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

Borong Yu\(^{a,b}\), Chentai Cao\(^{a,b}\), Pan Li\(^a\), Mei Mao\(^a\), Qiwen Xie\(^{c,*}\), Liangbao Yang\(^{a,b,**}\)

\(^a\) Institute of Intelligent Machines, Chinese Academy of Sciences, Hefei 230031, PR China
\(^b\) Department of Materials Science and Engineering, University of Science and Technology of China, Hefei 230026, PR China
\(^c\) Institute of Forensic of Anhui Public Security Department, Hefei 230061, PR China

ARTICLE INFO

Keywords:
Morphine
Human urine
Portable Raman spectrometer
Liquid-liquid micro-extraction (LLME)
SERS detection

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\(_4\)O\(_7\)) 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.
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 acclimation 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 seemingly 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/Na2B4O7), in order to guarantee the presence of free form of morphine in organic phase. And the mixture of chloroform (CHCl3) 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 (HAuCl4), sodium borohydride (NaBH4), silver nitrate (AgNO3), hydrogen nitrate (HNO3), ascobic acid (AA), isopropyl alcohol (iPrOH), chloroform (CHCl3), cyclohexane (CYH), N-butanol (n-BuOH), sodium borate (Na2B4O7), 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 Huaian 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 1% HAuCl4 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 NaBH4 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 1% HAuCl4 mixed with 10 mL of 0.1 M CTAB then added 125 μL of 0.008 M AgNO3 solution at 29 °C and followed by the addition of 100 μL of 2 M HNO3 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 further experiments.

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 Na2B4O7/KOH was added to the mixture until pH to about 9. Next, 0.1 mL of extractant (VCHCl₃: V𝑖PrOH = 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
indexed to MDMA [27,29]. Then, all intensities of the 714 and 809 cm\(^{-1}\) peaks from those 20 SERS spectra were plotted as the histogram in Fig. S1C and Fig. S1D. The value of statistical relative standard deviation (RSD) was less than 20% and respectively 10.4% and 8.3% as indicated. Above descriptions imply an excellent sensitivity and reproducibility of the prepared Au NRs platform.

On the basis of above work, such Au NRs platform was further taken to detect morphine. Morphine is a rigid molecule built of phenanthrene with an N-methyl piperidine ring and an O bridge, with two hydroxyl groups of some rotational freedom. And the molecular structure of morphine was shown in Fig. 1C [30]. In Fig. 1D, SERS spectrum was collected from 100 ppm of aqueous morphine and the normal Raman spectrum from standard morphine powder. The detailed description of Raman/SERS characteristic modes of morphine was shown in Table 1, which exhibited selective enhancement via SERS. Usually, the appearance of peaks at 443, 530, 608, and 628 cm\(^{-1}\) was attributed to morphine. However, the peak at 530 cm\(^{-1}\) was easily interfered by silicon (supporting substrate) because the laser has the ability of penetrating a certain depth of samples. In term of the sensitive Au NRs platform, the morphine at different concentrations ranging from 25 ppm to 1 ppm was measured in Fig. 1E, which demonstrated the feasibility of aqueous phase detection at low concentration. And then, a series of SERS spectra in aqueous solution were randomly collected from 25 ppm of morphine in different 20 spots (Fig. 1F). The 2D mapping presented clear fingerprint peaks of morphine molecules. The intensity of 443 and 628 cm\(^{-1}\) vibration data was shown to suggest good reproducibility (Fig. S2) and both the value of RSD was less than 20%. Since the excellent performance of Au NRs platform to aqueous morphine, it was expected to play good performance for identification of morphine in urine.

### 3.2. Direct detection of morphine in human urine

Urine is used to monitor drugs abuse in a noninvasive way, because of its ability to reflect drug consumption. Here, we randomly collected 50 specimen samples from 50 different drugs-free volunteers. The pH

---

**Table 1**

<table>
<thead>
<tr>
<th>Raman/cm(^{-1})</th>
<th>SERS/cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>443</td>
<td>443</td>
<td>(\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CCN)), (\delta(CCC))</td>
</tr>
<tr>
<td>490</td>
<td></td>
<td>(\delta(CH)), (\gamma(CCN)), (\delta(CNC)), (\delta(CCC)), (\delta(C-OH))</td>
</tr>
<tr>
<td>530</td>
<td>530</td>
<td>(\gamma(CCN)), (\delta(CH)), (\delta(CCC)), (\delta(CNC)), (\delta(COC)), (\delta(CH))</td>
</tr>
<tr>
<td>607</td>
<td>604</td>
<td>(\gamma(CH)), (\delta(CNC)), (\delta(CCC)), (\delta(C-OH)), (\delta(CH))</td>
</tr>
<tr>
<td>629</td>
<td>626</td>
<td>(\delta(CH)), (\delta(CH)), (\gamma(CCN)), (\delta(CNC)), (\delta(COC)), (\delta(C-OH))</td>
</tr>
<tr>
<td>675</td>
<td>669,703</td>
<td>(\delta(CH)), (\delta(CCC)), (\delta(CCN))</td>
</tr>
<tr>
<td>708</td>
<td></td>
<td>(\delta(CH)), (\delta(CCN)), (\delta(COC))</td>
</tr>
<tr>
<td>760</td>
<td></td>
<td>(\delta(CH)), (\gamma(CH)), (\delta(CCN)), (\delta(CNC)), (\delta(C-OH)), (\delta(CCC))</td>
</tr>
<tr>
<td>1030</td>
<td>1037</td>
<td>(\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH))</td>
</tr>
<tr>
<td>1087</td>
<td></td>
<td>(\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH))</td>
</tr>
<tr>
<td>1223</td>
<td></td>
<td>(\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH))</td>
</tr>
<tr>
<td>1282</td>
<td>1272</td>
<td>(\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH))</td>
</tr>
<tr>
<td>1330</td>
<td></td>
<td>(\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH))</td>
</tr>
<tr>
<td>1422</td>
<td></td>
<td>(\delta(CH)), (\delta(CH))</td>
</tr>
<tr>
<td>1636</td>
<td>1627</td>
<td>(\delta(CH)), (\delta(CH)), (\delta(CH)), (\delta(CH))</td>
</tr>
</tbody>
</table>

ν, stretching; δ, bending; γ, out-plane bending, δs, scissoring; δw, wagging; θr, rocking.

CTAB [28]. Fig. 1B showed the ultraviolet absorption spectrum of colloidal Au NRs. The longitudinal plasmon band was at 785 nm, which matched with the laser wavelength of portable Raman spectrometer. To preliminarily evaluate the sensitivity and of reproducibility the Au NRs platform, MDMA was chosen as the reporter molecule. First, the sensitivity was examined via different concentrations of MDMA. According to Fig. S1A, the corresponding intensity of SERS spectra decreased gradually as the concentrations varied from 25 ppm to 1 ppm and the detection limit reached 1 ppm. The detailed SERS spectra assignments of MDMA had been shown in Table S1. Second, the reproducibility was examined via randomly collected spectra from 20 different spots under the concentration of 10 ppm. The 2D mapping was illustrated in Fig. S1B. The obvious peaks (714 and 809 cm\(^{-1}\)) could be explicitly indexed to MDMA [27,29]. Then, all intensities of the 714 and 809 cm\(^{-1}\) peaks from those 20 SERS spectra were plotted as the histogram in Fig. S1C and Fig. S1D. The value of statistical relative standard deviation (RSD) was less than 20% and respectively 10.4% and 8.3% as indicated. Above descriptions imply an excellent sensitivity and reproducibility of the prepared Au NRs platform.

On the basis of above work, such Au NRs platform was further taken to detect morphine. Morphine is a rigid molecule built of phenanthrene with an N-methyl piperidine ring and an O bridge, with two hydroxyl groups of some rotational freedom. And the molecular structure of morphine was shown in Fig. 1C [30]. In Fig. 1D, SERS spectrum was collected from 100 ppm of aqueous morphine and the normal Raman spectrum from standard morphine powder. The detailed description of Raman/SERS characteristic modes of morphine was shown in Table 1, which exhibited selective enhancement via SERS. Usually, the appearance of peaks at 443, 530, 608, and 628 cm\(^{-1}\) was attributed to morphine. However, the peak at 530 cm\(^{-1}\) was easily interfered by silicon (supporting substrate) because the laser has the ability of penetrating a certain depth of samples. In term of the sensitive Au NRs platform, the morphine at different concentrations ranging from 25 ppm to 1 ppm was measured in Fig. 1E, which demonstrated the feasibility of aqueous phase detection at low concentration. And then, a series of SERS spectra in aqueous solution were randomly collected from 25 ppm of morphine in different 20 spots (Fig. 1F). The 2D mapping presented clear fingerprint peaks of morphine molecules. The intensity of 443 and 628 cm\(^{-1}\) vibration data was shown to suggest good reproducibility (Fig. S2) and both the value of RSD was less than 20%. Since the excellent performance of Au NRs platform to aqueous morphine, it was expected to play good performance for identification of morphine in urine.

### 3.2. Direct detection of morphine in human urine

Urine is used to monitor drugs abuse in a noninvasive way, because of its ability to reflect drug consumption. Here, we randomly collected 50 specimen samples from 50 different drugs-free volunteers. The pH
value of urine samples was in the range of 5.5–7.5 as shown in Table S2, mostly presenting faintly acid. At first, the directional SERS analysis was explored for the urine spiked with 25 ppm of morphine. The process of detection was shown in Fig. S3A and the SERS chip was constructed by Au NRs colloids. The collected 50 spectra were shown in Fig. S3B-F. From the results, we could observe that all of the urine samples showed strong fluorescence in the visible. Some spectra presented rich peaks with strong intensity and some spectra only showed silicon peak, which may be due to the inactive SERS chips after meeting the urine. But it was too difficult to identify SERS signals of morphine directly. We carefully compared the spiked urine with the blank urine (in Fig. S4), and the obvious peaks of spectra most derived from the components in urine [14,31]. Especially, urea is the main source of interference signals, which is the dominant nitrogen-containing component in urine. And there is no clear correlation between spectral peaks and the pH values. It is an impossible task to direct identification of morphine signals in urine without pretreatment, due to the strong fluorescence and the interferences from complex matrix. Therefore, it is necessary to rapidly separate and purify urine to reduce the interference signal and then improve the signal of morphine.

3.3. Efficient pretreatment of human urine via LLME

Here, LLME technique is used to couple with SERS detection for the advantages of simplicity, quickness, cost-effectiveness and a limited volume. On the basis of the ionization of acidic and basic drugs at various pHs, the chemical principle of differential extraction was utilized. Morphine is a zwitterion and tertiary amino coexists with phenolic hydroxyl. For the zwitterions (HX) and zwitterionic (HX±) species change with the external environment (Fig. S5). From the physicochemical parameters of morphine in Table S3, the lipophilicity profiles of log Doct vs. pH = 7.4 was 0.08, which indicates that the amount of morphine in organic phase is nearly the same compared to the water under such pH condition. When morphine molecules exist in a neutral form (HX°), the partition coefficient (log Poct) increases to 0.89[32]. Therefore, it’s necessary to adjust pH value to isoelectric point (pI) before micro-extraction. The value of pI can be roughly expressed as: pI = (pk1 + pK2)/2, where pk1 is the phenol pKa and pK2 is the amine pKa. And the value of pI approximately equals 9. Then buffer solution (KOH/NaB4O7) was used to regulate pH value (≈ 9) of urine samples, which can not only increases the affinity of morphine toward the organic phase, but also ensure the presence of free form of morphine in organic phase.

To find the most suitable extractant for LLME, we evaluated n-BuOH, as well as CHCl3 and CYH. The three organic solvents are commonly used for extraction of phenolic compounds with rapid evaporation. And according to the polarity of solvents, the order sequence is n-Bu-OH > CHCl3 > CYH. Firstly, aqueous morphine of 25 ppm was studied after pH adjustment. As shown in Fig. S6, the signals of morphine had better S/N ratios using n-BuOH as extractant compared to CHCl3 and CYH, which may attribute to the polarity of molecules and the hydrogen bonds between morphine and n-BuOH [33]. However, the application of n-butanol in urine samples could result in serious interference from urea, which may be due to the excessive extraction (Fig. S7). When CHCl3 was taken, the interference signals were reduced (Fig. S7). In order to improve the extraction efficiency and the intensity of analyte’s SERS signals, a small amount of iPrOH was added into the chloroform as part of the extractant. Indeed, the better spectrum was
obtained in comparison to single CHCl₃ for extraction (Fig. S7). Therefore, available urine spiked with morphine was vigorously extracted with CHCl₃/iPrOH (V/V=9:1) after buffer solution adjustment in the following experiments for LLME-SERS detection. And the schematic illustration about extracting morphine from urine was presented in Fig. 2.

In order to explore the sensitivity of the developed LLME-SERS method, different concentrations of morphine (from 25 ppm to 1 ppm) in urine were detected. From the results in Fig. 3A, the characteristic peaks of morphine could be clearly identified, even when 1 ppm of morphine was added. Afterwards, the randomly collected 5 urine samples from drugs-free volunteers spiked with morphine (10 ppm) and the positive results were shown in Fig. 3B, which prove the feasibility of such designed method. We believe that above works open vast possibilities for identification of urine from drug users.

3.4. Toward a rapid analyzer applied to urine samples from heroin users

A rapid SERS platform for detecting illicit drugs in human urine seriously depends on two important problems: the strategy for rapid separation and collection of drug molecules from human urine with the prevention of interferences; and the combination of high SERS-active nanostructures and analyte. So we developed LLME-SERS method for sensitive and simple determination of morphine in human urine and the Au NRs act as the active SERS substrate. To further verify the practical application of such proposed method, the specimen samples from heroin users (labeled HN-004 and HN-006) were demonstrated. The whole process for rapid detection was displayed in Fig. 4A and it just takes about 5–6 min to finish a single sample identification. From the results of SERS analysis in Fig. 4B and C, the characteristic peaks of morphine could be clearly identified. And the positive results were also proved by morphine diagnostic kit (the most common screening method, in Fig. S8). Moreover, LC/MS, one of the gold standard analytical tools at present, was also conducted to detect HN-004 and HN-006. The two urine samples from heroin users contained morphine and the concentration in HN-004 is lower than HN-006 on the basis of rough estimation of peaks area (Fig. S9). Therefore, SERS coupled with LLME can achieve simple and sensitive determination of zwitterionic morphine in urine from drug users.

4. Conclusions

In this article, a portable kit was developed for sensitive and simple determination of morphine in human urine via SERS coupled with LLME. Au NRs with strong optical extinction and spectral tunability of the longitudinal SPR were introduced to act as active SERS substrate for trace detection of morphine. On the basis that the detection limit of morphine in aqueous can reach less than 1 ppm, SERS method was further applied to urine samples spiked with morphine. The LLME technique was conducted to achieve rapid and efficient separation and concentration of morphine from human urine, further reducing the fluorescence signal and noise. The suitable conditions for LLME were explored on the basis of zwitterionic structure and physicochemical parameters of morphine. Finally, the buffer solution (KOH/NaB₄O₇) was used to regulate the pH value of urine samples first and then the mixture of CHCl₃ and iPrOH (V/V = 9:1) was taken to extract morphine.
from urine. Through the SERS-based method, the detection limit of morphine in human urine can still reach less than 1 ppm. Furthermore, the designed method was also taken advantage to identify the urine from heroin users. The good performance of such method illustrates the potential for on-site high-throughput analysis of morphine and LLME-SERS technique can also be extended to detect other analytes. In the future, our work will focus on developing the automated system, which can be used by police to perform measurements of urine from actual addicts for roadside testing.

Acknowledgements

The authors acknowledge the financial support from the Sci-tech Police Project of Anhui Province (1704d0802186), the National Science Foundation of China (21571180, 21505138 and 61605221); State Project For Essential Drug Research and Development (2018ZX09J18112).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2018.04.094.

References