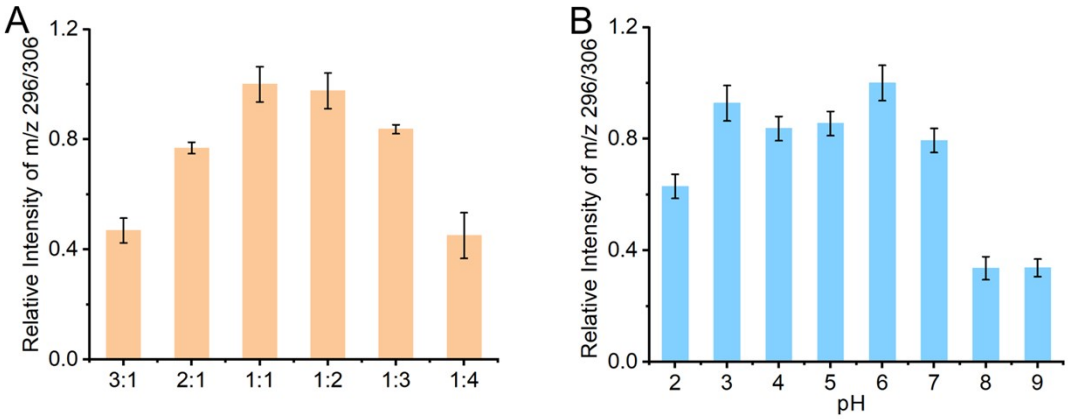

Fig. S8 Optimization of the spray solvent conditions for Fe_3O_4@CeO_2-GPC based iEESI-MS, (A) the different ratios of MeOH to H_2O and (B) the pH.

5. Quantitation of As(V) by iEESI-MS

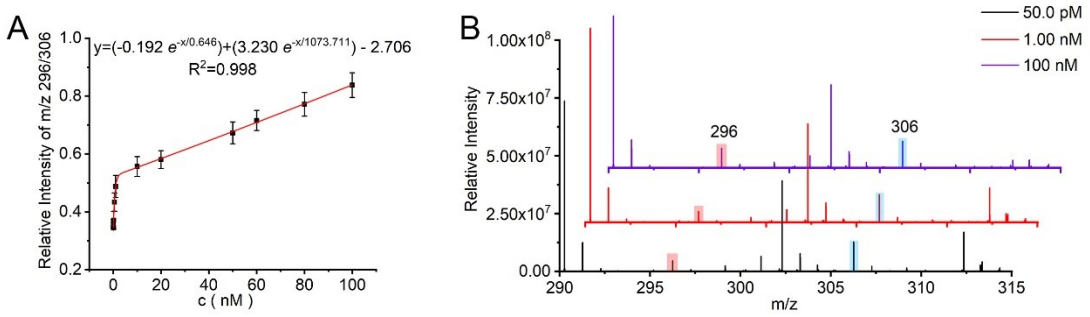

Fig. S9 (A) The standard curve between MS intensity ratio (m/z 296/306) and the concentration of As(V) from 50 pM to 100 nM by iEESI-MS; B. The representive MS spectra in A at 50.0 pM, 1.00 nM and 100 nM.

6. Calculation of LOD

According to the formula LOD=3.143 × S (S represents the standard deviation of 11 measurements,[6] Table S1), the LOD value was 27.22 pM, and the LOQ was 81.66 pM.

Table S1. Calculated the LOD and LOQ value.

Number	peak area ratios of 296/306	Average of peak area ratios of 296/306	SD
1	0.332		
2	0.310		
3	0.308		
4	0.332		
5	0.317		
6	0.318	0.320	8.66
7	0.310		
8	0.326		
9	0.327		
10	0.318		
11	0.324		

7. The selectivity test for As(V) and recovery tests

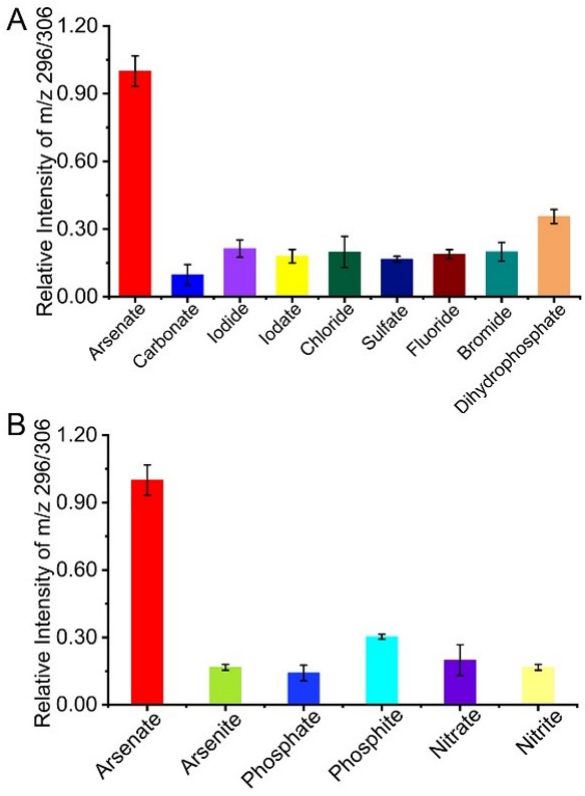

Fig. S10 (A) MS signal ratios of m/z 296/306 with As(V) and other possible interfering anions; (B) MS signal ratio of m/z 296/306, As(V) and other possible interfering anions of VA. (As(V) was 1.00 nM and other were all 100 nM).

Table S2. Recovery tests

Measured (pM)	Add (nM)	Found (nM)	Recovery	Average recovery	RSD (n=6)
27.22	20.0	21.3	1.07	1.05	8.00%
		19.4	0.970		
		19.6	0.980		
		20.8	1.04		
		21.6	1.08		
		23.8	1.19		
	60.0	63.2	1.05	1.02	4.39%
		65.3	1.09		
		58.2	0.970		
		59.1	0.985		
		61.3	1.02		
		60.5	1.01		
	80.0	70.4	0.881	0.977	10.51%
		71.2	0.891		
		75.5	0.942		
		74.3	0.929		
		89.3	1.12		
		88.1	1.10		

8. The standard curve of As(V) detected by ICP-MS

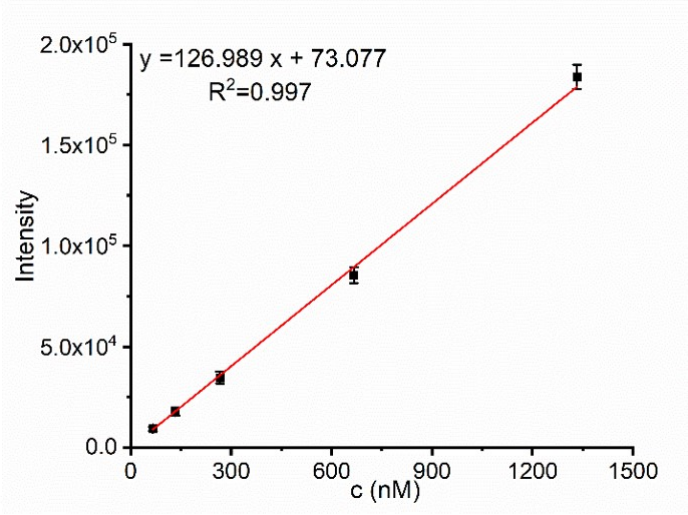

Fig. S11 The standard curve of As(V) detected by ICP-MS.

9. Detection of As(V) in different samples by ICP-MS

Table. S3 Detection of As(V) in food and water samples by ICP-MS.

Sample [a]	Added（nM）	Total found (nM)	As (V)	RSD (%)
Starch	200	237.52	37.52	1.22
Agar powder		232.77	32.77	1.30
Chitosan		206.25	6.25	1.02
Gelatin		228.99	28.99	2.25
Carrageenan		247.71	47.71	2.29
Da Ming Lake		210.01	10.01	1.27
Black Tiger Spring		213.56	13.56	1.26
Yellow River		215.24	15.24	1.18
Qushuiting Street		216.75	16.75	1.19
Tap water		213.76	13.76	1.10

[a] All samples were processed as mentioned in the experimental procedure.

References

1. Gao, Q.; Zhang, J.; Hong, G.; Ni, J., One-Pot Reaction to Synthesize PEG-Coated Hollow Magnetite Nanostructures with Excellent Magnetic Properties. *Journal of Nanoscience and Nanotechnology* **2010,** *10*, 6400–6406.
2. Zhang, J.; Yang, J.; Wang, J.; Ding, H.; Liu, Q.; Schubert, U.; Rui, Y.; Xu, J., Surface oxygen vacancies dominated CeO2 as efficient catalyst for imine synthesis: Influences of different cerium precursors. *Molecular Catalysis* **2017,** *443*, 131–138.
3. Cheng, A.; Tyne, R.; Kwok, Y. T.; Rees, L.; Craig, L.; Lapinee, C.; D'Arcy, M.; Weiss, D. J.; Salaün, P., Investigating Arsenic Contents in Surface and Drinking Water by Voltammetry and the Method of Standard Additions. *Journal of Chemical Education* **2016,** *93* (11), 1945-1950.
4. L″orincz, Á. M.; Szeifert, V.; Bartos, B.; Ligeti, E., New flow cytometry-basedmethod for the assessment of the antibacterial effect of immune cells and subcellular particles. *Journal of Leukocyte Biology* **2018,** *103*, 955–963.
5. Xiao, X.; He, E. J.; Lu, X. R.; Wu, L. J.; Fan, Y. Y.; Yu, H. Q., Evaluation of antibacterial activities of silver nanoparticles on culturability and cell viability of Escherichia coli. *Sci Total Environ* **2021,** *794*, 148765.
6. Yang, Y.; Wang, W.; Liu, H.; Tong, L.; Mu, X.; Chen, Z.; Tang, B., Sensitive Quantification of MicroRNA in Blood through Multi-amplification Toehold-Mediated DNA-Strand-Displacement Paper-Spray Mass Spectrometry (TSD-PS MS). *Angew Chem Int Ed Engl* **2022,** *61* (9), e202113051.