Rapid and Sensitive SERS Method for Determination of Ketamine in Urine

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Abstract

Surface enhanced Raman spectroscopy (SERS) has gradually proved to be a powerful tool with wide application in various fields. Here, a simple and rapid SERS method was developed for the determination of ketamine in urine based on silver aggregates as SERS substrate. Ketamine in urine were demonstrated by SERS technique with silver sol aggregated by 0.5 M NaBr solution. The limit of detection for ketamine in urine could be obtained as low as 7.5 ppm and a linear relationship for ketamine in urine between the Raman intensity and the concentrations was achieved in the range from 7.5 to 150 ppm ($R^2=0.977$). Additionally, the recovery of this method ranged from 95.7% to 104.9%, which laid a favorable foundation for the rapid and reliable quantitative detection of ketamine in urine. Therefore, this SERS approach with high sensitivity and simplicity has a great prospect for the real-world application of ketamine in urine.

Keywords: Surface enhanced Raman spectroscopy (SERS), drugs; ketamine, urine; silver sol.
Introduction

Ketamine is a clinically effective anesthetic derivate from Puppic Agent (PCP) and belongs to the NMDA (N-methyl-D aspartate) receptor antagonist. It is a synthetic sedative with rapid anesthesia which is always applied in the operation process of humans and animals. However, ketamine can cause psychedelic state, sedation and memory loss, and it is highly addictive with repeated abuse. After long-term use, it will damage to the nervous system, cardiovascular system, urinary system and digestive system, and it has been listed into controlled narcotic drugs by the United Nations. At present, some conventional methods for detecting ketamine have been reported and investigated including gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), and enzyme-linked immunosorbent assay (ELISA). Although the above-mentioned strategies are capable of high precision, the experiment require for large scale instrument and professional operator, which is not suitable for rapid detection on site. Therefore, it is of great significance to study a facile and accurate detection technique for ketamine.

In recent years, surface-enhanced Raman spectroscopy (SERS) has gradually become a powerful analytical technique, which has attracted the interest of many researchers. SERS technique inherits the high specificity of conventional Raman spectroscopy technology, and overcomes the problem of low sensitivity, leading to wide applications in various fields such as biology, medicine, materials, and food. The SERS spectrum has fingerprint characteristics, and the displacement in Raman spectra varied uniquely with the molecular structure. As a result, the presence and relative content of the chemical substances in the sample can be determined according to the Raman spectra. SERS spectroscopy provides fast, simple, repeatable, and more importantly, non-invasive qualitative and quantitative analysis, which eliminates the requirement for sample preparation. Based on this feature, some efforts has been devoted to the measurement of ketamine based on SERS techniques. For example, West et. al. reported
the application of Raman spectroscopy for the analysis of drugs of abuse in latent fingerprints that had been treated with powders and also subsequently lifted with adhesive tapes. However, only qualitative analysis of ketamine was conducted in the study and no quantitative analysis in real-world sample was investigated.

In this work, SERS technology was used to quantitatively analyze ketamine in aqueous solution and in urine based on silver sol as SERS substrate. The limit of detection for ketamine in urine could reach 7.5 ppm and a good linear relationship could be obtained in the concentration ranging from 7.5 to 150 ppm, which laid a good foundation for rapid on-site detection of ketamine. Additionally, high recovery rate of this SERS approach ensure the accuracy of the detection and analysis results.

**Experimental**

**Reagents and chemicals**

Silver nitrate (AgNO₃), sodium citrate were obtained from Sinopharm Chemical Reagent Co., Ltd., Ascorbic acid (AA), potassium iodide (KI), sodium chloride (NaCl) were obtained from West Long Chemical Co., Ltd., Sodium bromide (NaBr) was obtained by Tianjin Branch-Europe Chemical Reagent Co., Ltd., Cyclohexane (C₆H₁₂) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd., artificial urine was obtained from Shanghai Jingdu Biotechnology Co., Ltd. Deionized water was used for all procedures. All chemicals were used as received.

**Preparation of silver nanoparticles and samples**

Here, some modification have been carried out to the preparation methods of silver sol proposed by Qin et. al.¹⁹ The process in detail was described as following: first, AA (6.0×10⁻⁴ M, 125 mL) and sodium citrate (3.0×10⁻³ M, 125 mL) were mixed. The mixed solution was reacted to 30 °C in a water bath with gentle stirring, and then AgNO₃ (10⁻¹ M, 2.5 mL) was quickly added to the mixed solution. After stirring for 15 minutes, the mixed solution was heated to
The silver sol was synthesized by the method proposed by Qin et al. with some improvements. The morphology of the silver sol was characterized by SEM as displayed in Figure 1(a). The morphology of the silver sol nanoparticles is close to the sphere, and there is almost no nanowires and nanorods compared with the conventional silver sol. The reaction can be

Characterization and SERS measurements

Scanning electron microscopy (SEM) images were taken using a microscopy (model S-4800, Hitachi, Japan) with an acceleration voltage of 5.0 kV. Raman spectra were recorded by a portable laser Raman spectrometer (BWS415-785H, B&W Tek, Inc.). The excitation wavelength of the laser was 785 nm. The spectrometer provided that the spectral measurement range was 175~2000 cm\(^{-1}\) and spectral resolution was better than 3 cm\(^{-1}\). The laser power was 80 mW and integration time was 5 s unless otherwise stated.

Results and Discussion

Preparation of silver sol and selection of aggregate agent

The silver sol was synthesized by the method proposed by Qin et al. with some improvements. The morphology of the silver sol was characterized by SEM as displayed in Figure 1(a). The morphology of the silver sol nanoparticles is close to the sphere, and there is almost no nanowires and nanorods compared with the conventional silver sol. The reaction can be

A ketamine aqueous solution with a series of concentration of 0.5 ppm, 1 ppm, 2.5 ppm, 5 ppm, 7.5 ppm, 10 ppm, 25 ppm, 50 ppm and 75 ppm were prepared using ketamine hydrochloride injection (specification: 0.05 g/mL). The blank was deionized water. The preparation of the spiked urine sample is referred to the pretreatment method of Han et al. Sample solutions of ketamine into artificial urine with mass concentrations of 150 ppm, 75 ppm, 50 ppm, 25 ppm, 10 ppm, and 7.5 ppm were prepared. The spiked samples require a simple pretreatment process and the specific process is described as follows. Firstly, the solid NaCl was added to supersaturate the mixed solution. Then the mixture was extracted with cyclohexane and centrifuged at 10000 rpm for 2 min. Finally, the separated upper organic layer was taken out for experimental detection.

100 °C and boiled for 2 hours. The prepared sol was then cooled to room temperature and stored at 4 °C in the dark.
accomplished in half an hour with AA as reducing agent, which allows the nanoparticles insufficient to grow into other shaped nanoparticles. The particle size was analyzed from 100 random nanoparticles and the statistics results were depicted in Figure 1(b). The size of silver nanoparticles was concentrated between 52 and 70 nm with a uniform particle size.

In the process of synthesizing silver sol by chemical reduction method, citrate was employed as a stabilizer. However, citrate will prevent the analyte from adsorbing to the surface of silver nanoparticles during the detection process, resulting in reducing the SERS enhanced effect. Therefore, it is necessary to introduce an appropriate amount of inorganic salt as an aggregate agent to change the dispersion state of the nanoparticles in the sol, breaking the potential balance of the sol system and accumulating the sol particles, thereby generating more hot spots and obtaining more excellent SERS performance.\textsuperscript{21-23}

Here, three kinds of salt solutions, NaCl, NaBr and KI were selected as aggregation agents. The high adsorption performance of I\textsuperscript{-}, Cl\textsuperscript{-} and Br\textsuperscript{-} was wrapped on the surface of silver nanoparticles, which replaces the original citrate, which greatly enhances the Raman spectra. SERS detection of aqueous solution of ketamine with three different aggregation agents under the consistent conditions were carried out, as shown in Figure 2(a). The strong characteristic peaks were mainly located at 455, 604, 656 and 1050 cm\textsuperscript{-1} according to the literature.\textsuperscript{22} The results revealed that the best SERS behaviors were obtained by using NaBr as aggregation agent. It might because the exact strong absorption of Br\textsuperscript{-} on Ag nanoparticles for the detection of ketamine. The stronger affinity of other halide ion (I\textsuperscript{-}) on silver sol may prevent the sample molecule from binding with silver sol.\textsuperscript{24, 25} The difference in selecting the halide ion as best aggregation agent might be caused by the difference of sample molecules and detection system. Besides, from Figure 2(b), NaBr aqueous solution with a concentration of 0.5 M has the best enhancement effect. Therefore, 0.5 M NaBr aqueous solution was selected as the aggregation agent in the following experiment. Each time the detected droplets were mixed from the sample,
silver sol and NaBr solution in a volume ratio of 5:5:1. In the meantime, the photos of the ketamine, silver sol and NaBr solution in a cuvette (total volume: 4 mL) with different extent of aggregation were also presented in inset pictures in Figure 2 (c). It could be easily observed that the color of the solution varied significantly after the addition of the NaBr solution, and the color of the solution gradually changed as the concentration of the aggregation agent. The corresponding UV-vis absorption spectra were examined as displayed in Figure 2(c). We can conclude that the concentration of the aggregation agent in our study is within the controllable range, and the maximum concentration of the aggregation agent does not cause obvious excessive aggregation to sink. From Figure 2(d), when a mixed solution was added with a maximum concentration of NaBr, the laser can still pass through the solution and the scattered light could reach the detector without any block.

The repeatability and stability of the experiment are also an important parameter when performing SERS detection of ketamine.26. 27 Under the above-mentioned optimal detection conditions, the repeatability of SERS detection for ketamine aqueous solution at a concentration of 5 ppm was conducted, as demonstrated in Figure 3(a). It was clear that the SERS spectra have good consistency for 20 groups detection, which indicates that SERS detection has excellent stability. As presented in Figure 3(b), a low relative standard deviation (RSD) of 5.56% for the Raman characteristic peak at 656 cm\(^{-1}\) was calculated and obtained, which verifies splendid repeatability of this SERS method.

**SERS measurement for ketamine in aqueous solution and in urine**

The SERS spectra of different concentrations of ketamine aqueous solution were examined as shown in Figure. 4(a). It was apparent that the Raman peak intensity of ketamine increased with the increase of concentration. The SERS signal of ketamine could be clearly observed when the concentration was as low as 1 ppm, and the intensity was greater than 3 times of the signal-to-noise ratio. Therefore, the limit of detection limit (LOD) for ketamine in
aqueous solution could reach 1 ppm. Then, the Raman characteristic peak at 656 cm$^{-1}$ was selected as the analysis object, and the variation of Raman characteristic peak intensity with concentration of sample was plotted in Figure 4(b). As the sample concentration increases, the characteristic peak intensity also increases, but the increase degree is slower and slower. As the concentration of the sample increases, the ketamine molecule adsorbed on the surface of the silver nanoparticles might be saturated with the gradual increasing concentration of ketamine. A linear fitting was performed in the concentration range from 1 ppm to 10 ppm with a correlation coefficient ($R^2$) of 0.981, indicating that quantitative analysis for ketamine in water could be performed within this concentration range.

Similarly, the ketamine in urine was tested, and the SERS spectra of the samples at different concentrations were collected as shown in Figure 5(a). Here, artificial urine was selected, which is a solution that simulates the composition and pH of real urine. The spiked sample required some simple pre-treatment within 10 min and then mixed with silver sol and NaBr at the ratio of 5:5:1. As the concentration of the sample decreases, the Raman characteristic peak intensity at 656 cm$^{-1}$ also decreases. The low LOD for ketamine in urine could reach 7.5 ppm. The relationship between the concentration of ketamine in urine and the perk intensity at 656 cm$^{-1}$ was fitted as presented in Figure 5(b). It could be obtained a good linearity with $R^2$ of 0.977 in the concentration range from 7.5 ppm to 75 ppm for ketamine in urine. Additionally, to investigate whether other substances in real urine have an effect on the detection of ketamine. We took the real urine for preparing the spiked samples and tested the ketamine, as shown in Figure 5(c) and 5(d). The results of ketamine in real urine and artificial urine are similar without obvious difference. In fact, many other substances exist in real urine due to the different digestion processes of the individual. However, from the experimental results, other substances in real urine did not interfere with the detection of ketamine, which might be attributed to no Raman activity of other substances.
In order to verify the repeatability of the experiment and the accuracy of the fitting curve, the recovery rate was explored. In particular, the fitted curve and the peak intensity at 656 cm\(^{-1}\) was substituted back to determine the concentration, and the recovery rate was calculated, as listed in Table 1. The ketamine spiked samples were prepared at concentrations of 15 ppm, 80 ppm, and 100 ppm, respectively. **Three measurements were implemented to each spiked sample to calculate the spiked concentration.** The recovery rate was evaluated by the ratio of the calculated average peak intensity to the known spiked concentration, as described in Table 1. The recovery rate ranged from 95.7% to 104.9%, indicating the accuracy of the method for detecting ketamine in urine. The RSD of the recovery rate less than 5%, proving that this SERS method has great potential to achieve on-site and rapid detection of ketamine in urine.

**Conclusions**

In this paper, a facile and effective approach based on SERS technology was established to measure ketamine establishes. The main Raman characteristic peaks of ketamine are located at 455, 604, 656 and 1050 cm\(^{-1}\). Silver sol was performed as the SERS substrate with 0.5 M NaBr aqueous solution as aggregate agent to obtain excellent SERS behaviors. Under the optimal conditions, the sample, silver sol and NaBr with the volume ratio of 5:5:1 was mixed thoroughly for SERS measurement of ketamine in aqueous solution and in urine. The lower detection limit obtained in aqueous solution is 1 ppm, and the characteristic peak intensity at 656 cm\(^{-1}\) with the concentration of sample was fitted with a good linearity in the range of 1~10 ppm with \(R^2\) of 0.981. The LOD for ketamine in urine could reach as low as 7.5 ppm only with simple pre-treatment, and quantitative determination could be achieved in the range of 7.5~75 ppm with \(R^2\) of 0.977. Additionally, the recovery rate ranged from 95.6%~104.9% with RSD of 3.9%~4.6%, proving the accuracy and credibility of this SERS method. In summary, this method is expected to be applied in the rapid real-word detection and analysis of ketamine.

**Acknowledgements**
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References


Table 1. The recovery rate of ketamine in urine.

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Figure Captions

Fig. 1 a, The SEM image of the improved silver sol; b, The size distribution of the improved silver sol.

Fig. 2 a, SERS detection of ketamine with three different aggregation agents; b, SERS detection of ketamine with NaBr as aggregation agent with four different concentrations; c, The extinction spectra of Ag nanoparticles aggregated by NaBr solution with different concentrations; Inset: the corresponding pictures of Ag nanoparticles aggregated by NaBr solution with different concentrations; d, Ag nanoparticles aggregated by NaBr solution with maximum concentrations (1.5 M) under laser irradiation.
Fig. 3 The verification of experimental reproducibility; a, The SERS spectra of 20 groups sample solution repeated experiments at the concentration of 5 ppm under the same condition; b, The intensity distribution of Raman characteristic peak at 656 cm\(^{-1}\) of 20 groups sample solution.

Fig. 4 a, The SERS spectra of ketamine with concentrations of gradient descent in aqueous solution; b, The relationship between the Raman signal intensity and sample concentrations.
Fig. 5 a, The SERS spectra of ketamine with concentrations of gradient descent in artificial urine; b, The relationship between the Raman signal intensity and sample concentrations of ketamine in artificial urine; c, The SERS spectra of ketamine with concentrations of gradient descent in real urine; d, The relationship between the Raman signal intensity and sample concentrations of ketamine in real urine.