Rapid Detection of Dezocine in Biological Fluids
Based on SERS Technology

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Abstract

In this paper, a method based on surface enhanced Raman spectroscopy (SERS) technology for rapid detection of dezocine in urine and serum was established. Firstly, Ag colloid substrate was prepared and characterized. Then the Raman characteristic peaks of dezocine were assigned from both theoretical and experimental aspects. Finally, the Raman peak at 661 cm\(^{-1}\) was selected as its characteristic peak to perform SERS detection on dezocine in urine and serum, and the detection limit of dezocine in urine and serum was determined. The relationships between the characteristic peak intensity and the concentration of dezocine in urine and serum were fitted and the recovery rates were calculated. This rapid, accurate, non-destructive method establishes a good foundation for rapid on-site detection of dezocine in biological samples.

Keywords Surface Enhanced Raman Spectroscopy (SERS), Ag colloid, dezocine, urine, serum, rapid detection
Introduction

Dezocine (benzene morphine derivatives) is a new type of potent opioid analgesic, which belongs to opioid receptor agonist-antagonist drugs, and mainly stimulates κ receptors in the brain, brain stem and spinal cord. It has a certain antagonistic effect on the μ receptor, which has strong analgesia and slight sedation mainly used for postoperative analgesia, induction of general anesthesia and advanced analgesia. The drug possesses the advantages of good analgesic effect, fast onset, large safe dosage range, low adverse reactions, low dependence. It is widely used in clinical applications. With the increase in usage, the unreasonable application and adverse reactions of dezocine injection have also attracted more and more attention from clinicians. Although dezocine has mild adverse reactions and low addiction, its risk of addiction cannot be ignored and long-term use should be avoided. This medicine belongs to the second category of psychotropic drug administration, which needs to strengthen the review and monitoring of prescriptions and medical orders to prevent iatrogenic addiction.

To date, the current measurement methods for dezocine mainly include high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. Although these reported methods have high accuracy, sensitivity, and repeatability, these experiments are usually time-consuming and low concentration samples require extraction and concentration. Therefore, it is of great significance to develop a fast, accurate, and low-cost approach.

In response to the above problems, this paper proposes a method for rapid detection of dezocine in urine and serum based on surface enhanced Raman spectroscopy (SERS) technology. With the development of nanotechnology, the SERS technique based on gold or silver noble metal nanoparticles has overcome the weak signal of the traditional Raman spectroscopy and is widely applied in the field of pharmaceutical analysis. So far, to the best of our knowledge, the SERS technique has not been employed for the detection of dezocine. In this paper, dezocine in urine and serum were measured based on SERS technology, and this SERS method
establishes a reliable foundation for rapid on-site detection of dezocine.

**Experimental**

*Reagents and instruments*

Dezocine injection (specification: 1 mL/5 mg) was obtained from Yangtze River Pharmaceutical Group Co., Ltd. Silver nitrate (AgNO₃) and sodium citrate (C₆H₅Na₃O₇·2H₂O) were obtained from Sinopharm Chemical Reagent Co., Ltd. Ascorbic acid (AA), potassium iodide (KI), sodium chloride (NaCl) and sodium sulfate (Na₂SO₄) were obtained from West Long Chemical Co., Ltd. Sodium bromide (NaBr) was obtained from Tianjin Branch – Europe Chemical Reagent Co., Ltd. Cyclohexane (C₆H₁₂) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Artificial urine was obtained from ChuangFeng Technology Co., Ltd. Deionized water was used for all procedures.

Raman spectra were recorded with a portable laser Raman spectrometer (BWS415-785H, B&W Tek, Inc.). The excitation wavelength of the laser was 785 nm. The spectral measurement range was 68 - 2700 cm⁻¹ and the spectral resolution was better than 3 cm⁻¹. The laser power was 80 mW and the integration time was 5 s unless otherwise stated. Scanning electron microscopy (SEM) images were taken using a microscope (SU8010, Hitachi, Japan).

*Preparation of samples*

Preparation of dezocine standard aqueous solution: 250 μL of dezocine injection (1 mL/5 mg) was dissolved in ultrapure water and diluted to 5 mL to obtain a 250 μg/mL dezocine standard solution. A dezocine aqueous solution with a series of concentrations of 125, 100, 50, 25, and 10 μg/mL were prepared using dezocine standard solution and ultrapure water for experimental detection. The blank sample is ultrapure water.

Preparation of dezocine artificial urine samples: the pretreatment of the urine spiked sample is referred to by Han et al.¹⁰ Specifically, 0.9 mL of artificial urine was mixed with 0.1 mL of dezocine in a 1.5 mL centrifuge tube to prepare dezocine urine samples with different mass concentrations of 250, 200, 125, 100, 50, 25, and 15
5 μg/mL. Subsequently, solid NaCl was added to supersaturate the spiked urine samples. The mixture was extracted with 100 μL cyclohexane and centrifuged at 10000 r/min for 2 min. Finally, the separated upper organic layer was taken out for experimental detection. The blank sample is an artificial urine solution without dezocine.

Preparation of dezocine rat serum samples: the pretreatment of the rat serum spiked samples was modified from the method proposed by Zhang et al. In brief, rat serum stored at -20 °C was thawed in a 4 °C refrigerator, and then it was completely dissolved at room temperature. Finally, serum samples were diluted to 10% with ultrapure water and then added dezocine standard solution to prepare 250, 125, 100, 50, 25, and 20 μg/mL rat serum sample solutions. These serum samples were stored in a 4 °C refrigerator for experimental detection. The blank sample is a rat serum solution without dezocine.

Preparation of silver nanoparticles

In this experiment, some modifications have been carried out to the preparation methods of Ag colloid proposed by Qin et al. In concrete terms, ascorbic acid was regarded as reducing agent, sodium citrate as stabilizer, and silver nitrate was reduced to silver nanoparticles. Firstly, 250 mL solution containing 6.0×10⁻⁴ mol/L ascorbic acid and 3.0×10⁻³ mol/L sodium citrate was heated to 30 °C in a water bath at slow speed. Then, 25 mL of 0.1 mol/L AgNO₃ was added to the mixture rapidly, and the reaction was performed at 900 r/min stirring speed for 15 min. It was observed that the solution quickly changed from colorless to yellow, and then gradually changed to gray-green. Finally, the solution is heated to 100 °C and boiled for 2 h to promote the internal maturation of the particles and make the spherical nanoparticles more uniform. The synthetic sol was cooled to indoor temperature, and then stored at 4 °C to avoid light exposure.

SERS detection

The volume ratio of sample, silver colloid and coagulant was fully mixed at 4:4:1, and then SERS spectroscopy was performed. Each group was collected 5 times and averaged. It takes about 1 minute from the mixing of the sample, silver sol and
coagulant to the completion of the SERS detection. 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.

**Results and discussion**

*Characterization of Ag colloid*

Ag colloid nano substrate was prepared according to the above methods. A SEM image of the Ag colloid is presented in Fig. 1(a). It can be seen from the figure that the Ag colloid is mainly spherical and ellipsoidal nanoparticles. Compared with the classic spherical sol prepared by Lee et al. which contains rod-shaped and linear shaped particles, the colloid has a more uniform morphology. Obviously, anisotropic nanowires no longer exist in the Ag colloid, and the monodispersity and uniformity of the nanoparticles have improved. This is because ascorbic acid is more reductive than sodium citrate, and it takes only 15 minutes to complete the reduction reaction at room temperature, avoiding the nucleation reaction and growth process imbalance. The particle size statistics of 100 randomly selected silver nanoparticles were obtained, and the particle size distribution was depicted in Fig. 1(b). The particle size distribution of Ag nanoparticles is concentrated at 45-70 nm, and the range is narrow, with an average particle size of 56.27 ± 8.76 nm, which is in line with the Gaussian distribution. It can be observed that the prepared Ag colloid has good consistency in morphology and size.

*Attribution of dezocine Raman characteristic peak*

At present, the Raman spectroscopy of dezocine has not been reported, so the calculation of dezocine Raman spectroscopy can provide strong theoretical support for subsequent experiments. In this paper, Gaussian software was applied to construct the molecular model of dezocine, and the theoretical spectrum of dezocine was firstly calculated by density functional theory (DFT). The molecular structure was optimized using the mixed exchange interaction functional B3LYP and diffusion basis set 6-31+G (d, p), which were selected to describe the atomic orbital mathematically. The molecular structure, theoretical Raman spectra of dezocine and the SERS spectra
collected by experiment are exhibited in Fig. 2.

From Fig. 2 (b), it is apparent that the majority of the peaks in the theoretical spectra and SERS spectra are basically the same. The two most prominent characteristic peaks in the SERS spectra of dezocine, 534 cm\(^{-1}\) and 661 cm\(^{-1}\), are in good agreement with the theoretical vibration peaks. According to the visualization relationship between the Raman peak and the chemical bond vibration mode in Gauss View software, the characteristic peaks of dezocine were attributed and listed in Table 1. In the subsequent experiments, these two Raman peaks were regarded as characteristic peaks to detect dezocine qualitatively and quantitatively. In addition, the positions of the SERS characteristic peaks at 534 cm\(^{-1}\) and 661 cm\(^{-1}\) show slight frequency shifts between theoretical spectra and SERS spectra, which might be caused by the interaction between sample molecules and Ag nanoparticles during the SERS detection.

**Selection of coagulant**

In the process of Ag colloid synthesis, sodium citrate was used as a protective agent to enable nanoparticles to be suspended in water steadily. While the citrate was regarded as an obstacle to the contact between the molecules and silver nanoparticles, which reduced the enhancement effect. Therefore, an appropriate amount of inorganic salt was added as a coagulant to change the equilibrium state of the movement of nanoparticles and aggregate silver nanoparticles to generate more SERS hotspots to amplify the SERS signal. In general, a variety of anions are selected as coagulants to improve the SERS activity of silver nanoparticles. For different anions, the main difference is the affinity for silver nanoparticles. The known affinity order for silver nanoparticles is halogen ions (I\(^{-}\), Br\(^{-}\), Cl\(^{-}\)) > the citrate > NO\(_3^{-}\) > SO\(_4^{2-}\).\(^{15}\) In this paper, four representative inorganic salts, KI, NaCl, NaBr, and Na\(_2\)SO\(_4\), were selected to study the enhancement effect of different coagulants on the same concentration of dezocine aqueous solution, as displayed in Fig. 3 (a). It can be observed from the figure that the enhancement effect of NaBr is the strongest under the same detection conditions. Meanwhile, in addition to the types of coagulants, the concentration of
coagulants also has a certain influence on the enhancement effect. Therefore, a comparative study was conducted on NaBr solutions with different concentrations. As illustrated in Fig. 3 (b), when the concentration of NaBr is 0.75 mol/L, the enhancement effect is the best. Adding an appropriate amount of coagulant can cause the effective aggregation of nanoparticles, thereby enhancing the electromagnetic field at the gap, thus bringing better SERS performance. However, the coagulant should not be used in excess, because the excessive aggregation of nanoparticles will prevent the sample molecules from entering the hot spot area, thereby reducing the SERS intensity.\(^{16}\) Therefore, NaBr solution of 0.75 mol/L is selected as the coagulant in this experiment.

**Quantitative analysis of dezocine in aqueous solution**

In this work, through quantitative analyzation, establishing a fitting curve is aiming to directly calculate the concentration of the sample only by measuring the Raman intensity of the sample. The SERS spectra of dezocine in aqueous solution with gradient concentrations is exhibited in Fig. 4 (a). The Raman characteristic peaks at 534 cm\(^{-1}\) and 661 cm\(^{-1}\) could be identified even when the concentration of the aqueous solution was reduced to 10 \(\mu\)g/mL, where the peak intensity met 3 times of the signal-to-noise ratio.\(^{17}\) Therefore, the detection limit of dezocine aqueous solution sample is about 10 \(\mu\)g/mL. The Raman peak at 661 cm\(^{-1}\) was selected as the object of analysis, and the curve of the Raman intensity versus concentration of the dezocine aqueous solution was fitted, as presented in Fig. 4 (b). The curve equation is \(y=11494.5-11705.5e^{-x/121.4}\), and the correlation coefficient is 0.988.

**Quantitative analysis of dezocine in biological fluids**

In order to verify the feasibility of the SERS method for rapid detection of dezocine in biological fluids, we implemented the analysis of dezocine in two biological samples, urine and serum. The SERS spectra of dezocine in urine samples with gradient concentrations is depicted in Fig. 5 (a). While the concentration of dezocine in urine drops to 15 \(\mu\)g/mL, the Raman peaks located at 534 cm\(^{-1}\) and 661 cm\(^{-1}\) are still clearly visible. Taking the characteristic peak at 661 cm\(^{-1}\) as the target,
the curve of the characteristic peak intensity with concentration of dezocine in urine samples was obtained by fitting, as presented in Fig. 5 (b). The curve equation is $y=12106.2-11875.7e^{-x/314.9}$, and the correlation coefficient is 0.984. In addition, referring to our previous work, most of the impurities have been separated after centrifugation, so the impact of impurities on the SERS spectrum is reduced, and the results of artificial urine and actual urine are similar.

The SERS spectra of dezocine in serum samples with gradient concentrations is displayed in Fig. 6 (a). The weak Raman signal was still obtained from the spectra when the concentration was low to 20 μg/mL. The curve of the characteristic peak intensity with concentration of dezocine in serum samples was obtained by fitting, as exhibited in Figure 6 (b). The curve equation is $y=7317.8-7145.1e^{-x/201.2}$, and the correlation coefficient is 0.988. One thing to note, when detecting dezocine in water, urine and serum, although the detection target is same, the solvent is different. Therefore, the three equations have certain expressions difference due to the various absorption coefficient of the incident light for three solvents.

In fact, the detection limit of the dezocine is indeed not low enough. But we consider that this is not a problem with the silver nanostructure as SERS substrate. Because according to our previous work,18 the enhancement effect of silver colloid is excellent and the enhancement factor of silver colloid can reach as high as 5.4 × 10^6. We infer that it is the problem of the dezocine molecule itself. Raman spectra are generated due to changes in polarizability. The magnitude of the change in polarizability caused by molecular vibration can be qualitatively estimated by the difference in the shape of the electron cloud on both sides of the equilibrium position passed by the vibration. The greater the degree of difference, the greater the movement of the electron clouds relative to the skeleton. Thus the greater the polarization rate could be obtained and the stronger Raman scattering is exhibited at this time. As a result, the high limit of detection of dezocine might be attributed that the Raman activity of the sample molecule itself is too weak and the Raman signal is not strong enough.
To further verify the accuracy of the fitting curve, the spiked urine and serum samples were prepared separately, and the recovery rate and relative standard deviation (RSD) were calculated according to the fitting curves in Fig. 5 (b) and Fig. 6 (b), as listed in Table 2. As can be observed from the table, the recovery rate of dezocine-spiked urine samples ranges from 89.1% to 110.4%, and the RSD ranges from 1.90% to 8.22%. The recovery rate of dezocine-spiked serum samples ranges from 91.5% to 108.4%, and RSD ranges from 2.79% to 7.61%. The recovery rate of dezocine in urine and serum is similar and the RSDs are all less than 10%. Therefore, the method involved of SERS technique to detect dezocine in biological samples is reliable and applicable, which has great potential for rapid monitor of illegal drugs in real samples.

**Conclusion**

In this paper, a method for determining dezocine in urine and serum based on SERS technique has been developed. Ag colloid active substrate was prepared and characterized. The Raman characteristic peaks of dezocine were assigned from both theoretical and experimental aspects. The SERS spectra of the dezocine molecule are mainly located at 534 cm\(^{-1}\) and 661 cm\(^{-1}\). The Raman peak at 661 cm\(^{-1}\) was selected as the characteristic peak for SERS detection of dezocine in urine and serum. The limit of detection of dezocine in urine samples is 15 µg/mL. In the range of concentrations varied from 15 to 250 µg/mL, the relationship of the characteristic peak intensity and concentration of dezocine in urine can be expressed by the curve equation that is \(y=12106.2-11875.7e^{-x/314.94}\), and the correlation coefficient is 0.984. The recovery range is 89.1% to 110.4%, and the RSD ranges from 1.90% to 8.22%. The limit of detection of dezocine in serum samples is 20 µg/mL. In the range of concentrations varied from 20 to 250 µg/mL, the relationship of the characteristic peak intensity and concentration of dezocine in serum can be expressed by the curve equation that is \(y=7317.8-7145.1e^{-x/201.2}\), and the correlation coefficient is 0.988. The recovery range is 91.5% to 108.4%, and the RSD ranges from 2.79% to 7.61%. This rapid, accurate, non-destructive method established a good foundation for rapid detection of dezocine.
in biological fluids.

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### Table 1 Theoretical and experimental vibration frequencies of dezocine

| SERS spectra (cm\(^{-1}\)) | Theoretical spectra (cm\(^{-1}\)) | Assignments                                      |
|-----------------------------|----------------------------------|--------------------------------------------------|
| 381                         | 377                              | Seven carbon ring torsional vibration            |
| 534                         | 530                              | Benzene ring torsional vibration and C-C torsional vibration |
| 550                         | 546                              | Seven carbon ring torsional vibration            |
| 661                         | 668                              | Benzene ring torsional vibration and N-C torsional vibration |
| 807                         | 801                              | Seven carbon ring torsional vibration + C-C torsional vibration |
| 953                         | 954                              | Benzene ring torsional vibration                 |
| 1004                        | 1009                             | C-C stretching vibration + benzene ring torsional vibration |
| 1145                        | 1142                             | Benzene ring stretching vibration                 |

### Table 2 The recovery rate and RSDs of dezocine in urine and serum

|                | Dezocine sample in urine |               |                | Dezocine sample in serum |               |                |
|----------------|--------------------------|---------------|---------------|--------------------------|---------------|---------------|
|                | Times        | Spiked concentration (μg/mL) | Calculated concentration (μg/mL) | Recovery (%) | RSD (%) | Spiked concentration (μg/mL) | Calculated concentration (μg/mL) | Recovery (%) | RSD (%) |
|                | 1            | 265.79                  | 106.3          | 94.05                    | 94.2          | 248.82                  | 106.1          | 99.5          |
|                | 2            | 250                     | 272.34         | 108.9                    | 1.90          | 250                     | 265.32         | 3.28          |
|                | 3            | 275.99                  | 110.4          | 89.1                     | 8.22          | 260.57                  | 104.2          |               |
|                | Average      | 271.37                  | 108.5          | 95.9                     | 9.5           | 258.24                  | 103.3          |               |
|                | 1            | 100                     | 89.10          | 89.1                     | 8.22          | 125                     | 103.9          | 2.79          |
|                | 2            | 104.54                  | 104.5          | 95.9                     | 9.5           | 128.54                  | 102.8          |               |
|                | Average      | 104.5                   | 95.9           | 105.1                    |               | 131.31                  | 105.1          |               |
|                | 1            | 54.53                   | 109.1          | 54.53                    | 109.1         | 46.38                   | 92.7           |               |
|                | 2            | 50                      | 107.0          | 5.93                     |               | 50                      | 91.5           | 7.61          |
|                | 3            | 48.73                   | 97.5           | 48.73                    | 97.5          | 52.39                   | 104.7          |               |
|                | Average      | 52.26                   | 104.5          | 52.26                    | 104.5         | 48.17                   | 96.3           |               |
**Figure Captions**

**Fig. 1** (a) SEM image of Ag colloid; (b) size distribution of Ag colloid.

**Fig. 2** (a) Molecular structure of dezocine; (b) theoretical and experimental spectra of dezocine (The theoretical spectrum of dezocine and the SERS spectrum of an dezocine aqueous solution with a concentration of 125 g/mL).

**Fig. 3** (a) SERS spectra of dezocine with four different coagulants; (b) the characteristic peak intensity distribution of dezocine with different concentrations of NaBr solutions.
Fig. 4 (a) SERS spectra of dezocine in aqueous solution with gradient concentrations; (b) the relationship between the characteristic peak intensity and the concentrations of dezocine in aqueous solution.

Fig. 5 (a) SERS spectra of dezocine with gradient concentrations in urine, the blank spectrum is the spectrum of the blank urine; (b) the relationship between the characteristic peak intensity and the concentrations of dezocine in urine.

Fig. 6 (a) SERS spectra of dezocine with gradient concentrations in serum, the blank spectrum is the spectrum of the blank serum; (b) the relationship between the characteristic peak and the concentrations of dezocine in serum.
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