

Electronic Supplementary Information

Silicon nanohybrids-based SERS chip armed with an internal standard for broad-range, sensitive and reproducible simultaneous quantification of lead (II) and mercury (II) in real systems

Yu Shi^{‡,a}, Na Chen^{‡,a}, Yuanyuan Su^a, Houyu Wang^{*,a} and Yao He^{*,a}

^aJiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Institute of Functional Nano and Soft Materials (FUNSOM), Collaborative Innovation Center of Suzhou Nano Science and Technology, Soochow University, Suzhou, Jiangsu 215123, China.

*E-mail: hywang@suda.edu.cn; yaohe@suda.edu.cn

Table of Contents

1. Chemicals, reagents and instruments.....	S3
2. Calculation of SERS enhancement factor (EF).....	S4
3. Calculation of limit of detection (LOD).....	S5
4. SERS spectra of 4-ATP, ROX and FAM in the presence of Pb ²⁺ and Hg ²⁺	S6
5. Raman spectra of 4-ATP on the surface of Si@Ag NPs substrate and silicon wafer.....	S7
6. The linear fitting of the SERS intensities versus the logarithmic heavy metal ions concentration without the correction by IS signals	S8
7. Relativity of the developed SERS chip and ICP-MS.....	S9
8. Comparison of assay methods for monitoring heavy metal ions.....	S10
9. DNA sequences.....	S11
10. Determination of unknown Pb ²⁺ and Hg ²⁺ in real systems by SERS silicon chip and ICP-MS.....	S12
11. References.....	S13

1. Chemicals, reagents and instruments

Chemicals, Reagents. DNA oligonucleotides were synthesized and purified by Sangon Biotechnology (Shanghai, China), whose sequences were listed in Table S2. Hydrofluoric acid (HF, $\geq 40\%$), silver nitrate (AgNO_3 , $\geq 99.8\%$), hydrogen peroxide (H_2O_2 , $\geq 30\%$), sulphuric acid (H_2SO_4 , 98%), 4-Aminothiophenol (4-ATP, $\geq 97.0\%$), lead nitrate ($\text{Pb}(\text{NO}_3)_2$, $\geq 99.0\%$) and Mercury nitrate ($\text{Hg}(\text{NO}_3)_2$, $\geq 99.0\%$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Silicon (100) wafers (phosphate-doped, p-type, 0.01-0.02 Ω sensitivity) were bought from Hefei Kejing Materials Technology Co., Ltd. (Hefei, China). All chemicals were used without additional purification. All solutions were prepared in Milli-Q water (18.2 $\text{M}\Omega \text{ cm}^{-1}$) at room temperature. The real samples of industrial waste water were collected from Suzhou, Jiangsu Province, China.

Instruments. The characterization of the as-prepared SERS chip was conducted by a scanning electron microscopy (SEM) (FEI Quanta 200F), and an atomic force microscopy (AFM) equipped with Multi Mode V AFM (Veeco Corporation, USA). Inductively coupled plasma-mass spectrometry (ICP-MS) (Bruker Aurora M90) was utilized for the determination of heavy metal ions from real water samples. The Raman spectra was collected by a Raman microscope (HR800, Horiba Jobin Yvon, France) equipped with a He-Ne laser (633 nm, 20 mW, polarized 500:1) and a 100 \times objective (NA: 0.9). The collected Raman spectra was further analyzed by the LabSpec5 software. Industry waste water spiked with Pb^{2+} and Hg^{2+} were analysed by the developed silicon SERS chip coupled with a portable hand-held Raman instrument (NanoRam®, B&W Tek, USA) (laser wavelength: 785nm, laser power: 300 mW, laser stability $< 0.5 \text{ cm}^{-1}$, laser line width $< 2.0 \text{ cm}^{-1}$, and spectral resolution: $\sim 9 \text{ cm}^{-1}$).

2. Calculation of SERS enhancement factor (EF)

The EF value of the as-prepared SERS substrate was calculated through the following well-established equation:

$$EF = \frac{I_{SERS} \times N_{bulk}}{I_{bulk} \times N_{SERS}} \quad (1)$$

where, I_{SERS} and I_{bulk} are the intensity of the same Raman band for the SERS and bulk Raman spectra, respectively. N_{bulk} is the number of bulk molecules probed in a bulk sample, and N_{SERS} is the number of molecules adsorbed on the SERS substrate. Thus, for SERS examination, a certain volume V_{SERS} of 4-ATP aqueous solution was dispersed on an area of S_{SERS} at a concentration of C_{SERS} on the Si@Ag NPs substrates. On the contrary, a certain volume V_{bulk} of 4-ATP aqueous solution was dispersed on an area of S_{bulk} at a concentration of C_{bulk} on the clean silicon substrate. Both the substrates were dried in air. Foregoing equation thus comes:

$$EF = \frac{I_{SERS} \times N_{bulk}}{I_{bulk} \times N_{SERS}} \quad (2)$$

For objective calculation, Raman measurements were conducted under identical experimental conditions (laser wavelength, laser power, microscope objective/lenses, spectrometer, etc.).

Typically, 10 μ L of 10^{-4} M 4-ATP aqueous solution was dispersed on the clean silicon wafer, Si@Ag NPs substrate. The intensities of 1079 cm^{-1} were ~ 100 for the clean silicon wafer, ~ 12126 for the Si@Ag NPs substrate, respectively. All probe molecules within the laser spot were assumed to contribute to SERS signals. Based on the equation (2), the average EF values were calculated to be $\sim 2.0 \times 10^6$ for Si@Ag NPs.

3. Calculation of limit of detection (LOD)

The LOD was estimated without $\text{Pb}^{2+}/\text{Hg}^{2+}$ giving SERS signal at least three times higher than background (distilled water). The standard curve of $\text{Pb}^{2+}/\text{Hg}^{2+}$ was plotted as

$$Y = A + B \times \text{Log}_{10} X \quad (3)$$

where, A and B are the variable obtained via least-square root linear regression for the signal-concentration curve and variable Y represents the normalized SERS signal ($I_{\text{withPb}^{2+}} / I_{\text{IS}}$) at Pb^{2+} concentration of X ($C_{\text{Pb}^{2+}}$) or ($I_{\text{withHg}^{2+}} / I_{\text{IS}}$) at Hg^{2+} concentration of X ($C_{\text{Hg}^{2+}}$)

$$\text{When } Y = Y_{\text{blank}} + 3SD \quad (4)$$

where, SD is the standard deviation and Y_{blank} is the SERS signal of blank sample (distilled water).

The LOD is calculated as

$$LOD = 10^{[(Y_{\text{blank}} + 3SD)/Y_{\text{blank}} - A]/B} \quad (5)$$

SD is calculated according to the well-known formula:

$$SD = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^n (X_i - X_{\text{average}})^2} \quad (6)$$

where, n is the total number of the $\text{Pb}^{2+}/\text{Hg}^{2+}$ standard sample. X_i is the “ i ” sample of the series of measurements. X_{average} is the average value of the SERS signals obtained for the specific series of identical samples repeated n times.

4. SERS spectra of 4-ATP, ROX and FAM in the presence of Pb^{2+} and Hg^{2+}

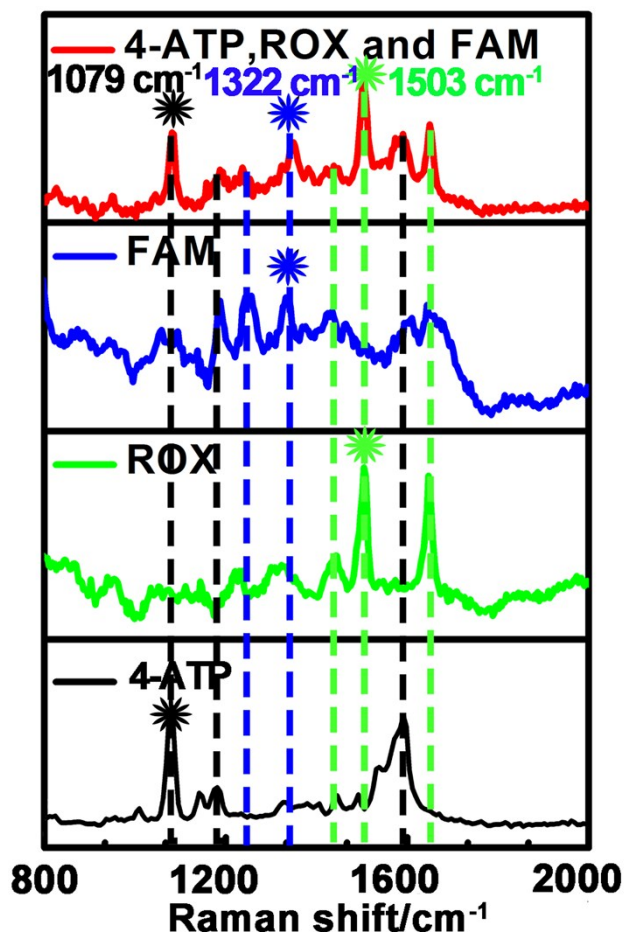


Figure S1. SERS spectra of 4-ATP, ROX and FAM in the presence of Pb^{2+} and Hg^{2+} . The three identifying bands from ROX, FAM, and 4-ATP (Internal Standard) are marked on the spectrum (multiplex detection of red line; FAM of blue line; ROX of green line; 4-ATP of black line).

As shown in Figure S1, in the coexistence of Pb^{2+} and Hg^{2+} , three typical Raman peaks of 1079 cm^{-1} , 1503 cm^{-1} and 1322 cm^{-1} respectively assigned to 4-ATP, ROX and FAM can be clearly observed without mutual interference.

5. Raman spectra of 4-ATP from the surface of Si@Ag NPs substrate and free silicon wafer

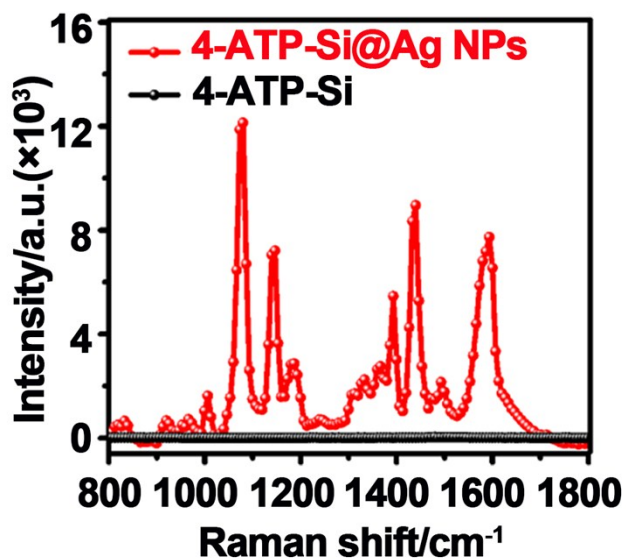


Figure S2. Raman spectra of 4-ATP from the surface of Si@Ag NPs substrate and free silicon wafer.

Raman signals in IS-Si@Ag NPs are clearly observed after the modification of 10^{-4} M 4-ATP (red line); comparatively, Raman signals in naked silicon wafer are undetectable under the same condition (black line).

6. The linear fitting of the SERS intensities versus the logarithmic heavy metal ions concentration without the correction by IS signals

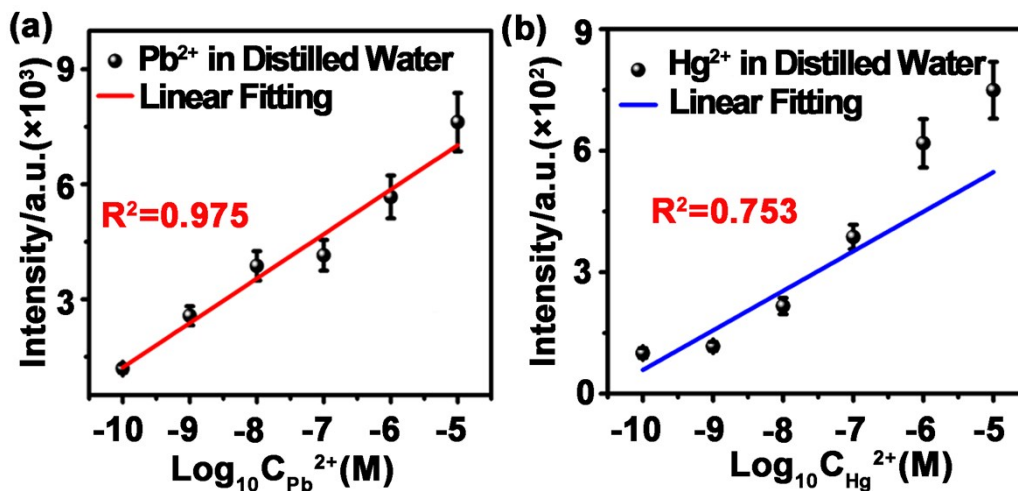


Figure S3. The linear fitting of the SERS intensities versus the logarithmic Pb²⁺ (a) and Hg²⁺ (b) concentration 1.0×10^{-10} to 1.0×10^{-5} M without the correction by IS signals.

Figure S3a shows the poorly linear relationship between the logarithmic concentration of Pb²⁺ and the SERS intensity of ROX at 1503 cm⁻¹, with a low correlation coefficient R^2 of 0.975. As shown in Figure S3b, a poor linearity exists between the SERS intensity of FAM at 1322 cm⁻¹ and the logarithmic concentrations of Hg²⁺, with a low correlation coefficient R^2 of 0.753.

7. Relativity of the developed SERS chip and ICP-MS

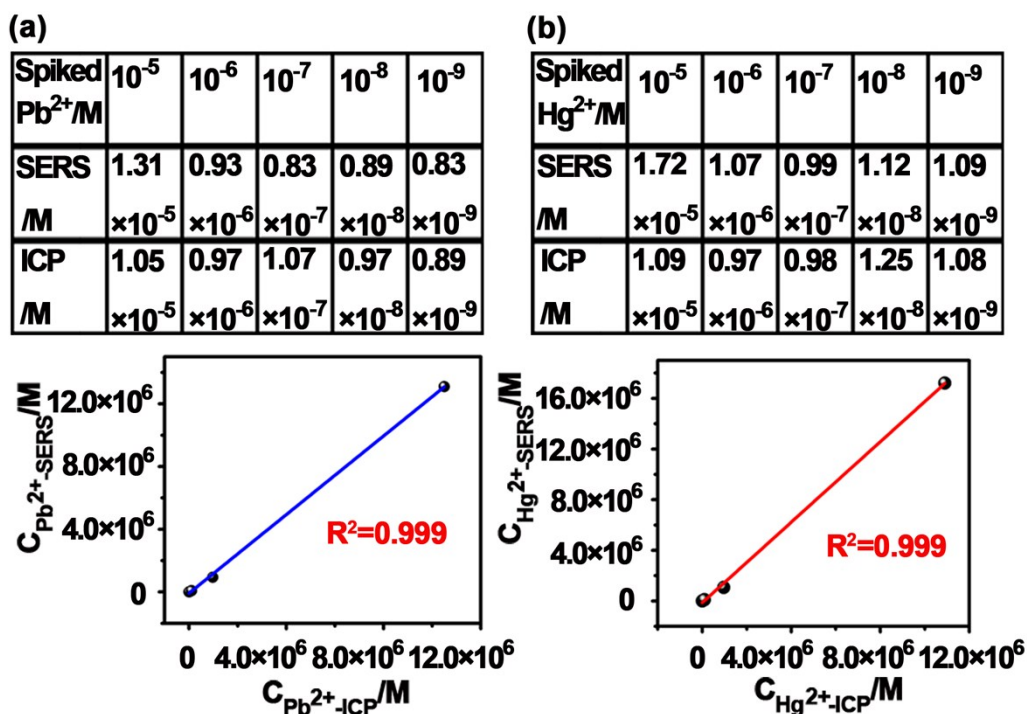


Figure S4. Correlation of Pb^{2+} and Hg^{2+} concentrations determined by our SERS silicon chip and the classic ICP-MS method.

According to the linear fitting, there is a good correlation between the SERS chip and ICP-MS, with the correlation coefficient R^2 of 0.999, indicating the Pb^{2+} and Hg^{2+} concentrations detected by the SERS chip were in good agreement with those determined by the ICP-MS strategy. For clear comparison, the values of Pb^{2+} and Hg^{2+} concentrations obtained from the standard addition analysis, determined by the ICP-MS and SERS methods are listed in the inset table.

8. Comparison of assay method for monitoring heavy metal ions

Table S1. Comparison of assay method for monitoring heavy metal ions.

Methods	Target ions	Linear range	Limit of detection	Internal standard	Ref.
Colorimetric	Hg(II)/Ag(I)/Cu(II)	0-200 μ M/0-200 μ M/0-200 μ M	0.19 μ M/1.69 μ M/1.40 μ M	No	1
Electrochemical method	Pb(II)/Ag(I)/Hg(II)	10pM-10 μ M/100nM-800nM/0.1nM-10 μ M	10pM/10nM/0.1nM	No	2
Electrochemical method	Pb(II)/Hg(II)	30pM-1 μ M /500pM-1 μ M	10pM/0.2nM	No	3
Electrochemical method	Pb(II)/Ba(II)	1nM-1 μ M/1nM-1 μ M	0.8nM/0.8nM	No	4
Fluorescence	Pb(II)/Hg(II)	0.5-30nM/10-200nM	300pM/5.0nM	No	5
Fluorescence	Hg(II)/Pb(II)	10pM-1 μ M/10 pM-1 μ M	51pM/27pM	No	6
Fluorescence	Cd(II)/Hg(II)/Pb(II)	0-1000nM/0-500nM/0-1000nM	4.8nM/2.0nM/0.1nM	No	7
Fluorescence	Hg(II)/Ag(I)	2.0nM-60 μ M/5.0nM-80 μ M	0.68nM/1.73nM	No	8
Fluorescence	Hg(II)/Cu(II)	0.2-2500nM/0.5-2500nM	0.1nM/0.3nM	No	9
SERS	Co(II)/Cu(II)	0-0.1 μ M/0-30 μ M	0.2nM/30nM	No	10
SERS	Hg(II)/Ag(I)	0.1-1000nM/10-1000nM	0.1 nM/1nM	No	11
SERS	Hg(II)/Ag(I)	6pM-1.5 nM/11pM-3nM	7.5pM/12.5pM	No	12
SERS	Pb(II)/Hg(II)	100pM-10 μ M/1nM-10 μ M	99.6pM/0.84nM	Yes	This work

9. DNA sequences

Table S2. DNA sequences.

DNA Name	Sequence (5'-3')
HS-B1-FAM	HS-(CH ₂) ₆ -TTCTTTCTTCCCCTTGTTTGTT-FAM
HS-17E-ROX	HS-(CH ₂) ₆ - TTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-ROX
17DS	ACTCACTATrAGGAAGAGATG

10. Determination of unknown Pb²⁺ and Hg²⁺ in real systems by SERS silicon chip and ICP-MS

Table S3. Determination of unknown Pb²⁺ and Hg²⁺ in real systems by SERS silicon chip and ICP-MS

Samples	Spiked Concentration (M)		SERS found (M)		Recovery (%)		ICP-MS found (M)	
	Pb ²⁺	Hg ²⁺	Pb ²⁺	Hg ²⁺	Pb ²⁺	Hg ²⁺	Pb ²⁺	Hg ²⁺
Industry waste water	0	0	1.12 × 10 ⁻⁶	1.01 × 10 ⁻⁶	-	-	0.97 × 10 ⁻⁶	0.94 × 10 ⁻⁶
	0	1 × 10 ⁻⁷	-	1.11 × 10 ⁻⁶	-	99.1	-	1.03 × 10 ⁻⁶
	1 × 10 ⁻⁷	0	1.22 × 10 ⁻⁶	-	99.5	-	1.05 × 10 ⁻⁶	-
	1 × 10 ⁻⁷	1 × 10 ⁻⁷	1.21 × 10 ⁻⁶	1.09 × 10 ⁻⁶	99.2	98.2	1.20 × 10 ⁻⁶	1.09 × 10 ⁻⁶

11. References

1. L. Liu and H. Lin, *Anal. Chem.*, 2014, **86**, 8829-8834.
2. Z. Lin, X. Li and H. B. Kraatz, *Anal. Chem.*, 2011, **83**, 6896-6901.
3. M. Zhang, L. Ge, S. Ge, M. Yan, J. Yu, J. Huang and S. Liu, *Biosens. Bioelectron.*, 2013, **41**, 544-550.
4. C. Yang, L. Liu, T. Zeng, D. Yang, Z. Yao, Y. Zhao and H. C. Wu, *Anal. Chem.*, 2013, **85**, 7302-7307.
5. C. W. Liu, C. C. Huang and H. T. Chang, *Anal. Chem.*, 2009, **81**, 2383-2387.
6. C. H. Chung, J. H. Kim, J. Jung and B. H. Chung, *Biosens. Bioelectron.*, 2013, **41**, 827-832.
7. P. J. J. Huang and J. Liu, *Anal. Chem.*, 2014, **86**, 5999-6005.
8. Z. X. Wang and S. N. Ding, *Anal. Chem.*, 2014, **86**, 7436-7445.
9. N. Zhang, Y. Si, Z. Sun, L. Chen, R. Li, Y. Qiao and H. Wang, *Anal. Chem.*, 2014, **86**, 11714-11721.
10. D. Tsoutsis, L. Guerrini, J. M. Hermida-Ramon, V. Giannini, L. M. Liz-Marzan, A. Wei and R. A. Alvarez-Puebla, *Nanoscale*, 2013, **5**, 5841-5846.
11. M. Liu, Z. Wang, S. Zong, H. Chen, D. Zhu, L. Wu, G. Hu and Y. Cui, *ACS Appl. Mater. Interfaces*, 2014, **6**, 7371-7379.
12. S. Li, L. Xu, W. Ma, H. Kuang, L. Wang and C. Xu, *Small*, 2015, **11**, 3435-3439.