

LABEL-FREE SERS-4,4'-DIPYRIDINE PROBE FOR THE DETECTION OF Hg²⁺ IN A COMPLEX SYSTEM OF MEDICAL INJECTION ****Hui Zhang^{1*}, Baoling Wang¹, Xiaoyi Liu¹, Hongxu Zhang¹, Jizhong Yan¹**

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A method was reported regarding a novel surface-enhanced Raman scattering probe to detect and quantify Hg²⁺ with reasonable specificity and selectivity. Highly selective and sensitive detection of Hg²⁺ in traditional Chinese medicine preparations was achieved by using silver nanoparticles as Raman substrate and 4,4'-dipyridine (Dpy) as the signal probe with the assistance of the polymerizing agent sodium chloride. Here, the 4,4'-dipyridine molecule was adsorbed on the surface of silver nanoparticles through Ag-N. Then, sodium chloride was supplemented to induce aggregation of silver nanoparticles, resulting in a significant enhancement of its signal. The presence of Hg²⁺ could make the 4,4'-dipyridine fall off from the surface of silver nanoparticles and reduce the signal. It showed an enviable detection speed with 10 ng/mL sensitivity. A good linear relationship between Raman spectral signal and Hg²⁺ concentration 50–1000 ng/mL ($R^2 = 0.9884$) was observed. The recoveries ranged from 97.47 to 100.07% for the detection of Qingkailing injection. The results indicated that the surface-enhanced Raman scattering-based 4,4'-Dipyridine probe method for detecting heavy metals could eliminate the interference of other small molecules in the complex system and perform significantly higher detection sensitivity than the pharmacopeia standard.

Keywords: heavy metal ion, Hg²⁺, surface-enhanced Raman scattering, silver nanoparticles, traditional Chinese medicine.

ЗОНД НА ОСНОВЕ ПОВЕРХНОСТНО-УСИЛЕННОГО КОМБИНАЦИОННОГО РАССЕЯНИЯ СВЕТА 4,4'-ДИПИРИДИНА БЕЗ МЕТКИ ДЛЯ ОБНАРУЖЕНИЯ ИОНОВ Hg²⁺ В СЛОЖНОЙ СИСТЕМЕ ДЛЯ МЕДИЦИНСКИХ ИНЪЕКЦИЙ**Hui Zhang^{*}, B. Wang, X. Liu, H. Zhang, J. Yan^{*}**

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Разработан зонд на основе поверхностно-усиленного комбинационного рассеяния для обнаружения и количественного определения ионов Hg²⁺. Высокоселективное и чувствительное обнаружение Hg²⁺ в препаратах традиционной китайской медицины достигнуто за счет использования наночастиц серебра в качестве КР-субстрата и 4,4'-дипиридина (Дру) в качестве сигнального зонда с помощью полимеризующего агента хлорида натрия. Молекула Дру адсорбируется на поверхности наночастиц серебра через Ag-N, для агрегации наночастиц серебра добавляется хлорид натрия, что приводит к значительному усилению его сигнала. Присутствие ионов Hg²⁺ может привести к разрыву между Дру и поверхностью наночастиц серебра и ослаблению сигнала. Метод показывает высокую скорость обнаружения с чувствительностью 10 нг/мл. Наблюдается линейная зависимость

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между спектральным сигналом комбинационного рассеяния света и концентрацией Hg^{2+} в диапазоне 50–1000 нг/мл ($R^2 = 0.9884$). Извлечение для обнаружения инъекции Цинкайлин 97.47–100.07%. Использование 4,4-дипиридинового КР-зонда для обнаружения тяжелых металлов может устранить вмешательство других малых молекул в сложную систему и обеспечить более высокую чувствительность обнаружения, чем фармакопейный стандарт.

Ключевые слова: ион тяжелого металла, ионы Hg^{2+} , поверхностно-усиленное комбинационное рассеяние света, наночастицы серебра, традиционная китайская медицина.

Introduction. Traditional Chinese medicine (TCM), as a unique medicine in China, plays an influential role in preventing and treating diseases. It makes outstanding contributions to the development of society and the progress of medical civilization [1, 2]. With the wide application of TCM, people are increasingly concerned about the safety of TCM, which has seriously hindered its development [3]. The residue of heavy metals and harmful elements are the crucial factors that can influence the safety and effectiveness of TCM. Heavy metals are non-biodegradable and have the characteristics of bio-accumulation. They enter the atmosphere, water, and soil and finally impose a threat to human health [4, 5]. The mercury ion (Hg^{2+}), one of the most toxic liquid heavy metal ions, can cause permanent damage to the brain and kidneys even at a low concentration [6, 7]. Thus, it is of high importance to develop a sensitive and rapid method of detecting Hg^{2+} .

Currently, numerous efficient analytical methods have been used to detect trace amounts of heavy metals in TCM. Including inductively coupled plasma mass spectrometry (ICP-MS) [8–10], atomic absorption spectrometry (AAS) [11–13], electrochemical methods [14], colorimetry/SERS [15], and fluorescence spectrometry [16]. However, most of these approaches still face some limitations, such as tedious sample preparation, non-environmentally friendly reagents, and expensive testing equipment unsuitable for field analysis [17]. In addition, with the requirements of E.P., J.P., and the renewal of the 2020 version of Ch.P., there is an urgent need to develop a simple, rapid, and sensitive method to detect heavy metals in Chinese medicine.

Surface-enhanced Raman scattering (SERS) is an analytical method that can reflect the specific structure of molecules and has been widely used in the fields of national defense and security [18, 19], food and environmental safety detection, and biomedicine for its low cost, high-specificity, and rapid detection [20–27]. Importantly, complicated sample pretreatment or expensive instrumentation is not required for SERS-based detection, and the entire Raman signal acquisition time is only a few tens of seconds [28, 29]. SERS usually requires gold, silver, or other nanomaterials as substrates to enhance the Raman signal of target molecules, such as pesticides, dyes, and some drugs that SERS can directly detect. However, for substances that do not have a Raman response, like heavy metal ions, substances with SERS activity are usually needed as a molecular probe for indirect detection, such as 4-MBA, rhodamine, acridine flavin, and other dyes [30–33]. Tan et al. [34] used gold nanoparticles functionalized with 4-mercaptobenzoic acid as SERS substrates to detect heavy metal ions in water. They achieved linear quantification of Pb^{2+} , Cd^{2+} , and As^{3+} concentrations in the range of 0.5–100 mg/L by the variation of the relative signal of Raman spectra. Thatai et al. [35] used SiO_2 -AuNCs as the SERS substrate and CV as the probe for Raman detection of Cd^{2+} , whereby the limit of detection (LOD) could reach 0.1 mg/L. Wang [15] et al., proposed a dual-mode colorimetric/SERS method – cinnabar and water with detection limits of 2 and 0.2 μM , respectively – based on controlled etching to determine Hg^{2+} in traditional Chinese medicine. It is known that cinnabar is a mineral medicine, and the main component is HgS , so the Hg^{2+} content is high. However, generally speaking, the composition of Chinese medicine injections is more complex and is affected by more interference, thus a more sensitive method is needed to detect the residues of heavy metal ions in Chinese medicine.

This work presented a method based on 4,4'-dipyridine (Dpy) functionalized AgNPs as substrates detecting Hg^{2+} in Chinese herbal injections. Here, silver nanoparticles (AgNPs) were synthesized with trisodium citrate and combined with a Dpy probe molecule for the indirect detection of Hg^{2+} . Dpy contains two pyridine rings, in which the N can bind to AgNPs and show Raman signals; however, the contact of Dpy molecules to the AgNPs can be effectively suppressed when Dpy is bound to Hg^{2+} , which results in a certain degree of decrease in the SERS signal of Dpy. Therefore, the concentration of Hg^{2+} can be detected indirectly based on the change of the SERS signal of Dpy. In situ analysis can be achieved using the present method with a portable micro-Raman spectrometer. Using the current form in combination with a portable micro-Raman spectrometer, rapid analysis in the field can be achieved.

Materials and methods. Dpy and hydrochloric acid (HCl, 35–38%) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China), and nitric acid (HNO_3 , $\geq 99.0\%$) was purchased from Yonghua Chemical Technology Co., Ltd. (Hangzhou, China). Silver nitrate (AgNO_3 , 99.9%) and trisodium citrate

($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, $\geq 99.0\%$) were purchased from Chengdu Kolon Chemical Co., Ltd. (Sichuan, China). The standard solutions of Hg^{2+} , Pb^{2+} , Cd^{2+} , Cu^{2+} , As^{3+} (No. GBW(E) 1000 $\mu\text{g/mL}$) were purchased from Beijing Zhongke Quality Inspection Biotechnology Co., Ltd. (Beijing, China). Qingkailing injection (No. 191128B1) was purchased from Shenwei Pharmaceutical Group Co., Ltd. The glassware used in the experiments was washed with aqua regia ($\text{HCl}/\text{HNO}_3 = 3:1$, V/V) and then rinsed with ultrapure water. All solutions were prepared with Milli-Q grade water ($18.2 \text{ M}\Omega \cdot \text{cm}$).

The morphologies of AgNPs were characterized by a Field emission scanning electron microscope (Nova NanoSEM450, FEI, USA). UV-Vis absorption of the AgNPs was recorded with an ultraviolet spectrophotometer (UV-2450 Shimadzu, Japan) from 200 to 800 nm. All Raman spectra were obtained with a portable Raman spectrometer (Prott-EZRamanB2, USA) equipped with an excitation wavelength of 785 nm and a 1-cm quartz cell. For each SERS spectrum, excitation was performed using a fixed laser power (100 mW) and a fixed spectral acquisition time (10 s) in the range of 250–2350 cm^{-1} .

An ultrasonic detector with a frequency of 35 kHz was used for accelerated dissolution (KQ-250DB, China), while an intelligent dual-control thermostatic magnetic stirrer was used to synthesize AgNPs (DF101S, China). The weighing was performed with an electronic balance (FA2204N, China). Thermo Fisher Scientific (Milli Q, China) provided ultrasound water for the experimental procedure.

The silver nanoparticles (AgNPs) in this experiment were synthesized according to the classical Lee-Meisel method with slight modifications [36]. Briefly, AgNO_3 (18 mg) was added to 100 mL of ultrapure water and heated to reflux under a constant magnetic stirrer. After the solution boiled, 2 mL of pre-prepared 1% trisodium citrate was quickly added, followed by continued heating for 20 min and then cooling to room temperature. Compared with the conventional methods, this synthesis method was non-polluting and time-saving. The AgNPs prepared by this method were greenish-yellow with an average size of about 50 nm.

Preparation of a standard Hg^{2+} solution includes the accurate drawing of 0.1 mL of 1000 $\mu\text{g/mL}$ Hg^{2+} standard solution in a 10-mL volumetric flask, followed by a dilution process with ultrapure water that is followed by a fixing of the volume to 10 mL to get 10 $\mu\text{g/mL}$ Hg^{2+} reserve solution. The appropriate mercury ion reserve solution was taken and diluted with ultrapure water to 50–1000 ng/mL of standard working solution for later use. A series of metal ion solutions such as Pb^{2+} , Cd^{2+} , Cu^{2+} , and As^{3+} (10 $\mu\text{g/mL}$) were prepared, and the selectivity of the system for Hg^{2+} was investigated under the same optimized conditions.

In all, 200 μL AgNPs, 200 μL Dpy, 150- μL sodium chloride, and 200 μL of different concentrations of Hg^{2+} were added sequentially to the cuvette and mixed thoroughly at room temperature for the SERS test. All samples were acquired using a portable Raman spectrometer. Each spectrum's acquisition time was set to 10 s and accumulated three times. The cosmic ray interference was removed with the self-contained algorithm.

A 10 mL size Qingkailing injection (QKL) was taken for analysis, while a specific concentration of standard Hg^{2+} solution was added to the sample for spiked recovery experiments. The obtained mixture was mixed thoroughly at room temperature, and then the mixture was spiked according to above.

The average value was obtained by comparing the data of five experiments, and the SERS spectra were plotted with Origin2019b. All spectra were background corrected using the selective nonlinear iterative clipping (SNIP) algorithm. The ratiometric approach was employed to quantify Hg^{2+} , whereby the ratio of intensities at the maximum of the peak was plotted against concentration.

Results and discussion. *Principle of Hg^{2+} detection based on 4,4'-dipyridine functionalized silver nanoparticles.* The principle of detecting Hg^{2+} based on the Dpy functionalized AgNPs probe was shown in Fig. 1, whereas Fig. 1A represents the simple process of AgNPs synthesis. Figure 1B was the characterization result of AgNPs, and Fig. 1C–F showed the schematic diagram of the principle of detecting Hg^{2+} with Dpy as the probe. The two pyridine rings on the Dpy molecule can be adsorbed on the surface of AgNPs through the Ag–N bonds. The addition of the agent sodium chloride can cause AgNPs to aggregate together (Fig. 1D), resulting in a strong SERS signal of Dpy at 1296 cm^{-1} . When Hg^{2+} was added to the system (Fig. 1E), the solid binding effect of N and Hg caused the Dpy to be stripped from the surface of AgNPs. In a specific concentration range of Hg^{2+} , the decrease of the signal was shown to be linear with the concentration, thus the SERS signal change of Dpy could be used to detect Hg^{2+} in the complex sample indirectly.

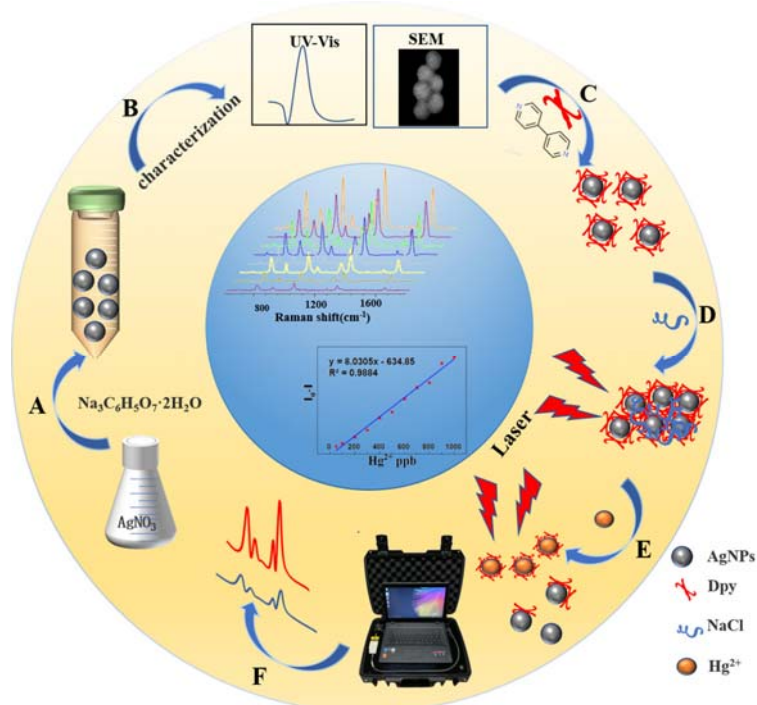


Fig. 1. Schematic diagram of Hg^{2+} detection based on 4,4'-dipyridine-functionalized AgNPs. Process (A) represented the synthesis process of AgNPs; (B) showed the UV-Vis absorption spectra of AgNPs and the scanning electron microscopy image; (C–F) showed the Raman mechanism of Hg^{2+} detection by 4,4'-dipyridine as a probe.

Optimization of the synthesis of silver nanoparticles. The preparation of SERS-active substrates is crucial for enhancing the Raman signals of the objective molecule. In order to obtain the perfect property of the probe and detect the content of heavy metal ions under optimal conditions, the necessary experimental parameters were investigated. Firstly, the amount of reducing agent and the reaction time were optimized in the synthesis of AgNPs by detecting the signal of Dpy (1.0×10^{-4} mol/L). Figure 2a showed the effect of different dosages of trisodium citrate dihydrate (TSC) on UV absorption. With increased TSC dosage, the UV absorption peaks of AgNPs were slightly blue-shifted, indicating the gradual decrease of their particle size. When the dosage of TSC reached 2 mL, the absorption peaks of AgNPs were the narrowest, which means that the homogeneity of the prepared AgNPs was better than in other conditions. Moreover, when it was higher than 2 mL, its UV absorption peak gradually broadened, and the size of AgNPs in the system varied greatly. As shown in Fig. 2b, the SERS signal of Dpy reached the maximum signal and was relatively stable when the TSC was 2 mL. The SERS signal could not achieve satisfactory results when the dosage was lower or higher than 2 mL, so the dosage of TSC was finally chosen to be 2 mL.

The reaction time is significant for the chemical synthesis of SERS nanoparticles. Here, the effect of different synthesis times from 3 to 27 min on the enhancement effect of AgNPs was investigated, and the AgNPs at different times were characterized by UV and Raman. As shown in Fig. 2c, the particle size of AgNPs gradually and uniformly decreased with the increase of the synthesis time. Figure 2d also showed that the AgNPs exhibited a strong Raman effect at 18 min; therefore, the synthesis time was finally selected at 18 min.

Characterization of silver nanoparticles. AgNPs were successfully fabricated and used as SERS substrates by our previously proposed method of silver nitrate reduction by trisodium citrate. Figure 3 displayed the UV-Vis spectra and SEM images of the AgNPs, respectively. As shown in Fig. 3a, the UV-Vis spectrum with an evident absorption band at 421 nm, presumably the AgNPs prepared by this method, were spherical. As shown in Fig. 3b, their shape was further confirmed via scanning electron microscope (SEM) images with an average particle size of 50 nm. The state of AgNPs in the system after adding the coagulant sodium chloride was observed in Fig. 3c. The more dispersed AgNPs were aggregated mainly due to Cl^- , the more Raman signal was able to be provided in the subsequent work.

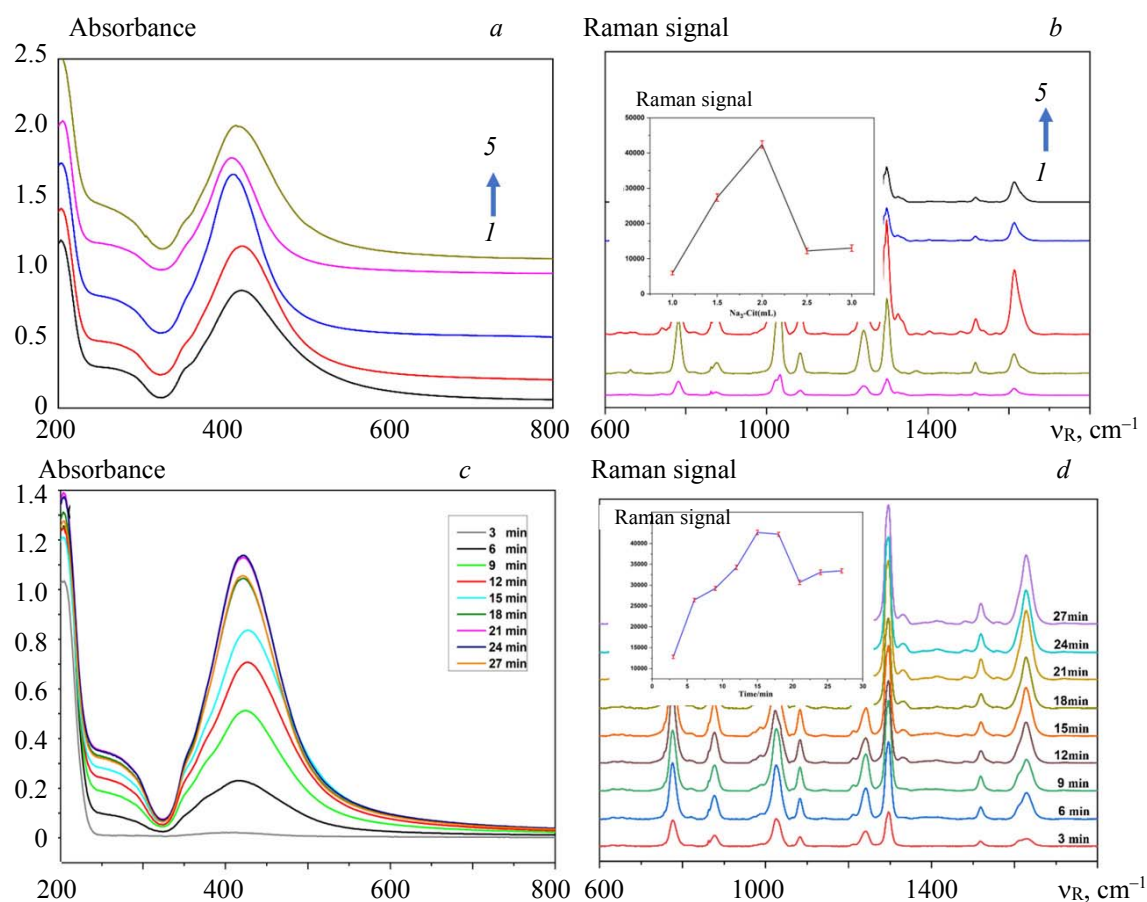


Fig. 2. (a) Effect of reducing agent dosage (trisodium citrate 1%) on the UV-Vis absorption spectra of AgNPs; (b) SERS of AgNPs + 4,4'-dipyridine + sodium chloride acquired with different addition amounts of trisodium citrate including 1.0 (1), 1.5 (2), 2.0 (3), 2.5 (4), and 3.0 (5). 2mL was determined as the additional amount of 1% trisodium citrate to synthesize AgNPs. (c) Effect of reaction time on the UV-Vis absorption spectra of AgNPs; (d) SERS of AgNPs + 4,4'-dipyridine + sodium chloride at different reaction times. 18 min was determined as the synthesis time of AgNPs.

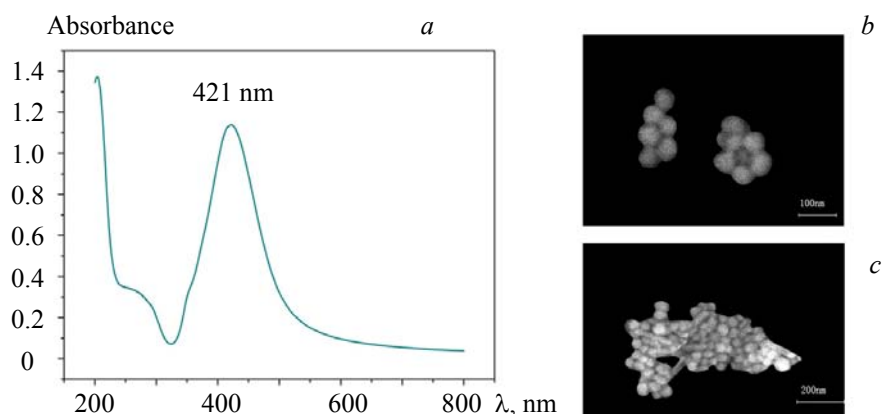


Fig. 3. (a) The UV-Vis spectra of AgNPs, (b) scanning electron microscopy images of AgNPs, and (c) scanning electron microscopy images of AgNPs added with sodium chloride.

Establishment of the Raman detection system of the 4,4'-dipyridine probes. In the pre-experiment, the necessary parameters were optimized, including the concentration of Dpy, the concentration of sodium chloride, and the addition order before implementing the quantitative detection.

First of all, the determination of the Dpy concentration was the key point of these experiments because it essentially decided the Raman performance. As shown in Fig. 4a, the prominent Raman scattering bands of Dpy were located at 766, 880, 1026, 1066, 1242, 1296, 1516, and 1612 cm^{-1} . Among these, the peak signal at 1296 cm^{-1} was more prominent and belonged to the C-H plane vibration on the pyridine ring. It has also been found that the peak signal was more stable here than in other Raman scattering bands and was selected as a characteristic peak for quantitative analysis. As shown in Fig. 4b, the Raman signal reached a maximum when the concentration of Dpy was 1.0×10^{-4} mol/L, and the SERS signal did not rise with the increase of Dpy concentration. This may be because the nano-silver particles were coated by the excessive Dpy molecular in the system, which resulted in the decrease of Raman enhancement.

Sodium chloride was selected as the aggregating material in the enhancement process. It increased the roughness of the substrate, which is an essential condition for the SERS effect. As shown in Fig. 4c, the different concentrations of sodium chloride have significantly influenced the SERS signal. With the increase of sodium chloride concentration, the Raman signal of Dpy at 1296 cm^{-1} increased and was followed by a decrease. When the sodium chloride solution was 0.1 mol/L, the Raman signal reached a maximum value.

The influence of the addition order of AgNPs, Dpy, and sodium chloride on the SERS signal was investigated after fixed Dpy and sodium chloride at their optimal concentrations. As shown in Fig. 4d, indicates the sequential addition of Dpy and Sodium chloride to AgNPs; indicates the sequential addition of Sodium chloride and Dpy to AgNPs. The results indicated that the effect of AgNPs binding to Dpy first and then aggregating by sodium chloride showed a stronger signal than the other addition order.

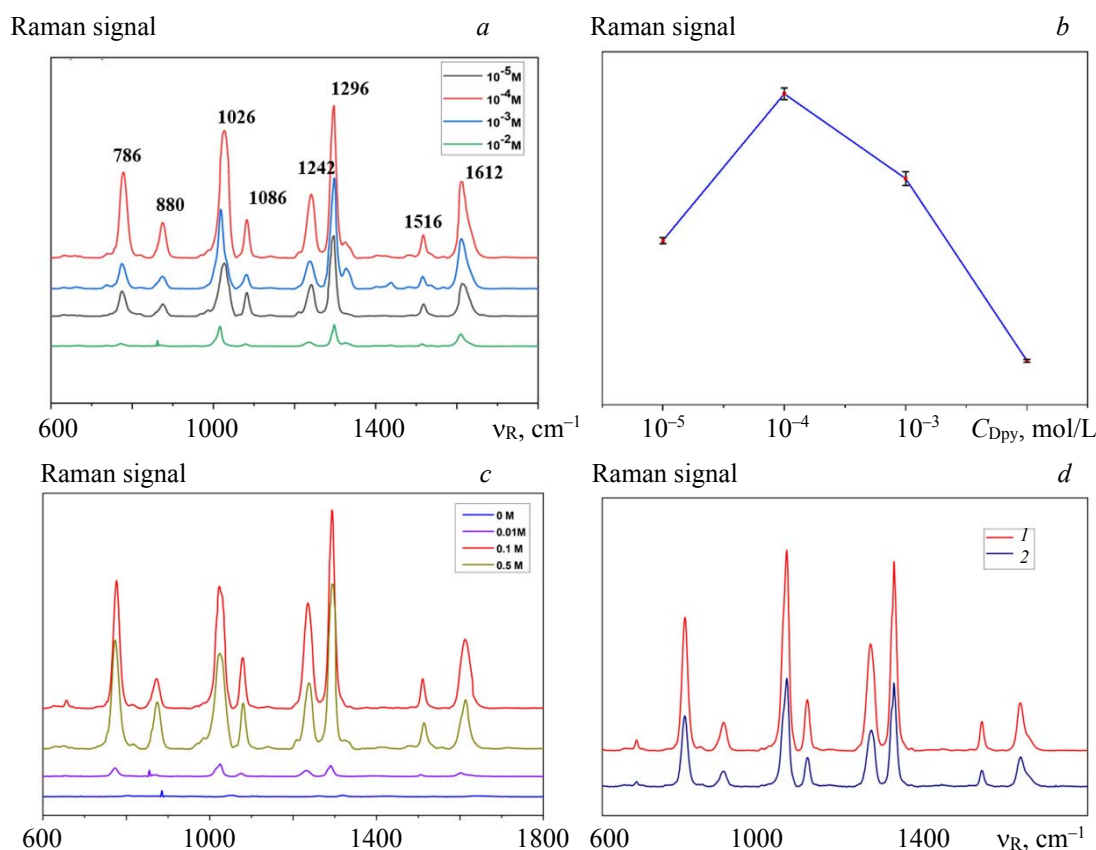


Fig. 4. (a) SERS of different concentrations of 4,4'-dipyridine. (b) Line chart of Raman signal at 1296 cm^{-1} for different concentrations of 4,4'-dipyridine. (c) The effect of different concentrations of sodium chloride on the Raman signal. (d) Effect of different addition sequences on the Raman signal. (1) AgNPs-4,4'-dipyridine-sodium chloride; (2) AgNPs-sodium chloride-4,4'-dipyridine.

Determination of Hg^{2+} . To explore the potential application of Dpy-functionalized AgNPs for the quantification of Hg^{2+} , we measured the Raman signal of Dpy by adding different concentrations of Hg^{2+} solutions under optimal experimental conditions. The concentration-dependent SERS spectra of Hg^{2+} from AgNPs-Dpy are shown in Fig. 5a. As the concentration of Hg^{2+} increases, the strong complexation between Hg^{2+} and Dpy probes leads to the stripping of the probe Dpy from the AgNPs, which decreases the SERS signal. Here, 1296 cm^{-1} was chosen for quantitative analysis, which was the vibration of the C-H plane of the pyridine ring. Figure 5b shows the trend of the enhanced SERS signal of Dpy at 1296 cm^{-1} with the change of Hg^{2+} concentration. The SERS signal displayed an excellent linear correlation with Hg^{2+} concentration ranging from 50 to 1000 ng/mL ($R^2 = 0.9884$). The LOD of this method for measuring Hg^{2+} is 10 ng/mL. It is worth noting that the SERS signal was expressed by $I_0 - I$, where I_0 and I are the SERS intensities of blank samples before and after the addition of Hg^{2+} , respectively.

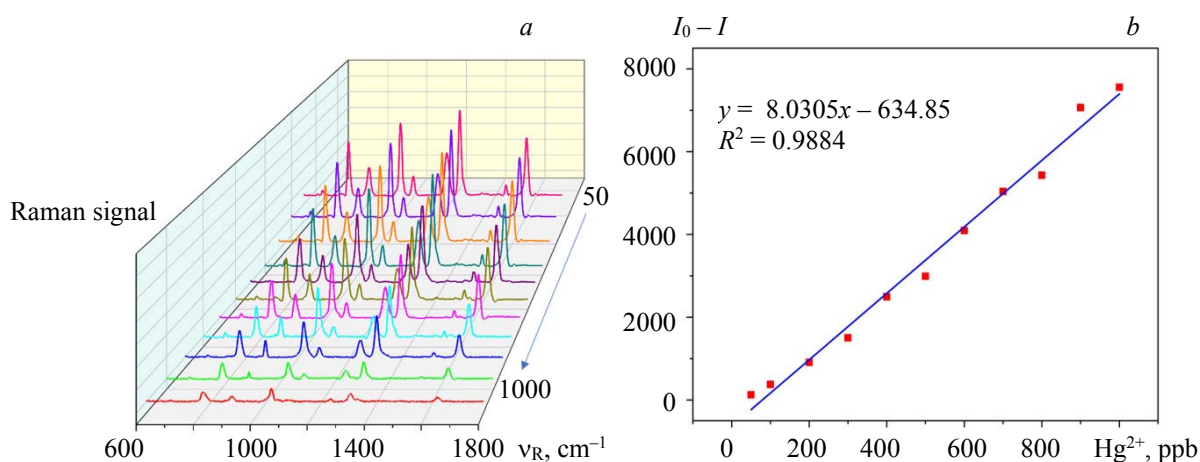


Fig. 5. (a) SERS of AgNPs+4-4'-dipyridine + sodium chloride + Hg^{2+} at concentrations of 50–1000 mg/mL. (b) The calibration curve of Hg^{2+} was established in the concentration range of 50–1000 mg/mL, and a good linear relationship was obtained. I_0 and I are the SERS intensity of the blank sample before and after the addition of Hg^{2+} , respectively.

Compared with previous studies, this method has a high sensitivity in detecting Hg^{2+} . As shown in Table 1, the SERS has better or comparable detection performance compared to other Hg^{2+} -specific based detection methods such as colorimetric, fluorescence, electrochemiluminescence, and AAS.

Selectivity, precision, repeatability, and stability. To evaluate the specificity of the method detecting Hg^{2+} , we detected four other heavy metal ions (Cu^{2+} , Cd^{2+} , Pb^{2+} , and As^{3+}) under the same detection conditions. The metal ion concentrations were all 10 ng/mL. The SERS signal (R/R_0) was used for comparison, where R_0 was the SERS signal of the blank sample and R was the SERS signal after the addition of heavy metals. As shown in Fig. 6a, the other four heavy metal ions had little effect on the SERS signal with $R/R_0 \geq 80\%$, while the value of Hg^{2+} was 6.55%, indicating that the method has a high selectivity for the detection of Hg^{2+} . According to a literature review [37], the stability constants of Hg-N bonds were much higher than those of other heavy metal ions. Therefore, the proposed AgNPs-based Dpy probe had good selectivity for detecting Hg^{2+} in a complex system.

The accuracy, reproducibility, and stability of the method were also investigated. Firstly, to demonstrate the accuracy of AgNPs, multiple Raman measurements were performed on the same batch of prepared AgNPs. Figure 6b showed the variation of the Raman peak signal at 1296 cm^{-1} for the same set of prepared AgNPs randomly ten times with $\text{RSD} = 3.81\%$, indicating that the prepared AgNPs had great precision. For reproducibility, six batches of AgNPs were examined with an RSD of 4.19% (Fig. 6c). As shown in Fig. 6d, the change of Dpy ($1.0 \times 10^{-4}\text{ mol/L}$) Raman signal during 1, 5, 10, 15, 20, and 25 days was stable with $\text{RSD} = 5.54\%$. The results indicated that this method performed with excellent specificity, high precision, strong repeatability, and good stability.

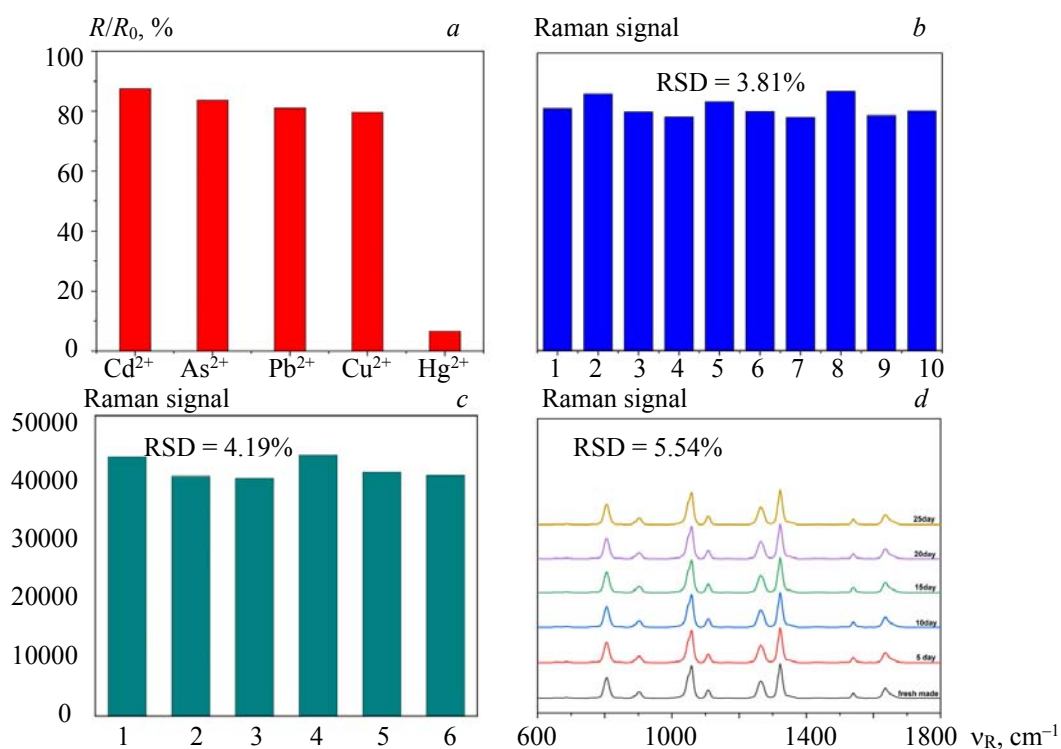


Fig. 6. (a) Selectivity of AgNPs + 4-4'-dipyridine + sodium chloride to detect Hg^{2+} (R_0 was the Raman signal of the blank sample, the R was the Raman signal after adding heavy metal ion samples). (b) SERS at 1296 cm^{-1} for 10 random measurements of the same batch of AgNPs. (c) Repeatability of different batches of AgNPs. (d) SERS of AgNPs + 4-4'-dipyridine + sodium chloride at different storage times.

Detection of Hg^{2+} in complex traditional Chinese medicine. Compared with other TCM preparations, injectables have the highest requirements in terms of safety evaluation. In this work, Qingkailing injection (QKL), listed in the 2020 Chinese Pharmacopoeia, was selected as a model of Chinese medicine. According to Pharmacopoeia, the content of heavy metals in QKL should be not more than 10 mg/kg . In order to exclude the influence of Chinese medicine components from the detection process and confirm the feasibility of Dpy functionalized AgNPs in the detection of Hg^{2+} , Raman analysis of Hg^{2+} in QKL was performed by the standard addition method (Table 2). The recovery experiments were conducted by determining Hg^{2+} spiked in the QKL sample with 200, 600, and 1000 ng/mL concentrations. The recoveries were in the range of 97.47–100.07%; therefore, the results indicated that the content of Hg^{2+} in QKL samples had been lower than 200 ng/mL , which complied with the safety evaluation requirements of Pharmacopoeia.

TABLE 1. Comparison of the Previously Reported Method with the Present Method for Detecting Hg^{2+}

Method	Linear range, $\mu\text{g/mL}$	Limit of detection, ng/mL	Reference
SERS	0.05–1.00	10	This work
SERS	0.5–100	100	[34]
Colorimetric-SERS	0.2–1.0	40	[15]
Colorimetry	0.2–2.0	15.6	[38]
UV-Vis-AuNPs	0.056–1.6	22.4	[39]
AAS	0.010–214	9	[40]
Electrochemical	5–110	250	[41]
Fluorescence	0–24	28	[42]

Note: SERS, surface-enhanced Raman scattering; UV-Vis, ultraviolet and visible spectrophotometry; AAS, atomic absorption spectroscopy.

TABLE 2. Real Detection and Recovery Test of Hg^{2+} in Qingkailing Injection ($n = 3$)

Sample	Spiked, ng/mL	Found (ng/mL) mean $\pm$ SD	Recovery, %
1	200	197.61 $\pm$ 2.67	98.81
2	600	584.82 $\pm$ 2.00	97.47
3	1000	1000.71 $\pm$ 1.47	100.07

Conclusions. In summary, a surface-enhanced Raman scattering method based on 4,4'-dipyridine functionalized AgNPs was established by portable Raman spectroscopy for the indirect determination of Hg^{2+} in complex systems. Following the previous experimental results, it was demonstrated that portable Raman has the advantage of comprehensive and holistic information acquisition compared with laser confocal Raman. The results illustrated that the surface-enhanced Raman scattering signal of 4,4'-dipyridine gradually decreased with the increase of Hg^{2+} , performing excellent linearity between 50 and 1000 ng/mL. The minimum limit of detection for the proposed method was 10 ng/mL, significantly lower than 0.2 mg/kg (approximately 2000 ng/mL) stipulated in the 2020 edition of the Chinese Pharmacopoeia. The practical application was investigated by determining Hg^{2+} in Qingkailing injection samples with spiked recoveries ranging from 97.47 to 100.07%. Compared with inductively coupled plasma mass spectrometry and atomic absorption spectroscopy methods, the 4,4'-dipyridine probe-based surface-enhanced Raman scattering detection method has the advantages of simple pre-processing, miniaturization of test samples, green and pollution-free, convenient, and sensitive detection, etc. It provides a new technique for detecting heavy metals in Chinese medicine products.

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