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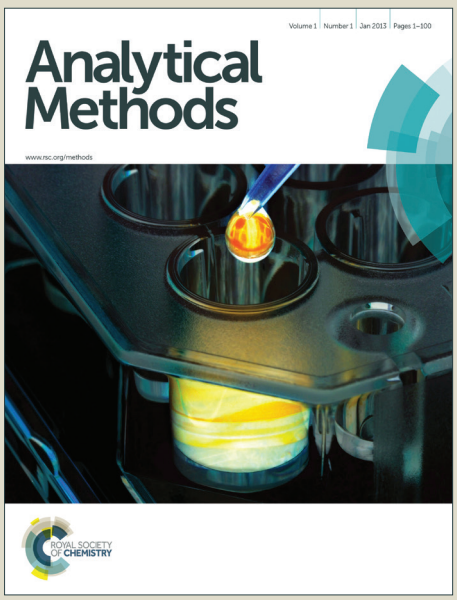

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ARTICLE

Rapid and simple detection of pethidine hydrochloride injection using surface-enhanced Raman spectroscopy based on silver aggregates

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A portable Raman spectrometer was used for rapid detection of the surface-enhanced Raman spectroscopy (SERS) of pethidine hydrochloride injection employing silver colloid as SERS active substrate. Different substrates and aggregation agents were investigated in order to explore the optimum conditions for SERS detection of pethidine hydrochloride injection. Under the optimum experimental conditions, an excellent reproducibility and stability of SERS detection was guaranteed. In addition, the limit of detection (LOD) for pethidine hydrochloride injection in water was low to 0.1 μg mL^{-1} with an analytical enhancement factor of 5.3×10^4, which was extremely below typical administered dosages (50 mg mL^{-1}). Finally, good linear relationship was obtained for pethidine hydrochloride injection in water at concentration ranges from 0.1 to 10 μg mL^{-1} (R^2=0.999), which lays a favourable foundation for the semi-quantitative analysis of the concentration of pethidine hydrochloride injection. In general, the capabilities reported here demonstrate that SERS method is convenient, rapid and efficient, which have good potential clinical applications for point-of-care detection and real-time monitoring.

Introduction

Pethidine hydrochloride ($C_{15}H_{21}NO_2$.HCl) injection, as one of the most common antalgic drugs, has been widely used for the clinical treatment by surgeon doctors in previous years.[1] Although pethidine hydrochloride injection is suitable for all kinds of pain, it is also an analogue of illicit drug which must abide strictly by national special management regulations to avoid possible abuse such as incorrect dosage, substitution of one drug for another, and infusion of a drug that was not actually prescribed by Intravenous (IV) therapy.[2, 3] Among them, overdose represents a noteworthy challenge to emergency room (ER) personnel, since it can bring about a variety of symptoms. What's more, if used illegally for a long time, mild cases will cause myocardial infarction, hypothermia, seizures, hallucinations, or arrhythmias and severe cases may produce toxicity or even to death.[1, 4, 5] Thus, hospitals' medication safety efforts will be primarily focused upon the monitoring of illicit drugs. The prescription should be retained for two years for future reference, and the colour of the prescription should be distinguished with other medicines as well. In addition, the illicit drugs must be detected frequently to provide effective guarantee for the strict control of them.

In present, to detect pethidine hydrochloride injection, various methods have been reported such as HPLC, [6, 7] GC, [8, 9] GC/MS[10] and spectrophotometry[11] etc., these methods are all capable of determining drug abuse and providing quantification analysis. However, among these methods, most usually require more complex pre-treatments as well as large-scale instruments,

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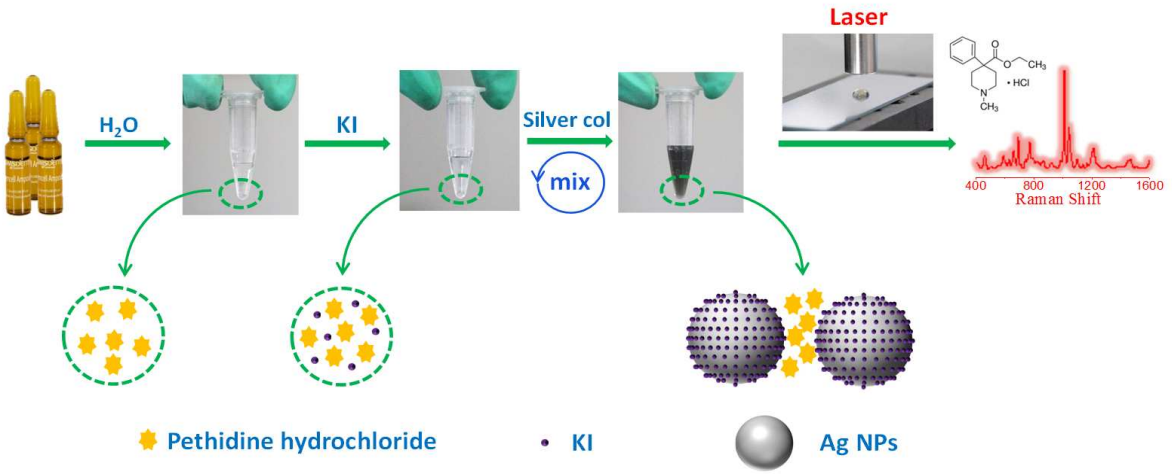

Fig. 1 Schematic Diagram of the Method Proposed for Pethidine Hydrochoride Injection Detection Process

which make the existing methods are not conducive for rapid and real-time determination. Therefore, it is of great realistic significance to develop a simple, sensitive and inexpensively method for the real-time identification of pethidine hydrochloride injection.

Since the first observation of an enhanced Raman signal of pyridine adsorbed on a roughened silver electrode by Fleischmann et al[12] in 1974, surface enhanced Raman spectroscopy (SERS) has been a powerful vibrational spectroscopy technique that allows for highly sensitive structural detection of low concentration analytes. [13, 14] Gold, silver and other metal nanostructures as SERS active substrate can significantly improve the enhancement effect, which mainly attributes to the amplification of electromagnetic fields generated by the excitation of localized surface plasmas on the rough surface of the metal.[15] Ag nanoparticles have been a focus of much research and more than six orders of magnitude of SERS enhancement was achieved routinely.[16] In addition, both Raman and SERS spectroscopic investigations can be performed without the interferences of water, thus these techniques can be put into used for the research of organic and biochemical substances in their natural environment.[17, 18] However, One major problem that arises in applying Raman spectroscopy is the normal Raman spectroscopy is quite weak and always obscured by fluorescence. Therefore, the SERS technique has been widely used in various fields including food safety, drug monitoring, bioanalysis and materials characterization, [19-24] resulting from its significant advantages including eminent specificity, narrow spectral band peaks and high sensitivity.[25, 26] Meanwhile, there is only a portable Raman spectrometer used in SERS detection, not large-scale equipment, which provides rapid detection for illicit drugs in convenient spot.

In this letter, we have developed a rapid and simple SERS method to detect pethidine hydrochloride injection utilizing the combination of a portable Raman spectrometer with silver colloid (Fig 1). The SERS enhance efficiency of several silver aggregates induced by different aggregation agents have been investigated. At the same time, a great repeatability and stability of the SERS detection were ensured. Further, the SERS spectra of pethidine hydrochloride injection at different concentrations in water were collected. Eventually, the potential application of SERS technology in quantitative measurement of pethidine hydrochloride injection was illustrated. The SERS detection method is reliable, fast and simple to operate, thus, it can be used for the monitoring of illicit drugs in realistic environment.

Experiment

Materials

Silver nitrate ($AgNO_3$) and sodium citrate were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), pethidine hydrochloride injection (50 mg mL^{-1}) was obtained from Yichang Humanwell Pharmaceutical co., Ltd. (Hubei, China), potassium iodide (KI), sodium chloride (NaCl) were obtained from Xilong Chemical Co., Ltd. (Beijing, China), sodium bromide (NaBr) was obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China), quartz wafer was obtained from Nantong optical technology co., Ltd. (Nantong, China), deionized water was used for all procedures. All chemicals were used as received.

Sample preparation

Pethidine hydrochloride standard stock solution (100 μg mL^{-1}) was prepared at first. A series of concentrations of standard pethidine hydrochloride solutions were prepared by diluting the stock solution with deionized water to obtain concentrations of 10, 7.5, 5, 2.5, 1, 0.5, 0.25, 0.1 μg mL^{-1}. Water is as blank control.

Preparation of silver colloid

Silver colloid was prepared according to the method of Lee and Meise.[27] Briefly, 45 mg of silver nitrate was added to 250 mL of deionized water, which was then brought to a boil in a flask under vigorous stirring. 5 mL of 1% sodium citrate was added, and the solution was keep boiling for 1 hour. The silver colloid was cooled naturally after the solution turned to greenish brown. Finally, the silver colloid was stored at 4 ºC.

SERS measurement

Raman spectra were recorded by a portable compact laser Raman Spectrometer BWS415-785H (B&W Tek, Inc.). The excitation wavelength of the laser is 785 nm and the Raman spectrum is over the range of 175 to 3200 cm^{-1} with a spectral resolution of better than 3 cm^{-1}. The beam was converged using the lens which the focal length is 6.8 mm, and the spot size of focus laser beam is about 10 μm in diameter. The spectral measurements were conducted with 5 s exposure time and laser power of 30 mW besides otherwise states, and the SERS spectra were collected in a certain period of time (<2 minutes), in which the intensity of Raman characteristic peak remains almost stable (Fig S1). Boxcar averaging was used to smooth the raw spectrum, and a baseline correction routine was performed to obtain the final spectrum with the background subtracted.

Results and discussion

The TEM image for Ag nanoparticles

The intensity of the Raman characteristic peak of pethidine hydrochloride injection usually depends on several parameters such as the type of substrate, substrate concentration, and aggregating agent. As illustrated in Fig 2, the Ag NPs were almost spherical and the average size of Ag NPs was between 30 and 40 nm.

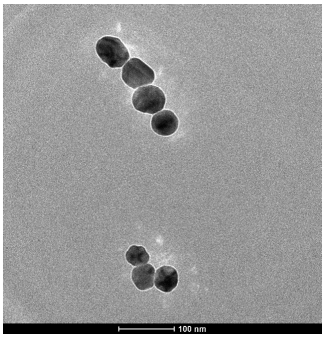

Fig. 2 The TEM image for Ag nanoparticles.

Firstly, compared with other new-type substrates, the preparation method of Ag nanoparticles is quite simple, and the Ag nanoparticles also provide observably SERS enhance efficiency, making the Ag nanoparticles meet the need for convenient, rapid and real-time detection. Afterwards, although previous studies have reported that the silver substrate presents a poor stability and reproducibility, the reason for this phenomenon mainly result from non-ideal conditions, such as high temperatures and harsh chemical environments during monitor processes [28, 29]. Whereas the laser power is 30 mW in this study, and each sample for detection is freshly prepared, moreover, the SERS spectra are collected in a certain period of time to avoid long time exposure under a laser heating effect (Figure S1). Thus the influences of the external conditions on the experiment are negligible. Finally, the focused laser beam has a spot size of 10 μm which is big enough to result a statistical mean value. Therefore, the Ag nanoparticles are chosen as SERS active substrate to detect pethidine hydrochloride injection.

The Raman spectra of different substrates and the normal Raman, SERS spectrum of pethidine hydrochloride injection

The substrate carrying silver colloid and samples will cause interference for the Raman spectrum of pethidine hydrochloride injection. Thus, the Raman spectra of three different substrates including ordinary glass slide, silicon wafer and aluminium foil tape were collected (Fig 3(a)). Strong Raman peaks at 300-500 cm^{-1} as well as 1000-2000 cm^{-1} were observed in the Raman spectrum of ordinary glass slide caused by the impurities (Na_2O and CaO), which is extraordinary easy to cause interference for the Raman spectrum of the pethidine hydrochloride injection; nevertheless, almost no obvious Raman peaks were observed in the Raman spectra of silicon wafer and aluminium foil tape, which won't cause any interference for the Raman spectrum of pethidine hydrochloride injection. However, the silicon wafer is not suitable for widely used in realistic detection for its high prices. Therefore, aluminium foil tape was selected as substrate for SERS detection due to its low cost as well as weaker interference.

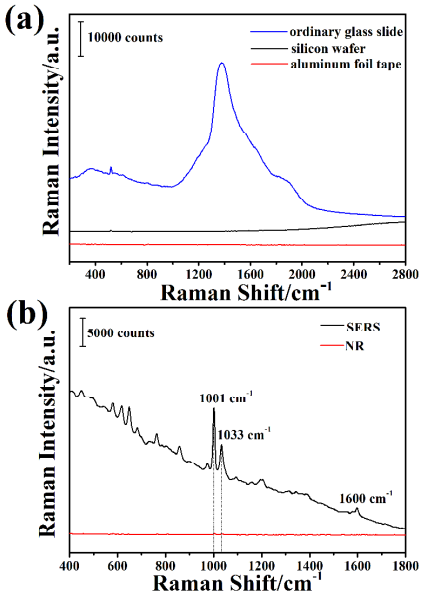

Fig. 3 Raman spectra of three different substrates (a): ordinary glass slide (blue line), silicon wafer (black line) and aluminum foil tape (red line), and normal Raman (red line) and SERS (black line) spectra of pethidine hydrochloride injection (50 mg mL^{-1}), respectively (b).

For comparison purposes, the normal Raman and SERS spectra of pethidine hydrochloride injection were measured by means of a portable Raman spectrometer. Proper volume of pethidine hydrochloride injection was dropt on the aluminum foil tape to measure its normal Raman spectrum; then adding silver colloid according to 1:1 proportion to measure its SERS spectrum. Finally, the corresponding Raman spectra were collected (Fig 3(b)).

As shown in Fig 3(b), no obvious Raman signal was observed without the addition of silver colloid. The reason to this phenomenon is the normal Raman signal of pethidine hydrochloride injection is dramatically weak, which illustrates that the normal Raman detection for pethidine hydrochloride injection is of little practical value. On the contrary, the intensity of many dominant vibration bands increases markedly in the SERS spectrum of pethidine hydrochloride injection, mainly distributed in the places such as 1001, 1033 and 1600 cm^{-1}, which are all attributed to phenyl modes.[1] In short, compared with normal Raman spectroscopy, SERS technology can provide much higher sensitivity, and the identical SERS spectral feature to that of normal Raman in both relative peak intensity and frequency suggests that SERS technology is competent for both qualitative and quantitative analysis. Therefore, it is of significant realistic meanings to use SERS technology in inspection of pethidine hydrochloride injection.

The effects of different aggregation agents on SERS enhance efficiency

As a kind of reducing reagent, sodium citrate plays a vital role in the synthesis of silver colloid.[30] Unfortunately, the citrates surrounding nanoparticles not only prevent sample molecules from adsorbing on Ag nanoparticles but also seriously limit the enhance efficiency of Ag nanoparticles. In general, if the Raman signal is quite weak when using original silver colloid as SERS substrate, it can be dramatically promoted by adding a proper amount of inorganic salt, which can make the samples more fully absorbed on the Ag nanoparticles.[31] However, there are observable differences on the SERS enhancement effect among the different inorganic salts in a particular experiment. Therefore, three kinds of inorganic salt including KI, NaCl and NaBr were chosen in this experiment in order to achieve remarkable SERS enhancement effect.

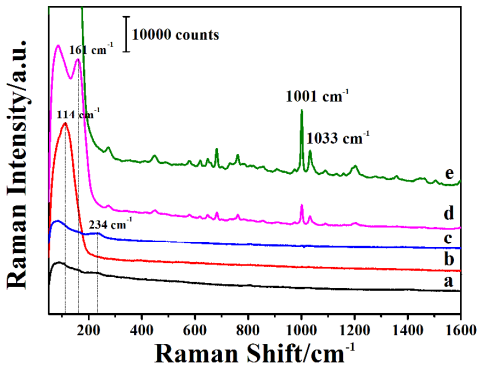

Fig. 4 SERS spectra of original silver colloid (a), KI with silver colloid (b), and SERS spectra of pethidine hydrochloride standard stock solution (100 μg mL^{-1}) with silver colloid induced by (c) NaCl, (d) NaBr, (e) KI. 50 μL pethidine hydrochloride standard stock solution were integrated with 10 μL KI, NaCl and NaBr solution, respectively, which concentrations in test are both 0.1M, then followed with 50 μL silver colloid for SERS detection.

The SERS spectra of pethidine hydrochloride standard stock solution with different aggregation agents were presented in Fig 4. It is obvious that SERS enhancement effect of KI as aggregation agent is most outstanding contrast with NaCl and NaBr, which mainly attributes to its types of bindings with Ag NPs[33]. At the same time, a strong Ag–I band at 114 cm^{-1} and clean background in the region of 50−1600 cm^{-1} were observed in the SERS spectrum of Ag colloid with KI compared with original silver colloid.[32] As a result, KI as an aggregation agent not only works best but also won't disturbed by the other impurity peaks. In addition, the influence on SERS enhancement effect of the samples prepared with different mixing procedures has been studied, and the highest enhance efficiency was obtained with the presence of pethidine hydrochloride injection when undergoing aggregation. The reason is that the pethidine hydrochloride molecules would have more opportunities to adsorb on those hot spots if they were present in the solution during the particle aggregating process.[34-36] Moreover, the SERS enhancement effect is best when the pethidine hydrochloride injection, KI and silver colloid in accordance with the ratio of 5:1:5 as well as under the neutral experimental environment (Figure S3) through practical research.

The ultraviolet absorption spectra of samples

The UV−visible absorption spectra of original silver colloid, silver colloid with KI and silver colloid with KI as well as pethidine hydrochloride standard stock solution (100 μg mL^{-1}) were measured (Figure 5).

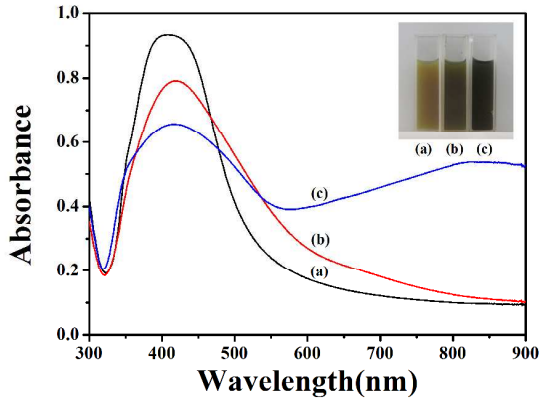

Fig. 5 The ultraviolet absorption spectra of the original silver colloid (a), silver colloid with KI (b) and silver colloid with KI as well as pethidine hydrochloride standard stock solution (100 μg mL^{-1}) (c).The ultraviolet absorption spectra were collected by the reagent's ratio of 5:1:5 reported above.

As shown in Figure 5, the UV−visible absorption band of original silver colloid was located at 409 nm (Figure 5(a)), which can be assigned to the plasma resonance absorption of single isolated silver nanoparticles in water. Moreover, the UV−visible absorption peak of the mixture solution of silver colloid with KI was slightly red-shifted to 419 nm (Figure 5(b)), and a change of colour from greenish brown to dark green of silver colloid, the reason for this phenomenon was that the I^- would replace the citrates surrounding nanoparticles due to its high adsorption

performance, which would decrease the stability of the citrate-stabilized Ag NPs, leading to aggregation of Ag NPs. [37, 38] When a certain amount of pethidine hydrochloride standard stock solution added, a new strongly broad UV−visible absorption peak in longer wavelength region was observed (Figure 5(c)) and the intense band at 419 nm was reduced to some extent. The peak of this stable new band was usually various from the added molecules and their concentration. To the pethidine hydrochloride injection-silver colloid system, this peak was at about 824 nm and it appears as a consequence of the pethidine hydrochloride absorption on the silver aggregates surface, which induced a variation in the surface charge density leading to the aggregation once again due to the chlorine ion it contains.[39, 40] However, although pethidine hydrochloride injection could also induce the aggregation of bare silver colloid, an apparent SERS signal from pethidine hydrochloride injection without KI as aggregation agent was not observed (Fig S2). Therefore, KI played an important role to active silver nanoparticle, and the Raman signal of the pethidine hydrochloride adsorbed on the Ag aggregates greatly increase was result from the aggregated Ag NPs produced a number of hotspots. In conclusion, the appearance of new peak is beneficial for the mixture solution to make a better match with the given laser excitation wavelength (785nm). [41]

The calculation of the enhancement factor (EF)

In order to determine the Raman enhancement of the silver aggregates, this can be quantitatively evaluated by the SERS enhancement factor (EF). Thus, the SERS spectrum of pethidine hydrochloride standard stock solution (100 μg mL^{-1}) and the normal Raman spectrum of pethidine hydrochloride injection (50 mg mL^{-1}) were collected (Fig 6). The enhancement factor can be calculated by the formula (1):

$$EF = (I_{SERS} / I_{RS}) (C_{RS} / C_{SERS}) \quad (1)$$

Where I_{SERS} and I_{RS} is the integrated peak intensity of the characteristic Raman band ($1001cm^{-1}$) of pethidine hydrochloride injection from SERS and normal Raman spectrum, respectively. C_{SERS} and C_{RS} is the concentration of pethidine hydrochloride injection used in the normal Raman and SERS measurement, respectively.

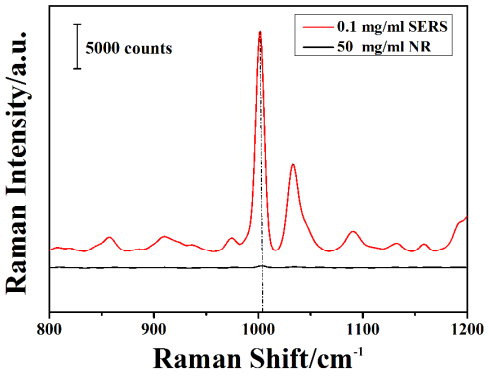

Fig. 6 SERS spectrum of pethidine hydrochloride standard stock solution (100 μg mL^{-1}) (red line) and normal Raman spectrum of pethidine hydrochloride injection (50 mg mL^{-1}) (black line). The Raman intensity at 1001 cm^{-1} was used to calculate EF values.

The peak intensity of normal Raman spectrum of 50 mg mL^{-1} pethidine hydrochloride injection is 238.8, while the prominent peak intensity of SERS spectrum of 100 μg mL^{-1} pethidine hydrochloride standard stock solution is 25089.8 (Fig 6). An approximately 5.3 × 10^4 fold of signal enhancement in the SERS spectrum was achieved by the formula (1). It suggests that the area of the SERS peak is at least 4 orders of magnitude higher than that of the normal Raman peak, and the proposed silver aggregates is potentially applicable for the rapid determination of pethidine hydrochloride injection.

The reproducibility of SERS detection

The reproducibility and stability play a vital role in SERS detection of pethidine hydrochloride standard stock solution. In order to study the stability and reproducibility of the SERS detection, 15 times repeated SERS measurement were conducted respectively based on the optimum experimental conditions. It should be point out that the small droplet on aluminium foil tape each time for detection was freshly prepared and the SERS spectra of pethidine hydrochloride standard stock solution (100 μg mL^{-1}) from 15 times repeated experiments were collected (Fig 7).

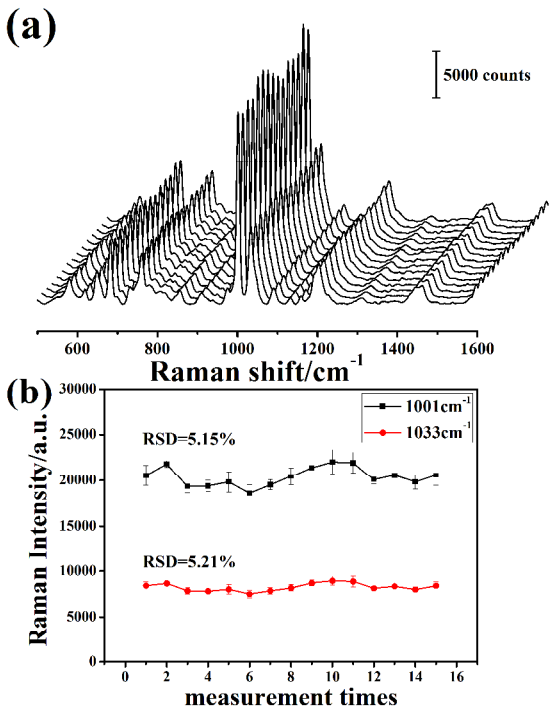

Fig. 7 The reproducibility and stability of SERS detection for pethidine hydrochloride standard stock solution (100 μg mL^{-1}) (a).The intensity distribution of the 1001 cm^{-1} peak (black line) as well as 1033cm^{-1} (red line), respectively (b). 25 μL freshly prepared mixture were spotted on the aluminum foil tape for SERS detection each time. SERS spectra were collected successively from 15 times repeated experiments and each spectrum represents the average value from five SERS spectra.

As displayed in Fig 7(a), the dominant SERS intensity peaks of pethidine hydrochloride standard stock solution at 1001 cm^{-1} as well as 1033 cm^{-1} were markedly clear; meanwhile, no SERS intensity variation was found from 15 times repeated experiments, indicating a good stability. Furthermore, the intensities at 1001 cm^{-1} and 1033 cm^{-1} were measured to quantitatively evaluate the reproducibility, as illustrated in Fig

7(b). The SERS intensity of pethidine hydrochloride standard stock solution remained almost constant over the 15 times repeated experiments, and the relative standard deviation (RSD) of the peak intensities at 1001 cm^{-1} and 1033 cm^{-1} were calculated to be 5.15% and 5.21%, respectively. It illustrates that the excellent stability and reproducibility of the SERS detection for pethidine hydrochloride injection were obtained. In addition, the brilliant reproducibility overcomes the obstacle of normal Raman spectroscopy and offers SERS as a reliable analytical tool for practical analysis.

The SERS spectra of pethidine hydrochloride injection in water at different concentrations

SERS spectra of pethidine hydrochloride injection in water at different concentrations were recorded using KI as aggregation agent, and each average SERS spectrum from five spots was collected under the optimal experimental conditions (Fig 8). The peak intensity of the strongest peak (1001 cm^{-1}) was chosen for the quantitative measurement of pethidine hydrochloride injection in water. As shown in Fig 8, the specific peak of pethidine hydrochloride injection at 1001 cm^{-1} increased concomitantly with increasing the corresponding concentration in water, and even at the 0.1 μg mL^{-1} level, the strongest signal at 1001 cm^{-1} can also clearly be collected, thus the detection limit for pethidine hydrochloride injection in water can be as low as 0.1 μg mL^{-1}, which was extremely below typical administered dosages (50 mg mL^{-1}), revealing the high sensitivity of the SERS technology.

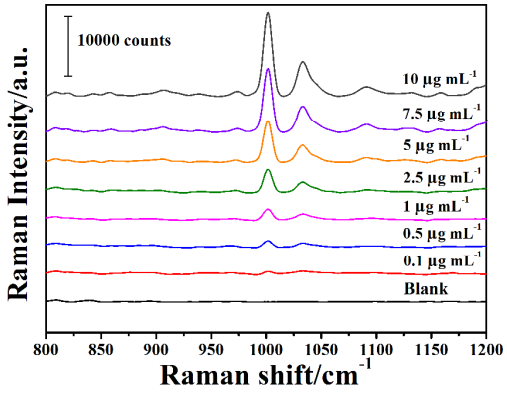

Fig. 8 SERS spectra of pethidine hydrochloride injection in water at various concentrations. Each spectrum represents the average value from five SERS spectra. The laser power is 50 mW and the exposure time is 10 s.

To investigate the relationship between SERS intensity and concentration of analyte, pethidine hydrochloride injection of each concentration was selected as a model analyte. Figure 9 plots the concentration of pethidine hydrochloride injection in water over the ranges of 0.1 ~ 10 μg mL^{-1} versus the intensity of the band at 1001 cm^{-1}. Within the concentration ranges of 0.1 to 10 μg mL^{-1}, the equation of the calibration curves y=1324.1+448.37x with R^2=0.999 was obtained through the linear fitting for data, where y and x represent the SERS intensity and the concentration of pethidine hydrochloride injection in water, respectively. All these results suggest that pethidine hydrochloride injection's concentration is linearly related to its SERS peak intensity. Obviously, the intensity of the characteristic peak of pethidine hydrochloride injection at 1001 cm^{-1} is linear increase with the enhancement of the corresponding concentrations in water. In brief, these results are forceful enough to provide a feasible approach to the detection of pethidine hydrochloride injection, indicating that the SERS spectroscopy presents a great application prospect in semi-quantitative analysis of pethidine hydrochloride injection in the future.

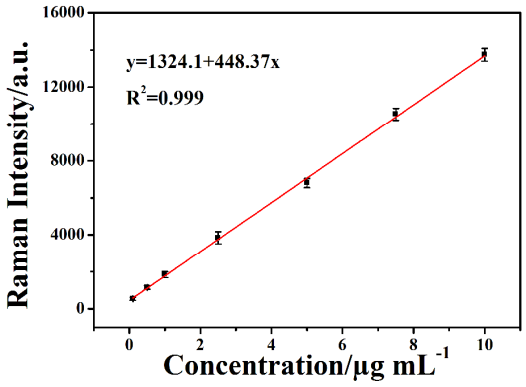

Fig. 9 Concentration dependence of the relative Raman intensity of the 1001 cm^{-1} SERS band of pethidine hydrochloride injection in water. Each data point represents the average value from five SERS spectra. Error bars show the relative standard deviations measured from five different spots.

Conclusions

In summary, great potential application prospect of the SERS technique is demonstrated by applying it to the identification of the pethidine hydrochloride injection in this paper. The interference for Raman spectra by using different substrates was researched, and aluminum foil tape was chosen as substrate for its low price and weakly interference. In addition, the enhancement performance of silver aggregates induced by NaCl, NaBr and KI has been compared, and the prominent enhancement effect was achieved using KI as aggregation agent. Furthermore, a great reproducibility and stability was guaranteed under the optimum experimental conditions, and the relative standard deviation (RSD) of peak intensities of pethidine hydrochloride standard stock solution at 1001 cm^{-1} and 1033 cm^{-1} were 5.15% and 5.21%, respectively. Moreover, the detection limit of pethidine hydrochloride injection in water can reach to 0.1 μg mL^{-1} with an enhancement factor of 5.3 × 10^4. Good linearity (R^2=0.999) was acquired at concentrations ranging from 0.1 to 10 μg mL^{-1} for Pethidine hydrochloride injection in water. Eventually, surface enhanced Raman spectroscopy is competent to meet the need for the reliable and rapid identification for on-site as well as real-time detection of pethidine hydrochloride injection, preferably to enhance the security of legal certificates.

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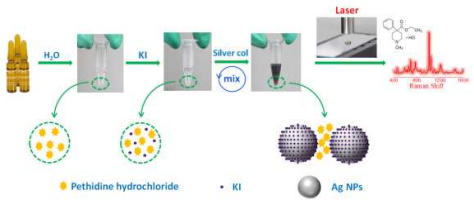

A rapid and simple SERS method for detecting pethidine hydrochloride injection utilizing silver aggregates as active substrate was developed.