Probing catecholamine neurotransmitters based on iron-coordination surface-enhanced resonance Raman spectroscopy label

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\textbf{A B S T R A C T}

Catecholamine (CA) is neurotransmitters of the biological amines class which play an important role in organism. However, it is quite difficult to realize sensitive and selective detection of CA in complex system. Here, we employ surface-enhanced resonance Raman spectroscopy (SERS) strategy, iron-nitrotriatomic acid functionalized PVP-Au NPs (Au-Fe(NTA)) as Raman label for rapid and sensitive detection of CA containing dopamine (DA), norepinephrine (NE) or epinephrine (EP) in complex serum. The Au NPs is sufficient to provide Raman enhancement and Fe-NTA label can rapidly trap CA molecules adjoining gold core to form NTA-Fe-CA resonant structure, which can amplify the signals of CA. More important, we successfully distinguish these three CA molecules in serum since surface-enhanced Raman spectroscopy (SERS) technique can provide fingerprint identification. Furthermore, the SERS signals of Au-O band from PVP stabilized Au NPs can be utilized as a stable internal calibration standard for quantitative detection of target. Additionally, we continue to investigate the binding constants between different CA molecules and functionalized substrate to evaluate the corresponding adsorption property. This SERS strategy is not only capable to offer exciting opportunities to selectively trap the analyte, but also strongly amplify the Raman signals of CA as well as achieve quantitative measurement.

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1. Introduction

Neurotransmitters are of vital importance to both the central and peripheral nervous system for transmission of information. Catecholamine (CA) with the structure of amino and catechol is one of the most significant biological amine neurotransmitters, which contains dopamine (DA), norepinephrine (NE) and epinephrine (EP). As a kind of important neurotransmitter, CA not only directly takes part in behavioral activities, but also relates to some functional diseases like disorder, Parkinson’s disease and depression [1–3]. There is increasing demand to concern and monitor the level of CA in the human body fluids such as serum and urine due to the special pharmacological property and physiological function [4]. Consequently, developing the sensitive strategy for the study of CA in complex system is of great significance. Typically, the detection of CA molecules (DA, NE or EP) in the complex is achieved through the methods of electrochemical detection [5,6], colorimetric biosensors [7], immunoassays [8,9] and HPLC [10]. Although some progress have been made, there still exists some disadvantages. An example is several techniques are time-consuming and require long pretreatment steps to enrich the sample. Moreover, the major problem of electrochemical detection is the coexistence of interferences, especially ascorbic acid (AA) [11]. Because the voltammetric responses of DA and AA with nearly same oxidation potential are overlapping, so that the analytes cannot be clearly distinguished [12]. These drawbacks can be acted as the driving force to propose the rapid and sensitive analytical method for detection of CA in complex biological fluids.

In recent years, surface-enhanced Raman spectroscopy (SERS) as a promising technology [13], has been widely applied in various fields involving living cells [14,15], food safety [16] and organic pollutants [17] for its intrinsic advantages of providing fingerprint identification and high sensitivity, requiring less volume of sample and less time-consuming [18,19]. More important, SERS is considered to have exceptional potential for employing in complex bio-fluids detection [20] for a number of reasons, one of which is...
that water has a very weak Raman scatter. However, in terms of CA neurotransmitters, the two main limitations of SERS are that the Raman activity of CA is relatively low and measurement of CA is quite complicated with low basal concentration (0.01–1 μM) and coexistent interferences, which cannot be overcome only by modifying substrates. Surface-enhanced resonance Raman spectroscopy (SERS) strategy is capable to offer exciting opportunities to selectively trap the analyte and strongly amplify the Raman signals by modifying a chromophore on the surface of SERS substrate [21,22]. The chromophore coupled with the localized surface plasmon of active nanostructure leads to spectral resonant and surface enhancements for overcoming above mentioned limitation.

On the other hand, it is worth noting CA with low level plays an important role in various physiological activities, thus the precise determination of analyte concentration is critical for biological sensing applications. Nonetheless, there are only a few reports focus on quantitative detection of CA [23–25]. To realize sensitive and reliable SERS quantitative detection, the two issues need to be addressed: firstly, a uniform SERS substrate is necessary to ensure the stability and reproducibility of the signal. Only on the basis of uniformity can achieve effective control of hotspots. Gwo and co-workers [26] designed self-assembled Au NPs super-lattices to realize quantitative detection. The second issue of quantitative analysis is related to the measurement of SERS signal. In addition to the intrinsic properties, the intensity of SERS signal is always affected by external conditions such as laser power and resolution of the instrument, etc. As a result, it is hard to reproduce SERS signal even the same sample, which brings a great challenge to quantitative detection. A common solution for this difficulty is to normalized relative intensity of analyte by using an internal reference [27] or internal standard [28].

Herein, by taking the advantage of chromophore feature amplifying the Raman signals of small molecules with weak Raman activity, we employ the SERS strategy to realize the rapid and high sensitive detection of CA molecules as well as distinction in complex serum specimen. In this report, the iron-nitrolactiatic acid (Fe-NTA) as a Raman label is modified on the surface of PVP stabilized Au NPs (PVP-Au). We reason that, upon addition of analytes (taking the DA as an example), DA molecule will transform from a non-resonant state to an electronic state because of the formation of NTA-Fe-DA chelate structure. The Au NPs can provide sufficient enhancement for Raman signal, and then Fe-NPs structures can rapidly trap the analytes adjoining the gold core to form the complex of NTA-Fe-DA resonant structure to realize highly sensitive and selective detection for CA molecules by using 633 nm laser. More important, due to the advantage of fingerprint identification of SERS, the spectra of CA molecules in serum can be differentiated successfully. Furthermore, the independent Raman signals of Au-O band of PVP stabilized Au NPs can be used as the internal reference for quantitative SERS detection of target molecules. In view of these three substances of CA molecules, all the normalized relative intensity of analyte-sensitive band versus the band of internal reference exhibits good linear response to the negative logarithm of the concentration. In short, this reported SERS strategy not only realizes the high sensitive and selective detection, but also obtains the quantitative measurement for CA molecules. Additionally, we study the adsorption constants about three kinds of analytes by means of this quantitative method.

2. Experimental section

2.1. Materials

Hydrogen tetrachloroaurate (HAuCl₄·4H₂O), iron(III) nitrate nonahydrate (Fe(NO₃)₃·9H₂O), sodium citrate, crystal violet (CV) were purchased from Shanghai Chemical Reagent Company. Dopamine hydrochloride and α, β-Nitrosoacetic acid (NTA) were obtained from Sigma-Aldrich, polyvinylpyrrolidone (PVP), epinephrine bitartrate and noradrenaline bitartrate monohydrate were supplied from Aladdin Company. Millipore water purification was used to produce ultrapure water (18.2 MΩ cm).

2.2. Instruments

Transmission electron microscopy images were collected on a FEI Tecnai G2 F20 S-TWIN high resolution transmission electron microscopy. The scanning electron microscopy images were obtained by a field-emission scanning electron microscope (Quanta 200FE). Ultraviolet–visible (UV–VIS) absorption spectra were taken on a Shimadzu UV-2550 spectrophotometer (Japan), and the background spectrum was deducted. Fourier transform infrared (FT-IR) spectra were performed on a Nexus-870 spectrophotometer. X-ray Photoelectron Spectroscopy (XPS) measurements were carried out by Thermo ESCALAB 250Xi (with a monochromatic Al Kα (1486.6 eV) excitation source). The zeta potentials were measured by Zeta-check, Microtrac (Germany). Raman spectra were recorded by LabRAM HR800 confocal microscope Raman system (Horiba JobinYvon) with a 633 nm He–Ne laser source.

2.3. Synthesis of PVP stabilized Au NPs

The synthesis of PVP stabilized Au NPs by seed growth method [29]. The preparation of Au seed nanoparticles were made by the citrate reduction of HAuCl₄·4H₂O [30]. Briefly, adding 1 mL of HAuCl₄·4H₂O (1% wt) to the rounded bottom flask of 250 mL, which injected into 99 mL deionized water, and then the solution was heat to boiling during vigorous stirring, adding into sodium citrate (1% wt) at the moment. The boiling solution was keep heating for 30 min continuously, and then let it cool.

The general procedure for seed growth process was as follows: putting 25 mL seed solution into 250 mL three-necked round bottom flask, and then adding 1 mL sodium citrate (1% wt), 1 mL PVP solution (1% wt) and 20 mL 2.5 mM NH₂OH·HCl into the solution, successively. Under the condition of stirring, 20 mL HAuCl₄ (1% wt) was injected into solution at the speed of 1 mL min⁻¹ by a peristaltic pump. The reaction was carried out at room temperature.

2.4. Preparation of Au-Fe(NTA) SERS substrate

The process of Fe-NTA modified on the surface of Au NPs on a basis of an approach which reported by Kayat [21]. Briefly, the formation of Fe-NTA was mixed with Fe(NO₃)₃·9H₂O (10⁻² M) and NTA (10⁻³ M) at the ratio of 1:1, and then 0.1 M NaOH was added to adjust the pH to 7, the solution of Fe-NTA was standing for 15 min. 1 mL PVP stabilized Au NPs was centrifuged and dispersed in 1 mL ultrapure water. Then the prepared Fe-NTA was mixed with re-dispersed PVP stabilized Au NPs at the volume ratio of 1:1. After centrifugation, discarding the excess Fe-NTA, and the sol precipitation in the bottom was left to use.

2.5. Samples preparation and SERS measurements

2.5.1. Samples preparation

In SERS detection of these three CA molecules, 3 μL sol of Au-Fe(NTA) prepared above was dropped on the clean Si wafer, and then the SERS functionalized substrate are dried in incubator at the temperature of 30 °C. To achieve detection of CA molecules in serum (the serum were obtained from CAS Hefei Cancer Hospital), a certain pretreatment method was prepared to process the serum sample. Briefly, 10 μM 10⁻³ M DA (NE or EP) was adding into 100 μM serum sample, and then mixed with 300 μM CH₃OH. The
mixture was centrifuged at 9000 r/min for 10 min to remove macro-molecular protein, leaving the supernatant liquid for further usage. The serum samples of binary and ternary mixtures of CA molecules were prepared similarly. Above 5 μL of each sample was dropped on the dry functionalized substrate.

2.5.2. SERS measurement

All samples were analyzed by LabRAM HR800 confocal microscope Raman system (Horiba JobinYvon) with a 633 nm He–Ne laser source. The lasers were focused by a LWD 50/0.5 NA objective lens, the laser spots had a diameter of about 1 μm with the laser powers of approximately 0.9 mW, and the accumulation time for each spectrum was 3 s.

3. Results and discussion

3.1. Characterization of uniform PVP stabilized Au NPs

In this report, we use the Fe-NTA as the signal amplifier to selectively trap CA molecules in the complex system, thus the surface feature of the Au NPs is of great significance to combine with the Fe-NTA in our test. Generally, the classical citrate-protected Au NPs originated from the method of Turkevich–Frens with negative charge ligands is not beneficial to the attachment of Fe-NTA with positive charge. Also, citrate-protected Au NPs is easy to aggregate with addition of analytes, resulting in the incredible and irreproducible SERS signals. Therefore, it is urgently need to explore greater stable Au NPs in the complex system for sensitive SERS detection. Guo et al. has reported the self-assembly large-scale monolayer of Au nanoparticles modified with PVP for SERS quantitative analysis with high sensitivity and reproducibility [31]. Another example is Zhou employed the PVP stabilized Au NPs for the pharmaceutical and medical diagnosis [11]. As a consequent, herein, we successfully synthesized the uniform PVP stabilized Au NPs through a strategy of kinetically controlled seeded growth. In the process of the seed growth, a thin layer of PVP can bind to Au NPs by partial replacement of the citrate ions on the surface of nanoparticles [31], which can be verified that the zeta potential value of citrate-protected Au NPs is much lower than that of PVP stabilized Au NPs (Fig. S1). The self-assembly of monolayer Au NPs arrays by solvent evaporation is mainly composed of two-stage: nucleus formation and crystal growth, which involves the attractive capillary forces and convective particles flux to govern the ordered array [32]. What’s more, order monolayer of nanoparticle is also controlled by the water evaporation rate properly. Here, PVP as a kind of surfactant will reduce the evaporation rate compared with pure water at the same conditions [33], meanwhile, its steric feature enables hard-sphere-like interactions between adjacent nanoparticles [34], which facilitates the formation of highly uniform monolayers array (Fig. 1a). As shown in Fig. 1b, the uniform PVP stabilized Au NPs with the diameter of 42.8 nm (Fig. S2a and c) are close-packed with a large area of monolayer distribution. By statistics of Au NPs from the TEM images, Fig. S2d presents the gap distance distribution of interparticle separations are mainly concentrated on 2–4 nm range, which can act as ideal SERS active substrates with excellent uniformity and high sensitivity.

Hence, the dye crystal violet (CV), a typical SERS target analyte, is selected as the reported molecule to evaluate the SERS performance of the substrate. The SERS characteristic spectra of CV with a concentration range from $10^{-6}$ M to $10^{-8}$ M are exhibited in Fig. S3. Considering the reproducibility of substrate, a series of SERS spectra ($10^{-7}$ M CV) are collected from randomly 50 sites in 2D presentation. The main vibrations bands of CV molecules were listed in Table S1 [35]. The intensity of primary fingerprint peaks from 50 spots of SERS data exhibits good reproducibility in Fig. 1c. In order to further verify the uniformity of the substrate, Fig. 1d shows SERS area mapping of $10^{-7}$ M CV with the area of 20 μm × 20 μm from the band at 1621 cm$^{-1}$, demonstrating the uniform SERS substrates. This uniform substrate ensures a good reliability, so that it can efficiently combine with Fe-NTA label and then capture the target molecule from complex specimen.
3.2. SERRS sensing for CA molecules

The signal sensing strategy for SERRS detection of the three CA molecules is shown in Fig. 2a. Typically, it is very difficult to directly detect CA by using PVP stabilized Au NPs because of the intrinsic small Raman cross section of CA (Fig. 2b2–d2, green line). Nonetheless, many previous works have reported that complexes of Fe(III)-catechol indicates the features of special stability and ligand-to-metal charge transfer, bringing CA complex with a high Raman cross section [36]. In this report, consequently we decorate the Au NPs with Fe-NTA through Au–N bonds as functionalized substrate (Fig. S4). The complex of NTA-Fe-CA has special stability because of the formation of chelate ring. Therefore, the functionalized substrate can not only capture CA molecules, but also provide enhanced Raman signal readout (Fig. 2a). In the absence of Fe-NTA molecules, the CA molecules demonstrate the weak Raman bands due to the non-resonant state. As is shown in Fig. 2(b2–d2, red line), upon addition of CA, the strong Raman signals are clearly observed. The signal-on effect is due to the formation of NTA-Fe-CA complex, which induces the transformation of CA molecules from the non-resonant state to electronic resonant state. The formed three complexes can be detected since the new absorption peak around 600 nm are appeared in UV–vis absorption spectra (Fig. 2b3–d3, red line) in comparison with three CA molecules and Fe-NTA complex.
Here, there are several advantages for detection of CA. Typically, the localized surface plasmon of Au NPs is ascribed to the plasmonic absorption in the range of 525–650 nm. Moreover, the electronic resonance of NTA-Fe-CA complex is match with 633 nm laser, as revealed in Fig. 2(b3–d3). Thus, the resonances bring the high electromagnetic (EM) field enhancement for sensitive detection of CA. In addition, Fe-NTA modification can improve the selectivity toward the CA, owing to the Fe-NTA label not only holds higher affinities for binding Au NPs but also brings the CA molecule closer to the NPs surface to trap CA with the formation of resonant state.

Furthermore, we studied the influence of pH value on detection of CA (Fig. S5, take DA as an example). When the pH value is 3, there is no obvious absorption band in ultraviolet range. However, with the increase of pH value, the new absorption peaks appear in the UV–vis range and then the absorption peaks are red-shifted, reaching the maximum at 613 nm when the pH value is 7. What’s more, the absorption bands are blue-shifted when the pH is higher than 7, which leads to the formation of iron hydroxide precipitation. Therefore, Fe-NTA modified on the Au NPs can provide the selectivity and stability as well as capture the CA proximal to the NPs surface under the pH = 7. In addition to UV–vis absorption spectra, FT-IR is also used to represent the combination of Fe-NTA with DA (Fig. S6). As a consequence, both of UV–vis absorption spectra and FT-IR spectra demonstrated the formation of resonant structure NTA-Fe-CA. In the next detection, our measurements would be performed under neutral conditions. However, in SERS detection process, the performance of the substrate is important for the reliability of the experimental results. In order to consider the effect of SERS detection about CA molecules, first a series of tests to understand the sensitivity, reproducibility and selectivity of the functionalized substrate are performed. Compared with blank sample of the functionalized substrate, the characteristic bands of CA molecules could be clearly identified, and all the RSD values of CA peak ascribed to $\nu_{13b}$ are lower than 15% with the concentration of $10^{-7} $ M (Fig. S7). Hence, the results above show the functionalized substrate with high sensitivity and reproducibility. In real biological fluids, interference problem is a key issue such as amino acid, glucose, AA, etc, thus, it is a great challenge to detect target molecule in complex system not only depending on detection limits and sensitivity, but also lying in selectivity. Furthermore, in order to illustrate the selectivity (here take DA as an example), Fig. S8 exhibits the strong SERS signal of NTA-Fe-DA at 1481 cm$^{-1}$, while other interferences like AA, various amino acids and glucose have not demonstrated the distinct peak at 1481 cm$^{-1}$. In addition, the stability of the presented SERS sensor was shown in Fig. S9. Therefore, we can conclude the functionalized substrate can selectively trap the target molecules from the complex specimen.

3.3. Detection of CA analytes in serum systems

The level of CA molecules including DA, NE and EP is related with the diagnosis of the diseases, therefore, it is encouraging to develop a method to detect and distinguish the CA from mixture sample. As presented in Fig. 3b, there is little difference among the SERS spectra of DA, NE and EP owing to their similar molecular structures, while the Raman shift and related intensities of charac-
teristic bands have slightly fluctuated [37]. To differentiate these three chemicals more intuitively, the further analysis using principal component analysis (PCA) is carried out on the basis of SERRS spectral data of DA, NE and EP. PCA is a simplified data set technique, which is often used to reduce the dimensionality of the data set, maintaining the largest contribution to the data set [38]. We first altogether collected 30 spectra in 10 different runs of experiments to DA, NE and EP, respectively. As shown in Fig. S10a, these three molecules DA, NE and EP can be clearly distinguished from independent SERRS measurements with 2D PCA. We further study the ability of the functionalized substrate for the detection of serum spiked with DA molecules. The serum from CAS Hefei Cancer Hospital and schematic procedures of pretreatment are exhibited in Fig. S11. The SERRS spectra of DA, serum and serum spiked with DA are shown in Fig. 3c. The bands located at 724 and 745 cm⁻¹ are attributed to v(C=H) of coenzyme A and adenine, the peak of 1445 is assigned to CH₂ bending mode of collagen and phospholipids, and 1580 cm⁻¹ is identified with C=C bending mode of various amino acid [39]. From the SERRS spectra of DA in serum sample, we can observe the characteristic peaks of DA can be clearly distinguished from that of serum sample. What’s more, both NE and EP in serum sample can also be detected successfully (Fig. 3d). As a consequence, the CA with similar structure can be clearly distinguished from mixture serum through SERRS technique combined with PCA (Fig. S10b). In addition, we further study the SERRS spectra of the binary and ternary mixtures of CA molecules (Fig. S10c). The difference is clearly observed in 2D PCA (Fig. S10d). Therefore, our data convincingly show that the CA molecules in mixture serum can be successfully distinguished by SERRS method combined with PCA.

3.4. Quantitative detection of CA molecules

The functionalized substrate with high reproducibility and stability provides the considerable advantages for quantitative detection. However, the absolute intensity of SERS signal is easy to be disturbed and varied by instrumental factors and other intrinsic properties. Therefore, it’s difficult to make quantitative SERS measurements utilizing the absolute intensity. Fortunately, in our functionalized substrate, the position of Au-O band derived from PVP stabilized Au NPs is stable with the alteration of the concentra-
function of the CA molecules, such that the SERS signal from Au-O can be considered as a normalized band to calibrate the SERS signals obtained from CA molecules for quantitative detection. As indicated in Fig. S12, the XPS spectra exhibit the bonding between Au and O atom. Fig. 4a shows the interaction mode between Au NPs and PVP molecule, and the corresponding SERS band locates at 228 cm\(^{-1}\), which is capable to distinguish from that of blank substrate. In Fig. 4b, the SERS spectra of DA (8.3 × 10\(^{-6}\) M) are from independent 10 times measurements. It is obvious that the absolute intensity of characteristic bands at 1481 (corresponding to benzene mode \(\nu_{19a}\) of CA) and 228 cm\(^{-1}\) are quite volatile even for the same concentration. Nevertheless, the variation of the peak intensity ratios at \(I(1481\text{ cm}^{-1})/I(228\text{ cm}^{-1})\) is quite slight (Fig. 4c). Due to the stable vibration, the band at 228 cm\(^{-1}\) from the functionalized substrate can be selected as stable normalization band. In addition, 228 cm\(^{-1}\) of Au-O band in the lower wavenumber region is an ideal vibration mode as a stable internal calibration standard for quantitative measurement without any overlap of the SERS signal from CA molecules. Thus, we can define a normalized DA SERS intensity by the ratio of SERS intensities from DA and Au-O band. The SERS spectra of different concentrations of DA are shown in Fig. 4d. Normalized relative intensity of DA exhibits good linear response as demonstrated in Fig. 4e. The regression equation of DA is \(I_R = -(0.824 \times \log[c]+5.4083\), and the linear correlation coefficient is 0.99855. The linear relationship between \(I(1481\text{ cm}^{-1})/I(228\text{ cm}^{-1})\) and –log[DA] can be achieved for quantitative detection in the range of 556 nM to 10 µM. Similarly, we define the normalized SERS intensity of NE and EP by \(I(1487\text{ cm}^{-1})/I(228\text{ cm}^{-1})\) and \(I(1485\text{ cm}^{-1})/I(228\text{ cm}^{-1})\), respectively. Fig. S13 shows the quantitative detection NE in the range of 125 nM to 10 µM, and for EP is in the range of 200 nM to 9.09 µM. According to the results above, our detection strategy can achieve quantitative SERS measurement for CA molecules.

3.5. Determination for binding constants of CA molecules with functionalized substrates

In this report, the functionalized substrate can selectively capture the target molecules owing to the formation of resonant structure. In other words, the binding process of CA molecules with the functionalized substrates is attributed to chemical adsorption. On the basis of the close-packed PVP stabilized Au NPs dispersed
by a large area of monolayer distribution, herein, theoretically we consider this adsorption process following the Langmuir model. SERRS spectra of CA molecules at various concentration are studied to determine the adsorption constant and binding strength with the functionalized substrate. Marker bands of the individual CA molecules were chosen from the V13b in order to obtain the corresponding binding constants. The adsorption constant [40] related to the interaction between the functionalized substrate and adsorbate can be deduced from the Langmuir adsorption model according to the expression.

\[ R_c = \frac{l_g}{l_0} = \frac{K_{ad}[c]}{1 + K_{ad}[c]} \]  

(1)

Here, \( R_c \) represents the coverage rate of the functionalized substrate by CA molecules and \([c]\) is concentration of the analyte. Due to the uniform substrate, we approximately consider that the SERS intensity depends on the number of molecules adsorbed on the substrate surface. Hence, \( R_c \) is proportional to SERS intensities \( l_g/l_0 \) where \( l_g \) is normalized relative intensity at a concentration of CA and \( l_0 \) is SERS intensity of saturation coverage on the substrate surface under this concentration.

By simple conversion, Eq (1) can be expressed as

\[ [c]/l_g = \frac{[c]}{l_0} + 1/K_{ad}[l_g] \]  

(2)

It exhibits good linear response between the ratio of concentration to the normalized relative intensity and the concentration of analyte (Fig. 5b, d and f). The adsorption constant \( K_{ad} \) was calculated as follows: \( K_{ad}(DA) = 2.84 \times 10^{3} M^{-1}, K_{ad}(NE) = 1.02 \times 10^{2} M^{-1}, K_{ad}(EP) = 6.03 \times 10^{3} M^{-1} \). According to the value of \( K_{ad} \), we can conclude there is slight difference of the absorption properties between the three molecules. Therefore, this strategy can reflect the sensitive detection of CA molecules in complex system.

4. Conclusion

In summary, we have demonstrated the SERRS strategy for sensitive and selective analysis of CA molecules. By functionalizing the Fe-NTA label on the PVP stabilized Au NPs, both the CA response and SERS activity are integrated into the SERRS sensor Au-(FeNTA)). The self-assembly PVP stabilized Au NPs as active substrates provide high reproducibility. The Fe-NTA label has rapidly trapped the CA molecules adjoining the gold core to form the complex of NTAFe-DA resonant structure, thereby making the SERRS nanosensors suitable for the highly sensitive and selective detection of CA molecules by using 633 nm laser. Moreover, the three CA molecules spiked into serum sample can also be determined and distinguished by using SERRS technique combined with PCA successfully. Most important, using the Au-O band from PVP stabilized Au NPs as the stable internal calibration standard, we realize the quantitative measurement of CA molecules. Additionally, on the basis of data and adsorption process of CA molecules on the substrate with Langmuir model, we obtain the affinity of CA towards functionalized substrates with slight differences. We believe this proposed SERRS strategy shows great potential in qualitative and quantitative detection of CA involved in complex specimens.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.snb.2018.04.117.

References

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Binbin Zhou received his BS degree from Anhui University in China in 2013 and he is currently doing PhD thesis with Professor Liangbao Yang at University of Science and Technology of China. Her research interests focus on designing acupuncture needle as a SERS active platform to amplify signals of biological molecules.

Xiangdu Tan received his PhD degree in Materials Physics and Chemistry (2014) from the University of Science and Technology of China. He is currently an associate professor in the Institute of Intelligent Machines, Chinese Academy of Sciences, Hefei. His work was focused on the design and construction of complex structure SERS substrates for applications in environmental detection and catalytic monitoring.

Meilong Ge received his BS degree from Anhui University in China in 2016 and she is currently a master student with Professor Liangbao Yang at University of Science and Technology of China. Her research interests focus on designing and preparation of SERS composite substrates.

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Jinhui Liu currently works as a senior scientist and the director at Institute of Intelligent Machines, Chinese Academy of Sciences, China. He was a visiting scholar in Brown University (1986–1988) and Polytechnic University (1988–1989) in USA; a senior visiting scholar in the University of Cincinnati in USA (1993–1994) and in Toyohashi University of Technology in Japan (1996). He was appointed as the chief scientist of National Major Project of Fundamental Research (973 Project) from 2010 to 2015. His research addresses problems in drinking water purification, environmental pollutant detection, plasmonic nanodevice and SERS sensor fabrication.