

Analytical Methods

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A table of contents entry

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- Text: one sentence, of maximum 20 words, highlighting the novelty of the work.

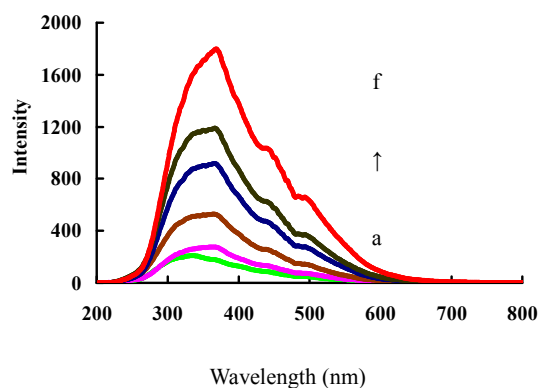


Figure 1 RRS spectra of the SeH₂-Cu(II)-tartrate system

a: 0.4 mmol/L CuSO₄ -0.0492 mol/L KNaC₄H₄O₆-0.46 mol/L H₂SO₄-36 mg/mL NaBH₄; b: a+0.025 mg/L Se;
c: a+0.25 mg/L Se; d: a+0.5 mg/L Se; e: a+0.75 mg/L Se; f: a+1.5 mg/L Se.

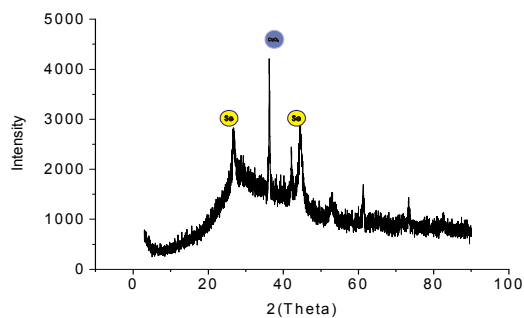


Fig. 2 X-ray diffraction graphs for the Se-Cu₂O

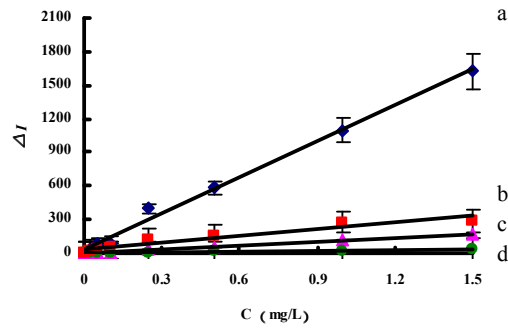


Fig. 3 Working curve

a: 374nm (F-7000); b: 405nm (F95S); c: 465nm (F95S); d: 515nm (F95S).

Highlighting the novelty of the work:

A 3.3 ng/mL Se can be determined by RRS, based on SeH_2 reducing Cu(II) to produce $\text{Cu}_2\text{O-Se}$ particles.

A novel hydride generation spectral method for trace Se based on resonance Rayleigh scattering of Cu₂O-Se nanoparticles

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Se(IV) was reduced by NaBH₄ to form SeH₂ gas that reduce Cu(II) to produce Cu₂O-Se particles with a strong resonance Rayleigh scattering (RRS) peak at 374 nm. The RRS intensity was linear to the Se(IV) concentration from 0.008 to 1.5 mg/L, with a detection limit of 3.3 μg/L.

Se is an essential trace element in human body. In nature, the main existence forms are +4, +6, 0 and -2^[1]. Se cannot be synthesized by the body, and it must be absorbed from the outside. In recent years, epidemiological studies have shown that adequate intake of Se is beneficial to humans that can improve the ability of anticancer^[2-4]. But when the Se intake overtops 400 μg/d, it will be harmful to human body. Reverse, a lower Se (50 μg/d) was ingested long time that is also harmful to human body^[5, 6]. Thus, it is important to set up a simple, sensitive, economic and rapid method to detect Se.

At present, several methods have been reported to detect Se, including fluorescence spectrometry^[7-9], atomic absorption spectrometry^[10-12], chromatography-mass spectrometry^[13-15], ICP-mass spectrometry^[16], and electrochemical method^[17-19]. Atomic spectroscopy has the advantages of high sensitivity and good selectivity, but the substrate can influence the determination of Se in complex samples. Hydride generation (HG) technology can separate efficiently the analyte from substrate, with high selectivity and strong enrichment effect, it has been coupled with several detection techniques including atomic spectroscopy^[20-26]. The HG-atomic spectrometry for Se has the advantages of high sensitivity and high selectivity. Mendez combined HG with atomic absorption spectrometry and atomic fluorescence to determine the content of selenium in the samples^[1], with high sensitivity and selectivity, but the equipment cost is high^[10]. Resonance Rayleigh scattering (RRS) possesses some advantages including simplicity and rapidity, high sensitivity and low-cost using simple fluorescence meter such as model F95S, and has been employed for the analysis of inorganic and organic compounds^[27, 28], with good results. The RRS method for Se has been reported by Liu research group^[29] that combined iodide with cation dye such as Victoria blue 4R to determine Se as low as ng/mL. The sensitivity is high, and the Se should be pre-separated from the complicated samples. To improve the selectivity, highly selective reactions such as immuno-reaction and aptamer reaction have been utilized in RRS analysis^[30-32]. However, the substrate interference exists still and the analyte should be separated from the samples. To our knowledge, there is no report about HG-RRS method for Se, combined organically

the hydride nanoreaction with the separation technique. In this work, NaBH₄ reduced Se(IV) to hydrogen selenide that reacted with Cu²⁺ in the absorption solution to form Cu₂O-Se particles with resonance Rayleigh scattering effect. Thus, a new HG spectral method was developed for sensitive detection of Se.

In H₂SO₄ medium, NaBH₄ is a strong reducing agent that generate new ecological hydrogen [H] to reduce Se(IV) to form SeH₂ (Figure 1S). Using CuSO₄-potassium sodium tartrate (KNaC₄H₄O₆) as absorption solution, Cu²⁺ was reduced by SeH₂ to form particles that resulted in the RRS intensity increasing. Owing to the potential of Se⁴⁺ being higher than the Cu²⁺ (equation 5 and 7)^[1], there is no Se⁴⁺ in the system, and the products include Cu₂O and Se particles, that is, Cu₂O-Se composite particles. The Se(IV) in sample has two contributions in enhancement of RRS intensity. One hand, the generated SeH₂ reduced Cu(II) to the Cu₂O particles that enhanced the RRS intensity. Other hand, the SeH₂ was oxidized to the Se particles that also enhanced the RRS intensity. When Se(IV) concentration increased, the RRS intensity increased duo to more Cu₂O-Se particles forming. Thus, a new and sensitive RRS method for the detection of Se(IV) was set up. The main reactions are as follows,

$$\text{NaBH}_4 + \text{H}^+ + 3\text{H}_2\text{O} \rightarrow 8[\text{H}] + \text{Na}^+ + \text{H}_3\text{BO}_3 \quad (1)$$

$$\text{Se}^{4+} + [\text{H}] \rightarrow \text{Se}^0 \quad (2)$$

$$\text{Se}^0 + [\text{H}] \rightarrow \text{SeH}_2 \uparrow \quad (3)$$

$$2\text{Cu}^{2+} + \text{SeH}_2 + \text{H}_2\text{O} \rightarrow (\text{Cu}_2\text{O} + \text{Se}) + 4\text{H}^+ \quad (4)$$

$$\text{HSeO}_3^- + 6\text{e}^- + 7\text{H}^+ \leftrightarrow \text{H}_2\text{Se} + 3\text{H}_2\text{O} \quad 0.386 \text{ V} \quad (5)$$

$$\text{Se} + 2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2\text{Se} \quad -0.369 \text{ V} \quad (6)$$

$$\text{Cu}^{2+} + \text{e}^- \leftrightarrow \text{Cu}^+ \quad 0.153 \text{ V} \quad (7)$$

The RRS spectra of Cu₂O-Se particles showed that it has a strong RRS peak at 374 nm (Figure 1). The RRS intensity $I_{374\text{nm}}$ increased linearly with the concentration of Se(IV) increased, and it was chosen for detection of Se(IV). The RRS spectra of SeH₂-tartrate system have two RRS peaks at 283 nm and 375 nm (See, Fig. 2S). The two peaks are ascribed to the maximal emission of the lamp. The blank value is big that it is owing to the scattering of other particles such as H₂O molecules and the light source of equipment is very strong. The signals increased with the increase of Se(IV) concentration that indicated Se particles forming from the SeH₂ decomposed. Although the sensitivity of SeH₂-tartrate system is also high, the accuracy and linear relationship between the RRS intensity and Se concentration are all bad. There are Se particles in the Se(IV) absorption solution system because the Se(IV) was reduced by SeH₂ to form Se (Fig. 3S). This is also demonstrated that there are Se particles in the SeH₂-Cu(II)-tartrate system. The Cu₂O particle RRS spectra in the glucose-Fehling reagent-Au nanoparticle catalytic system obtained as in Figure 4S. There are two RRS peaks at 365 nm and about 525 nm. It is different with the RRS spectra of Cu₂O-Se nanoparticles

owing to the influence of particle size and shape, lamp and free molecular absorption.

According to the procedure, the sample solution was obtained, then taken 1.0 mL sample solution in a 1.5 mL centrifuge tube, and centrifuged for 30min at 12000 rpm, and the supernatant discarded. Then, added 1.0 mL water in the centrifuge tube, and dispersed with ultrasonic for 30 min, and the process was repeated 2 times. After that, 10 μ L the solution was dropped on the silicon wafer and made it dry naturally. The samples were put in a scanning electron microscope to obtain the scanning electron micrograph (Fig. 5S). The size of Cu₂O-Se composite particles distributed from 40 nm to 150 nm, with an average size of 80 nm for the SeH₂-CuSO₄-KNaC₄H₄O₆ absorption system.

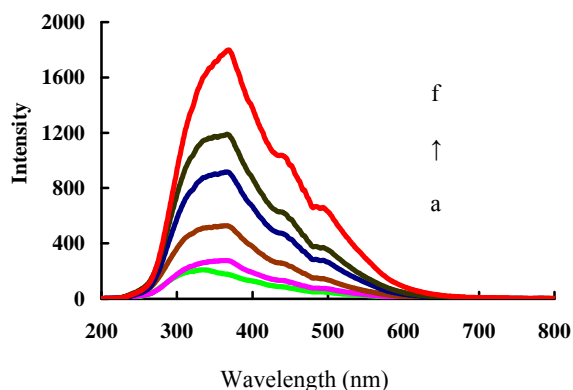


Figure 1 RRS spectra of the SeH₂-Cu(II)-tartrate system a: 0.4 mmol/L CuSO₄-0.0492 mol/L KNaC₄H₄O₆-0.46 mol/L H₂SO₄-36 mg/mL NaBH₄; b: a+0.025 mg/L Se; c: a+0.25 mg/L Se; d: a+0.5 mg/L Se; e: a+0.75 mg/L Se; f: a+1.5 mg/L Se.

According to the procedure, we obtained the reaction solution, then centrifuged and dried the solution, and it was used to do X-ray diffraction with a scanning range of 0.0-90.0°(2 θ). The Figure 2 showed that there are Cu₂O-Se mixed-crystals with five diffraction peaks at 36.2°, 42.1°, 52.9°, 61.2° and 73.3° being ascribed to Cu₂O^[32], and two peaks at 26.4° and 45.02° being ascribed to Se^[33]. The strongest peak at 36.2° indicated the content of Cu₂O is high.

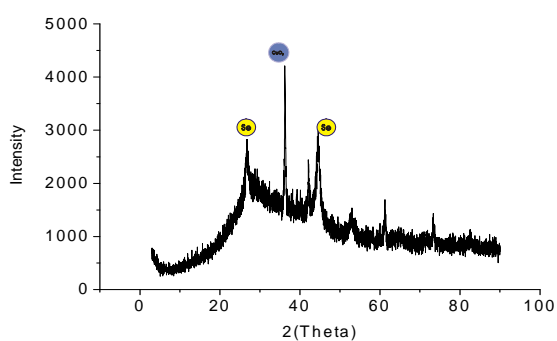


Fig. 2 X-ray diffraction graphs for the Se-Cu₂O

There is a maximal absorption peak at 300 nm for the Cu(II)-tartrate complex. When the Se (IV) was added, the Cu²⁺ was reduced by the generated SeH₂ to form Cu₂O-Se particles that also has a strong surface plasmon absorption peak at 300 nm (Fig. 6S). The peak increased with the concentration of Se (IV)

increased due to more particles forming. The increased value is linear to Se(IV) concentration in the range of 0.1-1.5 mg/L, with a regression equation is $\Delta A_{300nm} = 0.0585C + 0.0037$. However, the sensitivity is low.

Effect of H₂SO₄ concentration was considered. The ΔI_{374nm} increased as the H₂SO₄ concentration increased from 0 to 1.15 mol/L. When the H₂SO₄ concentration was 0.46 mol/L, the ΔI_{374nm} was maximal. Thereafter the ΔI_{374nm} was stabilized gradually with the H₂SO₄ concentration increased (Fig. 7S). Thus, a 0.46 mol/L H₂SO₄ was chosen for use. The ΔI_{374nm} increased as the NaBH₄ concentration is from 18 to 36 mg/mL. When the NaBH₄ concentration was 36 mg/mL, the ΔI_{374nm} was maximal. Thereafter the ΔI_{374nm} decreased with the NaBH₄ concentration increased (Fig. 8S). Therefore, a 36 mg/mL NaBH₄ was selected. The effect of CuSO₄ concentration was tested (Fig. 9S). The ΔI_{374nm} increased with the CuSO₄ concentration in the range of 0-0.4 mmol/L due to formation of more Cu₂O nano-particles. When the CuSO₄ concentration was 0.4 mmol/L, the ΔI_{374nm} was maximal due to formation of most Cu₂O nano-particles. Thereafter the ΔI_{374nm} decreased with the CuSO₄ concentration increased slowly, owing to the Cu₂O nano-particles hold constant and the free molecular absorption of Cu(II) enhanced that caused the intensity decreased. Therefore, a 0.4 mmol/L CuSO₄ was selected. Effect of KNaC₄H₄O₆ concentration (0-0.0984 mol/L) was considered, and the ΔI_{374nm} gradually increased as the KNaC₄H₄O₆ concentration increased. When the concentration was 0.0492 mol/L, the ΔI_{374nm} was maximal (Fig. 10S). Thus, a 0.0492 mol/L KNaC₄H₄O₆ was chosen.

According to the procedure, the influences of coexistent substances (CES) on the detection of 0.5 mg/L Se (6.33 μ mol/L Se) were examined, with a relative error of $\pm 10\%$. The Hg²⁺, Cu²⁺, Ni²⁺, Co²⁺ and Zn²⁺ have interferences, because of they generated sediment when adding NaBH₄ made hydrogen selenide capture or decomposition^[26]. Therefore, a 100 μ L 1% K₃Fe(CN)₆ was added in hydride generation reaction solution to eliminate the disturbance of those metal ions (Table 1S).

Under the optimal conditions, the working curves between Se concentration (mg/L) and the ΔI_{374nm} was drawn (Fig. 3). The ΔI_{374nm} value is proportional to the Se concentration (C) in the range of 0.008 to 1.5 mg/L in 20 mL of hydride reaction solution, with a regression equations of $\Delta I_{374nm} = 1070.3 C + 10.7$, a correlation coefficient of 0.9946, and a detection limit of 3.3 ng/mL Se. The reproducibility of the CuSO₄-KNaC₄H₄O₆ system was considered. The I_{374nm} of three concentrations of 0 mg/L, 0.5 mg/L, 0.75 mg/L Se(IV) was examined, and the RSD was 4.3%, 1.2% and 4.7% respectively. The RRS intensity was recorded on a very low-cost fluorescence spectrophotometer (model of F95S) for CuSO₄-KNaC₄H₄O₆ absorption solution, and the working curves were drawn (Fig. 3). The ΔI_{405nm} , ΔI_{465nm} and ΔI_{515nm} values are all proportional to the Se concentration (C) in the range of 0.025 to 1.5 mg/L. The results showed that the F-7000 fluorescence spectrophotometer has high sensitivity, but the instrument is expensive (about 220,000 Yuan). While the sensitivity of F95S fluorescence spectrophotometer is low, their working curves are good and the price is very low (about 16,000 Yuan).

According to the procedure, a 5.0 mL tea sample solution was taken to the reaction bottle to determine the Se content. Then a 100 μ L 0.1 g/L Se standard solution was added in the tea sample

to obtain the recovery. The results showed that the relative standard deviations were in the range of 3.5%-7.8%, the recoveries were in the range of 99.4%-109.7% (Table 2S).

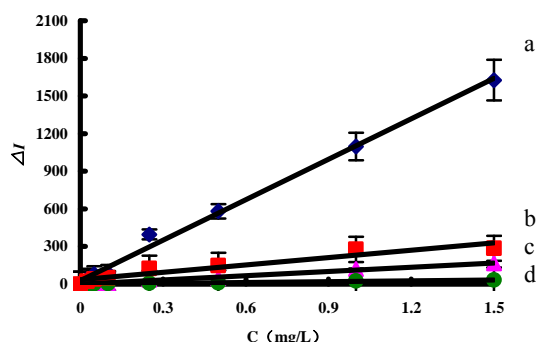


Fig. 3 Working curve
a: 374nm (F-7000); b: 405nm (F95S); c: 465nm (F95S); d:
515nm (F95S).

In summary, in 0.46 mol/L H_2SO_4 medium, the Se(IV) was reduced by $NaBH_4$ to form SeH_2 that was trapped by $CuSO_4-KNaC_4H_4O_4$ absorption solution. In which, the Cu^{2+} was reduced by SeH_2 to form Cu_2O-Se particles that RRS spectra were studied in details. Thereby a new HG-RRS method was developed to detect the trace amounts of Se, with high sensitivity, good selectivity, simplicity, and low-cost.

Acknowledgements

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