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Sensitive and simple determination of zwitterionic morphine in human urine based on liquid-liquid micro-extraction coupled with surface-enhanced Raman spectroscopy

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ABSTRACT

Morphine, a kind of illicit drugs, is also one of the main heroin metabolites. In consideration of a noninvasive way to monitor and identify drug abuse during forensic cases, the urine samples are usually detected. Here, colloidal gold nanorods (Au NRs) were introduced to act as active substrate, because of the strong optical extinction and spectral tunability of the longitudinal surface plasmon resonance (SPR). Thus, well surface-enhanced Raman spectra of morphine even at low concentrations could be obtained by portable Raman spectrometer. For the complex matrix environment of urine, liquid-liquid micro-extraction (LLME), a simple and inexpensive pretreatment, was employed to avoid the interferences. And then, the coupled surface-enhanced Raman spectroscopy (SERS) can give full play to the advantages of high sensitivity and unique spectroscopic fingerprint. According to the zwitterionic structure and physicochemical parameters of morphine molecules, the pH value of urine sample was adjusted to about 9 by buffer solution (KOH/NaB_4O_7) and the mixture of chloroform and isopropyl alcohol (V/V = 9:1) was chosen as extractant. Moreover, such pretreatment was proved to be appropriate for separation and concentration of morphine from urine. The developed LLME-SERS method could provide a detection limit less than 1 ppm in the human urine environment and the whole process of detection just needed take 5–6 min. What's more, the results of urine samples from heroin users exhibited application value of the proposed technique. The excellent performance makes it promising to become a rapid, reliable, and on-spot analyzer, especially for public safety and healthcare.

1. Introduction

Morphine, a kind of illicit drugs, is usually detected as an indicator of heroin abuse [1]. And heroin abuse is considered to be a serious criminal act [2]. It is mainly responsible for the cases of poisoning and death, especially in patients with a history of drug addiction or abuse for pain relief [3]. Among the many techniques of detection, surface-enhanced Raman spectroscopy (SERS) has become one of the most widely pursued spectroscopic tools with the advantages of high sensitivity and unique spectroscopic fingerprint. The sensitive determination of controlled substances (such as amphetamines [4,5], cocaine [6], 3,4-methylenedioxymethamphetamine (MDMA) [5], mephedrone [7] and ketamine [8]) have been applied to solve actual problems. For morphine molecule, its Raman and SERS spectra were explored in details. However, there is very few study to provide a method to achieve morphine detection at low concentrations (< 10 ppm) [9–11]. Kline et al. reported the optimization of SERS conditions for implementation into a microfluidic device for drug detection, in which morphine, cocaine, and methamphetamine (MA) were chosen as test analytes [12]. From the results, we knew that morphine usually represented a limit of detection 1–2 orders of magnitude lower than cocaine or methamphetamine. And there is difficulty to get the SERS spectra of morphine with high signal-to-noise (S/N) ratio at low concentrations. Herein, the good performance and reliable gold nanorods (Au NRs) was introduced as the effective SERS substrate by us. Au NRs could exhibit strong optical extinction in the range of visible and near-infrared (NIR) wavelengths and the geometrical anisotropy components ensure spectral tunability of the longitudinal surface plasmon resonance (SPR) [13].

Furthermore, SERS have outstanding potential in face of bio-fluids, because their major component (water) is the weak Raman scatterer.

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For urine, it can be collected in a noninvasive way and can also be used to monitor and identify of heroin abuse. Morphine, one of the main heroin metabolites, is excreted into it through the kidney. Nevertheless, the direct detection of morphine in urine samples is almost an impossible task, which is contributed to the strong fluorescence and the interferences from complex components. The nitrogen-containing compounds in urine, such as urea and uric acid, are sensitive for Raman spectroscopic methods, which can seriously affect the determination of analytes [14,15]. Nuntawong et al. developed acidulation treatments to the specimen samples and the product of urea nitrate would eventually precipitate. The remaining dissolved methamphetamine/amphetamine in the urine specimens therefore was enhanced for the SERS analyses, without any interference from the urea [16]. However, the urine is of complex matrix and only removal of urea is seemly not enough for practical detection. The rapid separation and purification of morphine from urine is perhaps of great importance to solve such problem.

At present, the number of publications related to SERS combined techniques is increasing year by year and the combined techniques are helpful for high-performance detection and characterization [17]. In order to detect analytes sensitively and accurately in complex environment by SERS without matrix interference, one way is to use capturing techniques. The captures include antibody [18], aptamer [19] and molecular imprinting [20–22], which enable the selectivity of SERS detection for targets molecules. However, capturing techniques are the costly, complicated and time-consuming sample preparation processes. Another way is to combine separation techniques. Microfluidic device is one of the representatives [23]. In addition, phase separation, thin layer chromatography (TLC) [24] and high pressure liquid chromatography (HPLC) [25] are also been successfully applied to overcome limitations and enhance capabilities for SERS characterization. Among them, phase separation seems to be a good choice in consideration of simple sample preparation and on-site detection procedures. Liquid-liquid micro-extraction (LLME) technique is of typical phase separation with the advantages of simplicity, quickness, cost-effectiveness and a limited volume, which promises a helpful pretreatment process [6,26,27]. Due to the distinct distribution of specific analyte to the different phases, analyte can be separated and enriched from a matrix thus increase the sensitivity compared to direct analysis using SERS, especially in the trace analysis of a complex matrix.

In this study, Au NRs was constructed to act as active SERS chips and the identification of morphine from human urine was based on LLME-SERS by portable Raman spectrometer. Tertiary amino coexists with phenolic hydroxyl in the morphine molecule. So the pH value of urine sample was adjusted to about 9 by buffer solution (KOH/NaB_4O_7), in order to guarantee the presence of free form of morphine in organic phase. And the mixture of chloroform ($CHCl_3$) and isopropyl alcohol (iPrOH) (V/V = 9:1) was chosen as extractant. The detection limit of morphine in human urine could reach less than 1 ppm after such pretreatment, which ought to meet actual demand. What's more, the developed LLME-SERS method had been successfully applied to the urine samples from heroin addicts and the results were also verified by morphine diagnostic kit (colloidal gold method) and liquid chromatography-mass spectrometry (LC/MS). It is indicated that the developed LLME-SERS promises to have good application in practical detection from complex environment.

2. Experiment section

2.1. Chemicals and materials

Cetyltrimethyl ammonium bromide (CTAB), chloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), hydrogen nitrate (HNO_3), ascorbic acid (AA), isopropyl alcohol (iPrOH), chloroform ($CHCl_3$), cyclohexane (CYH), N-butanol (n-BuOH), sodium borate ($Na_2B_4O_7$), and potassium hydroxide (KOH) sodium chloride (NaCl) were analytical grade and obtained from Shanghai Reagent Co. without further treatment. Accurate pH test paper (0.5–5.0) and (5.5–9.0) purchased from Hangzhou Shisan Science CO., LTD. Morphine test kits (colloidal gold) were purchased from Shanghai Venture Bio-tech Co. LTD. Standard morphine and MDMA were provided by physical evidence identification center of Anhui Provincial Public Security Bureau. Normal human urine samples were randomly collected from volunteers in cancer hospital, Chinese Academy of Sciences, Hefei. The urine samples of drug addicts were from Huainan Public Security Bureau.

2.2. Characterizations

The morphology and microstructure of Au NRs were characterized by field-emission scanning electron microscopy (FESEM, Sirion 200). UV–vis absorption spectra were collected using a Shimadzu UV-2550 spectrophotometer made in Japan. Raman spectra were recorded on portable Raman spectrometer BWS465–785 s. The laser power focused on the sample decays to 80% of the original laser intensity. In order to avoid signal overflow, integration time was settled to 5 s for direct detection of urine samples. And in term of different signal-to-noise (S/N) ratios, the integration time of MDMA and morphine detection was 10 s and 15 s respectively. LC/MS was conducted by Q-TOF 6550, Agilent. Allure PFP Propyl (100 mm*2.1 mm*5 μm) was chosen as the liquid-phase column. The mobile phase was composed of acetonitrile and the mixture (20 mmol/L ammonium acetate and 0.1% formic acid buffer) and the volume ratio is 70:30. The flow rate was 200 μL min^{-1}.

2.3. Synthesis of Au NRs

The uniform Au NRs were prepared by a typical seed-growth method previously developed by Nikoobakht and El-Sayed [28]. The process was described as following: (1) Au nanoparticle seeds. 103 μL of 1w% $HAuCl_4$ was mixed with 10 mL of 0.1 M CTAB solution at 29 °C under magnetic stirring in a small beaker. And then 60 μL of freshly prepared $NaBH_4$ solution (10 mM) was added quickly. Next, after 2 min stirring, the solution was settled. (2) Growth procedures. A conical flasks with 206 μL of 1w% $HAuCl_4$ mixed with 10 mL of 0.1 M CTAB then added 125 μL of 0.008 M $AgNO_3$ solution at 29 °C and followed by the addition of 100 μL of 2 M HNO_3 and 60 μL of 0.1 M ascorbic acid. Last, 12 μL of Au nanoparticle seed was injected into the solution. Finally, Au NRs with longitudinal surface plasmon resonance at 785 nm were obtained after 4 h settled.

2.4. Urine collection and micro-extraction of morphine from human urine

Human urine samples were collected in 50 mL falcon tubes and briefly kept at ~4 °C immediately following collection. Then samples were transported to the laboratory, and stored at − 80 °C within 2 h. Prior to analysis, specimen samples were thawed and centrifuged, and the supernatant was collected for the next experiment.

To simulate the authentic urine of drug addicts, 0.4 mL of human urine mixed with 0.4 mL of drug solution with certain concentration in 1.5 mL EP tube. Then, buffer solution comprised with $Na_2B_4O_7$/KOH was added to the mixture until pH to about 9. Next, 0.1 mL of extractant ($VCHCl_3$: V_{iPrOH} = 9:1) was added to extract and separate morphine from human urine. And tubes were hermetical, shook violently at room temperature and then centrifuged 5 min at 6000 rpm. The supernatants were discarded by aspiration and inferior organic phase were transferred for further experiments.

3. Results and discussion

3.1. Characterization of Au NRs and SERS performance

The uniform Au NRs (in Fig. 1A) were successfully synthesized by a typical seed-growth method in the presence of the cationic surfactant