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Sensitive and selective SERS probe for Hg(II) detection using aminated ring-close structure of Rhodamine 6G

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ABSTRACT

A sensitive surface–enhanced Raman scattering (SERS) probe for selective detection of Hg(II) was designed and prepared by incorporating the well-known Rhodamine 6G and ethylenediamine into one molecule which is a aminated ring-close structure of Rhodamine 6G (R-NH_2). Due to the presence of oxygen and nitrogen functional groups of R-NH_2, Hg(II) would combined with two R-NH_2 molecules. The probe molecule R-NH_2 displays a highly selectively response of SERS enhancement towards 1 nM Hg(II) and featured SERS response with a broad pH span. The results establish the well-fitted linearity function of the SERS intensity with the concentration of Hg(II) in aqueous solution, suggesting the possibility for real-time detection of Hg(II) and the convenience for potential application.

1. Introduction

Mercury contamination is widespread and arises from a variety of natural sources [1]. The neurotoxic coordination compounds formed between Hg(II) and protein cause serious irreversible neurological damage to brain, and also induce severe damages to the environment [2]. Thus, the pollution by Hg(II) ions will have severe effects on human health and the environment. Recently, several methods for determnation of Hg(II) have been reported, regarding spectrophotometry [3], atomic absorption spectrometry [4], inductively coupled plasma–mass spectros-copy (ICP–MS) [5], flourescence quenching [6] and SPR sensor [7]. Although these methods have good performance in the determination of Hg(II), a more accurate, ultrasensitive and highly selective method is an important part of analytical chemistry.

SERS is a well known technique. SERS has the advantage of clarifying chemical identity, and environmental pollutants adsorbed on metallic surfaces. Analysis of spectral features has provided detailed information on interfacial structures, adsorption mechanisms, and surface reactions. Rhodamine 6G (R6G) and its derivatives are excellent probes for SERS spectroscopy, because of their special molecular structure, strong absorption in the visible. Recently, many groups have synthesized R6G-derived Schiff [8,9], compound ferrocene [10], ferrocenyl groups R6G-derivative [11] and BSA-15-R6G-derivative [12] as fluorescent probe for selectively detection of metal ions. Itamar Willner and co-workers also demonstrated the use of SPR sensor though stabilizing formation of Hemin/G-Quadruplexes for toxic Hg(II) sensitive detection.

Here, we try to use aminated ring-close structure of Rhodamine 6G (denoted as R-NH_2 which is synthesized through Michael additive reaction [13,14]) as SERS probe for sensitively and selectively detection of Hg(II). The R6G has been widely used as SERS probe since it contains two functional groups, a dibenzopyrene xanthene and a carboxyphenyl group tilted by about 90° with respect to the xanthene ring [15] and exhibits a boost of Raman intensity. Then, firstly, the R-NH_2 not only continues strong Raman signal of R6G, but also possesses functional groups of chemical affinity, providing dominant combined position for selectively detection of Hg(II). Second, based on the specific chemical reaction between R-NH_2 and Hg(II), the Raman spectral features of mixture may provide detailed information about adsorption mechanisms. Furthermore, SERS intensity of R-NH_2 is much stronger due to combination of the electromagnetic enhancement with an electronic resonance [16] and has a much bigger scattering cross section than other interesting molecular probes [17]. Moreover, compared to fluorescence probe, SERS probe is high sensitivity and multiplex detection capability due to molecular narrow spectra [18]. In addition, we develop the R-NH_2 as SERS probe molecule adsorbed on Ag–Au alloy NPs (see the Figs. 1S and 2S in Supporting information), in which stimulates more efforts for fabricating Raman active molecule.

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2. Experimental

2.1. Reagents

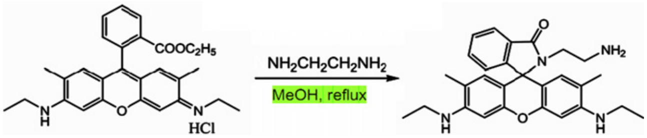
R6G, ethylenediamine ($NH_2CH_2CH_2NH_2$), ethanol (EtOH), acetonitrile (CH_3CN), silver nitrate ($AgNO_3$), polyvinylpyrrolidone (PVP $Mw=55{,}000$), trisodium citrate, chloroauric acid ($HAuCl_4$), mercuric nitrate $Hg(NO_3)_2$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All of these chemicals were analytical grade and were used without further purification.

2.2. Synthesis of R-NH_2

R-NH_2 modified by ethylenediamine were synthesized by R6G (958 mg, 2 mmol) was dissolved in 20 mL of hot ethanol, followed by addition of ethylenediamine (0.7 mL, 10 mmol). The reaction mixture was refluxed for 8 h till the color of the solution was changed from red to light pink. The reaction solution was cooled to room temperature, and the precipitated washed three times with 30 mL of cold ethanol. Crude product was purified by recrystallization from acetonitrile (CH_3CN) to give R-NH_2 white–pink 807 mg in 88.5% yield. ($M+H^+$): $m/z=457$.

2.3. Preparation of Ag–Au alloy

Ag NPs were synthesized by adding 10 mL 1×10^{-2} mol/L $AgNO_3$ into 90 mL distilled water of a 200 mL three-necked flask. When the solution have been heated to boiled and added into 3 mL 1% trisodium citrate solution for heating 1 h. Au–Ag alloy was synthesized by adding 2 mL 4.85×10^{-3} mol/L $HAuCl_4$ into conical flask containing 15 mL DMF, 10 mL Ag seed and 0.1 g PVP($Mw=550{,}000$). The final molar rations of Au–Ag were 1:1. The solution was heated in oil bath at 100 °C for 3 h and the sample was collected and washed centrifugally three times using distilled water and ethanol to get rid of organic material PVP. The collected substrate with rough surface and cavity structure provided much more “hot spots” to SERS probes.



Scheme 1. Synthesize routine for R-NH_2 through Michael additive reaction between precursor R6G and ethylenediamine.

2.4. Preparation of SERS substrate

First, the probe molecule R-NH_2 has been mixed and stirred with the Au–Ag alloy NPs. The concentration of probe molecule was 10 ppm and the volume ration between molecule and Au–Ag alloy NPs was 1:9. Second, different concentrations of Hg(II) were added into the mixture, respectively. Then, the mixture was collected and centrifuged. Meanwhile, with increasing Hg(II), more and more molecules have been combined, thus the SERS signal of probe molecule was decreased.

2.5. Preparation of FTIR sample

The FTIR of solid was described with the transmittance and the FTIR of derivatives solution was described with absorption; however, both of two descriptions or measurements could indicated the functional groups of derivatives. The solid sample was mixed with the KBr solid, then, mixture was pestled, tabletted and examined with FTIR measurements. This FTIR was the transmittance measurements. The liquid sample was dropped into the KBr solid, then, mixture was stoved, pestled and tabletted, finally examined with FTIR measurements, and this FTIR was the absorption measurements.

2.6. Apparatus

The Ag–Au alloy was characterized by field-emission scanning microscopy (FE-SEM, Sirion 200) with energy dispersive X-ray spectroscopy (EDS, OXFORD), and UV–vis absorption spectra for solution phase were recorded with a Shimadzu UV-2550 spectrometer. Fourier-transform IR (FTIR) spectra were obtained using a Nicolet-8700 spectrophotometer. Raman spectra were carried out on a LabRAM HR800 con-focal microscope Raman system (Horiba Jobin Yvon). The structure of Rhodamine 6G-derivative (R-NH_2) was determined with a ProteomeX-LTQ mass spectrometer employing a regular ESI source setup.

3. Results and discussion

The probe molecule R-NH_2 was facilely synthesized from Rhodamine 6G by a one-step reaction (Scheme 1) which is similar to the former studies [19–21]. The solid evidence came from the ESI–MS spectrum (Fig. 1a), the unique peak at m/z 457.22 affirmed the product was pure. As shown in Fig. 1b, in comparison to the precursor molecule, the band at 1685 cm^{-1} (red line) was attributed to the $V_{C=O}$ stretch vibration of the annular tertiary amides, formed between the –COO (1725 cm^{-1}) of R6G and $NH_2CH_2CH_2NH_2$.

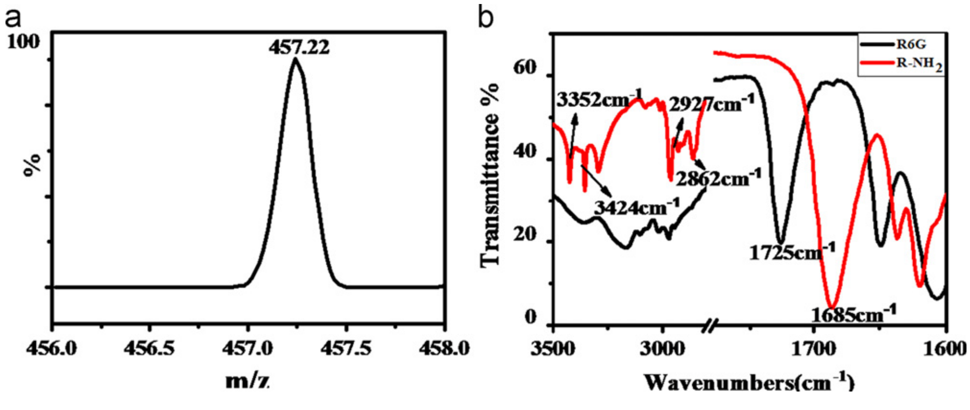


Fig. 1. (a) The ESI–MS of R-NH_2 ($M+H^+$): $m/z=457$ and (b) FTIR of R6G (black line) and R-NH_2 (red line) of solid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The bands in the range from 3500 cm^{-1} to 3200 cm^{-1} (red line) were attributed to primary amide asymmetrical stretching vibration, which showed the formation of free $–NH_2$. The bands at 2927 cm^{-1} and at 2862 cm^{-1} can be assigned to $–CH_2CH_2$ asymmetrical and symmetrical stretching vibrations, respectively. The results of ESI–MS and FTIR confirmed the molecule were the aminated ring-close structure of R6G.

The bands at 230 nm and 296 nm of R-NH_2 were also attributed to benzene B band and new spectra band R which was resulted from linking benzene with auxochromic groups $–NH_2$, –CO (Fig. 2). Because the π-systems in two chromophores of R6G are not conjugated, the strong UV–vis in aqueous solution reaches a maximum at 525 nm and a vibration shoulder at around 470 nm [22]. However, the R-NH_2, in comparison with the precursor R6G at pH ≈ 7, was absence of the band at 525 nm and the shoulder band. It might be account for the R-NH_2 broke the non-rigidity plane of R6G, then, the disappearance of non-rigidity plane gave rise to the trans-annular conjugate effect of derivative.

Interestingly, we found the color of R-NH_2 solution changed from colorless to strong yellow when the pH value is lower than 6.0 (the inset shown in Fig. 3a). At the same time, the UV–vis spectra of R-NH_2 appeared a new band at 525 nm as shown in Fig. 3a. Based on the results of above, we concluded that pH had a significant role in controlling the color of R-NH_2 solution and intensity of band at 525 nm, which is well consistent with the previous work of Dujols and co-workers [23]. It is well known that R6G-derivative exists isomers with different spectroscopic properties, its ring-closed molecular form is colorless and the ring-opened molecular form displays an intense yellow or red color [24]. As seen in Fig. 3b, due to more molecules have been broken and recovered the molecular structure of R6G with increasing pH, the intensity of band at 525 nm increased. The FTIR-spectra of R-NH_2 aqueous solution under neutral and acidic conditions are shown in Fig. 4. In comparison to the derivative noted under neutral condition, the band (red line) at 1668 cm^{-1} was attributed to intermediate amides $V_{C=O}$ stretch vibration of R-NH_2 at pH = 6. Annular amides $V_{C=O}$ stretch vibration frequency will change with different ring numbers, as shown Fig. 4 (red line), ring-close structure has been broken, so the $V_{C=O}$ stretch vibration changed from at 1685 cm^{-1} to 1668 cm^{-1}. The band at 1606 cm^{-1} was attributed to intermediate amides N–H transformation vibration due to tertiary amides has not N–H transformation vibration. Because this was FTIR-spectra of derivatives $H_2O–CH_3CN$ solution which was different from the solid-FTIR, the OH-stretch vibration overlapped with N–H stretch vibration. The FTIR-spectra also evidenced the transformation of molecular structure before and after addition of H^+.

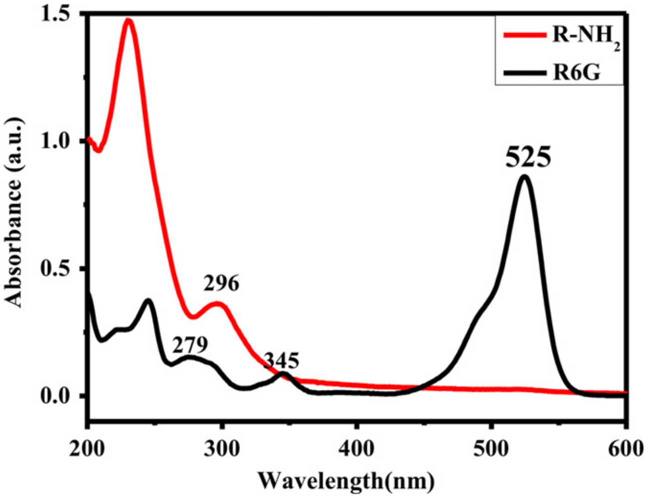


Fig. 2. UV–vis absorption spectra of 1×10^{-5} mol/L R-NH_2 solution (red line) and 1×10^{-5} mol/L R6G solution (black line), respectively. $H_2O:CH_3CN = 1:1$ (V/V) at pH ≈ 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The change of pH caused a new UV–vis at 525 nm and made the R-NH_2 solution became strong yellow color. We conclude that the ring-close structure and effect of trans-annular conjugate of R-NH_2 has been broken, and the non-rigidity plane of R6G has been recovered. As shown in Scheme 2, this ring-open mechanism was ascribed to the electron transfer and effect of hydrogen band. Both the Oxygen and the nitrogen with strong electro-negativity can combine with hydrogen; on the other hand, electron transfer was much easier to form stable structure. However, the addition of OH^- has given rise to R-NH_2 solution colorless and resulted in the disappearance of UV–vis at 525 nm, in which confirmed this process of open-ring was reversible. This open-ring mechanism was different from the pioneering work of Czarnik and co-workers that was irreversible.

As shown in Fig. 5, the absorbance was significantly enhanced from 0.97 to 0.4 at 527 nm upon gradual addition of Hg(II) content at pH = 6, suggesting the most of R-NH_2 molecules have been combined with Hg(II). To explore the optimum conditions for Hg(II) detection, the different pH condition for detection of Hg(II) have been researched (see the Figs. S3 and S4 of Supporting information).

We have demonstrated a sensitive and selective SERS probe, based on citrate-capped gold nanoparticles, for trivalent chromium detection [25]. In this study, aminated ring-close structure of R-NH_2 as SERS probe for sensitively and selectively detection of Hg(II) was used. The mechanism was proposed in Scheme 3. In comparison to

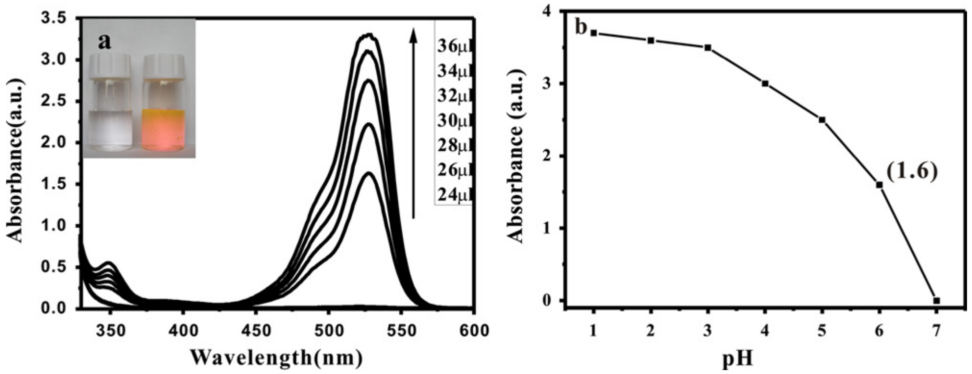


Fig. 3. (a) The evolution of UV–vis absorption spectra with addition different equivalent H^+ (0.1 mol/L HCl, 24 μL–36 μL, respectively) into 4 ml 1×10^{-5} mol/L R-NH_2 $H_2O:CH_3CN = 1:1$(V/V) solution and inset is the color change and (b) The intensity evolution of band 527 nm with different pH. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

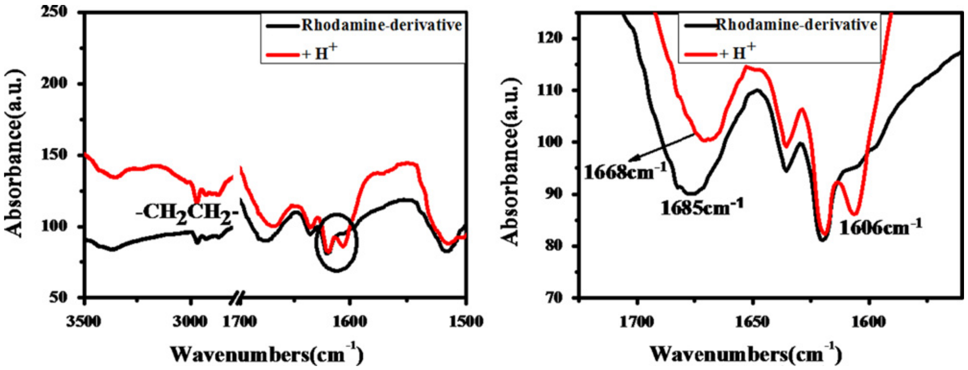
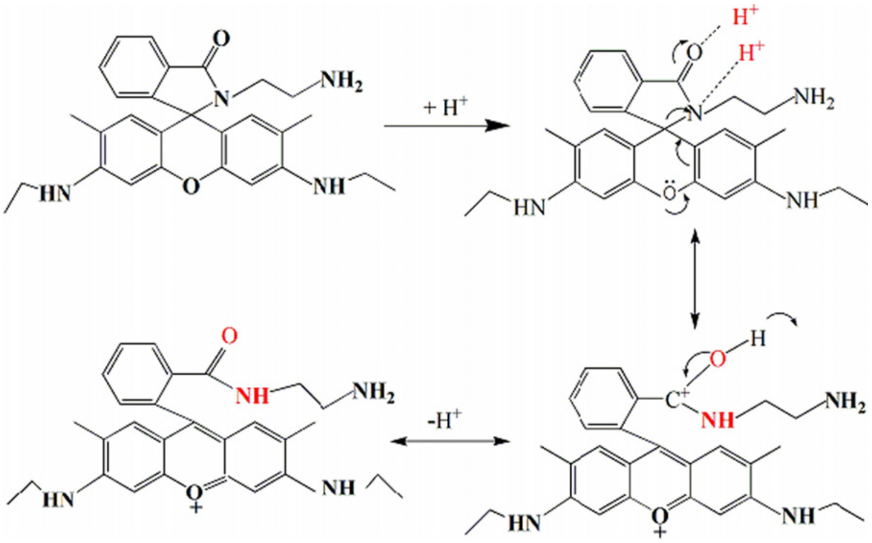


Fig. 4. The FTIR-spectra of derivative aqueous solution (1×10^{-5} mol/L) under neutral and acidic condition, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Scheme 2. The mechanism of electron transfer and effect of hydrogen band.

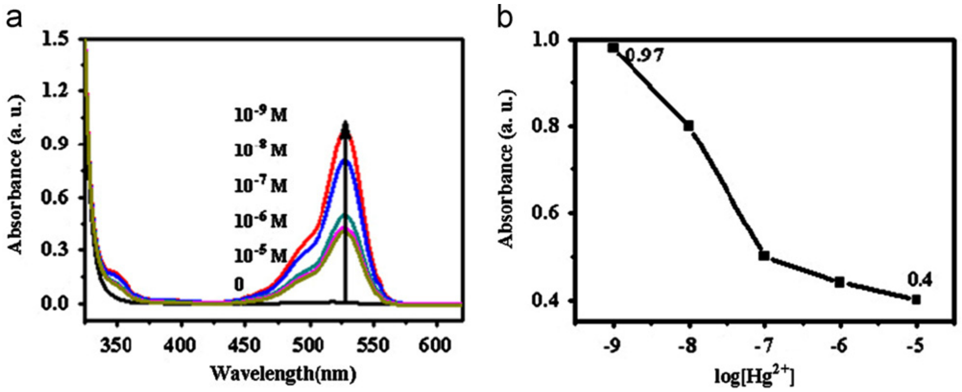
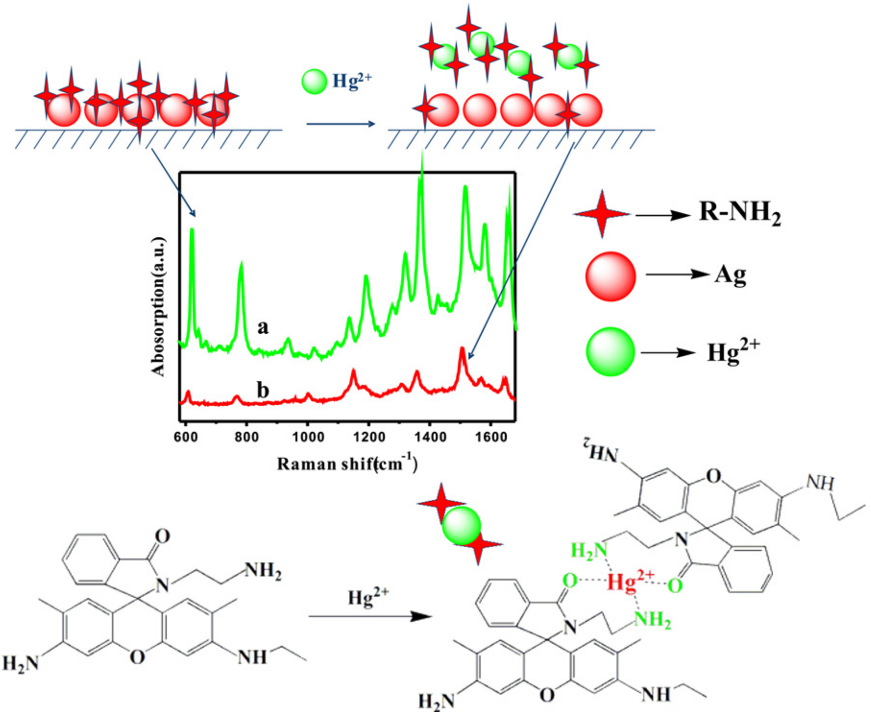


Fig. 5. The serial evolution of UV–vis absorption spectra (a) increasing Hg(II) (100 μL, respectively) into 4 mL 1×10^{-5} mol/L R-NH_2 solution (H_2O:CH_3CN=1:1V/V) and (b) the changing tendency of intensity of band at 527 nm at pH=6. The result was recorded every 5 min after adding Hg(II).

acid condition, R-NH_2 retained the structure of aminated ring-close structure under neutral condition, which made the distance between C=O and –NH_2 be short. Due to this free –NH_2 has been far away five-ring, then addition of Hg(II) can easily be combined with C=O and –NH_2 and not break the ring-close structure. Furthermore, oxygen and nitrogen were elements with lone pair electrons, which can easily be formed coordination band and feedback coordination band with Hg(II). With increasing Hg(II), more and more molecules have been combined, hence, the SERS intensity of probes decreased. On the other hand, absorbance of R-NH_2 was decrement with increasing of Hg(II) concentration, in which indicated that complexion with Hg(II) reduced the light absorption attributable to xanthene moiety. Meanwhile, the combination of the electromagnetic enhancement with electronic resonance for probe molecule was much weaker, so the decrement of Raman signal of probe molecule was also because of the reduction of “resonance effect” by the complexion with Hg(II). As shown in Scheme 3, the green line was the single SERS signal of probe, the red line was the



Scheme 3. The mechanism of chelation: in the presence of Hg(II) groups C=O and –NH$_2$ combined with Hg^{2+}, so two probe molecules (10^{-5} mol/L R-NH$_2$) have combined with one Hg(II) (*n*/*n*=2:1,*N*/*N*=2:1), forming Hg(II) four coordination compound. The SERS measurement of R-NH$_2$ was recorded every 5 min after adding Hg^{2+}.

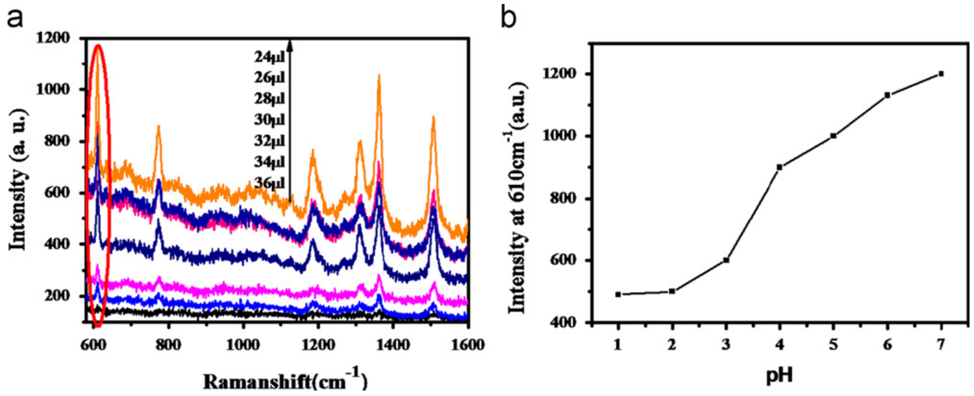


Fig. 6. The evolution of SERS of 1×10^{-5} mol/L R-NH$_2$ with the increase equivalent H$^+$ (0.1 mol/L HCl), respectively, and (b) the intensity change tendency of band at 610 cm^{-1} with different pH. The SERS measurement of R-NH$_2$ was recorded every 1 min after adding H$^+$.

SERS signal of complex of probe molecule with Hg(II). In addition, the SERS signal of probe gave rise to a litter blue shift. It should be noted that the charge transfer would occur in the touch surface between N and Ag with strong chemical absorption and chemical reaction [26].

The coordinative reaction of Hg(II) ion with the ring-close structure of R6G and the resultant complex structure were further confirmed by the ESI–MS spectroscopy at pH=7. After the Hg(II) ion were mixed with the SERS probe molecule in the solution, the mixture Hg(R-NH$_2$)$_2$ peak at m/z=1113.54 (see Fig. S5 in Supporting information) was detected, suggesting the formation of Hg(II) four coordinative compound in the mixture. This data also showed that the C=O and –NH$_2$ exhibited strong coordinative ability with metal ions and thus was widely used in selectivity detection of metal ions.

As shown in Fig. 6a, in comparison with normal SERS of R-NH$_2$, the SERS of R-NH$_2$ under acid solution was lack of the bands at 1575 cm^{-1} and 1651 cm^{-1} (Fig. 6a). The absence of two bands was because the charged derivative molecules absorbed on Ag–Au NPs surface induced intermolecular and intercluster interaction [27] though the H$^+$-induced open-ring has the similar structure with R6G. The evolution of SERS of R-NH$_2$ with the increasing pH was shown in Fig. 6b. The result was that molecules were parallel with Ag substrate, which made less molecules fall to the "hot spot"; the other was that protonated –NH$_2$ cannot combine with substrate, in which it made the SERS intensity decrease. CH$_3$CN solution of R-NH$_2$ was approaching neutral, addition of H$^+$ broke the ring-close structure of R-NH$_2$ and made the free –NH$_2$ protonated, then, the protonated –NH$_2$ cannot well combine with the Ag–Au NPs, in which most of molecules were possibly parallel with the Ag substrate. On the basis of the surface selection rule, when molecules are perpendicular on the substrate, the enhanced signal is stronger [28], consequently, with increasing pH, much more molecules were protonated and the enhancement signal was also much lower.

As shown in Fig. 7a, with increasing Hg(II), the R-NH$_2$ would be soluble and then be trapped by Hg(II), so the intensity of probe

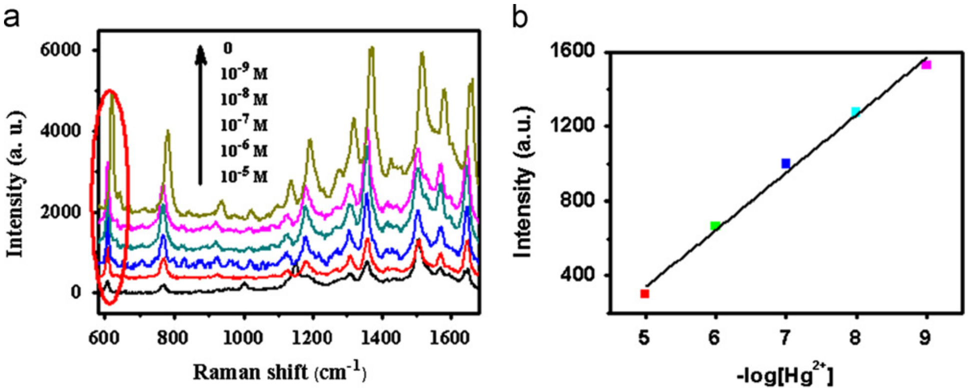


Fig. 7. (a) The evolution of SERS of 10^{-5} mol/L R-NH$_2$ and (b) the intensity change tendency of band at 610 cm^{-1} with increase Hg(II) concentration including 10^{-9} mol/L, 10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L,10^{-5} mol/L and 0 mol/L. All Raman spectra were recorded with a 532 nm laser with 1 mW power and all SERS measurement of R-NH$_2$ was recorded every 5 min after adding Hg(II).

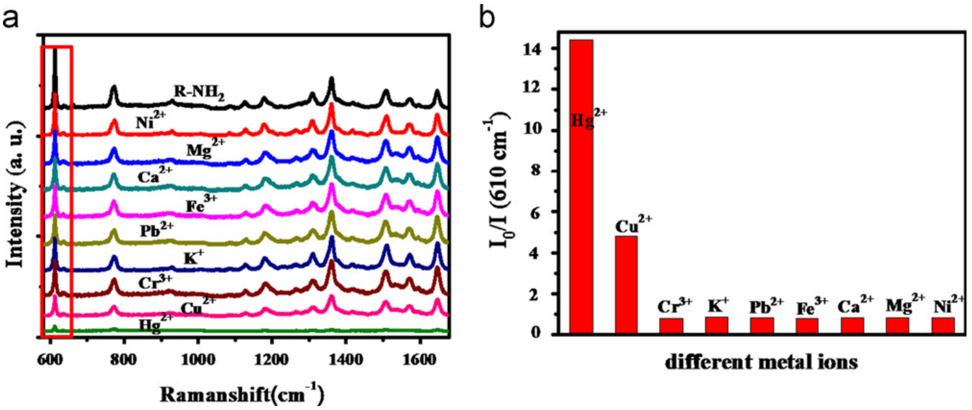


Fig. 8. (a) The selectivity evolution of SERS of probe with a serial of 1×10^{-5} mol/L metal ions in CH$_3$CN phosphate buffer (pH=6.9) and (b) the SERS intensity evolution (I_0/I) of R-NH$_2$ band at 610 cm^{-1} (I_0 and I represent the Raman intensity at 610 cm^{-1} in the absence and presence, respectively) with different mental ions.

molecule decreased. Based on the above mechanism and results, the strong band at 610 cm^{-1} was chosen to observe the changing tendency. The data indicated that the SERS intensity of band at 610 cm^{-1} obviously decreases with Hg(II) concentration from 10^{-9} mol/L to 10^{-5} mol/L and displays a correlation coefficient $R=0.9919$ and a corresponding standard deviation $SD=1.3850$ in Fig. 7b. The lower concentrations were examined (see the Fig. S6 in Supporting information). Fig. S6 showed when the lower concentrations Hg(II) (0.1 nM, 0.01 nM, 0.001 nM) were added into SERS probe system, the Raman intensity of SERS probe has few alternations compared with that in the present of 1 nM Hg(II); thus, the result indicated that this proposed assay has a detection limit of 1 nM.

To examine the selectivity of probe molecule, in CH$_3$CN-phosphate buffer (pH=6.9), a series of metal ions including 10^{-5} mol/L Cr^{3+}, Cu^{2+} K^{+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Na^{+}, Ni^{2+} and Fe^{3+} were used to evaluate the chelated properties of R-NH$_2$. As seen in Fig. 8b, only the addition of Hg(II) led to the large alteration of SERS of R-NH$_2$. Cu^{2+} also caused a little alteration. Hg(II) has a vacancy 6s circle and two nitrogen provide two lone pair-electrons for 6s circle, meanwhile, the formation of feedback π bond between Hg(II) and oxygen was further more beneficial for forming Hg(II) four coordination compound than other metal ions. This chelating process suggested a selective method for detection of Hg(II) by SERS measurement, meanwhile, at least two factors contribute to selectivity towards Hg(II): (i) R-NH$_2$, a very suitable coordination geometry conformation of bichelating ligands, the nitrogen–oxygen affinity character and amide deprotonation ability of mercuric [29], (ii) Hg(II), larger radius, R-NH$_2$ are sufficient to chelate Hg(II), forming Hg(II) coordination complexes and exhibit high selectivity for Hg(II) over other metal ions.

4. Conclusions

In summary, a simple and easy-to-prepare sensitive surface-enhanced Raman scattering (SERS) probe for selective detection of Hg(II) by incorporating the Rhodamine 6G and ethylenediamine into one molecule was prepared and structurally characterized. We further discussed the principle of color changing of R-NH$_2$, came up with possible open-ring mechanism and chose the optimum pH for detection of Hg(II). The current R6G-derivative SERS probe can afford Hg(II)-selective Raman enhancement and shows detection limits as low as the 1 nM level. Because of the suitable coordination geometry conformation of bi-chelating ligands, nitrogen–oxygen affinity character and amide deprotonation ability of mercuric, R-NH$_2$ were sufficient to exhibit high selectivity for Hg(II) over other metal ions.

Acknowledgements

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.01.013.

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