Designing of ordered two-dimensional gold nanoparticles film for cocaine detection in human urine using surface-enhanced Raman spectroscopy

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Key words: surface-enhanced Raman spectroscopy; cocaine; detection; human urine

Abstract

A novel and rapid method to detect cocaine in human urine has been developed by using self-assembly ordered two dimensional (2D) gold nanoparticles (GNPs) film as surface-enhanced Raman spectroscopy (SERS) substrates. In order to obtain high sensitivity, uniformity, and reproducibility of SERS platform, quasi-spherical, uniform GNPs were firstly synthesis using seed growth method, in which the GNPs have been functionalized with CTAB to form orderly close-packed GNPs film as SERS substrate. Importantly, the high-performance GNPs on solid substrates can produce a high yield of sub-10-nm gaps which can generate gigantic signals enhancement for analytes adsorbed GNPs surface. In view of the complex component of human urine, we develop a rapid and efficient pretreatment strategy for separation and purification of cocaine by using hexane extraction in real human urine samples within 3 min. With the advantages of better extraction rates (>75%) examined by ultraperformance liquid chromatography (UPLC) and the excellent signal-to-noise ratio detected by SERS, our pretreatment procedure can efficiently lower the interference of complex biological components in urine. To reach on-spot analyzer, a handheld Raman spectrometer was used for feasible SERS detection of cocaine in real human urine. The favorable results demonstrated our pretreatment strategy combined with SERS platform will be a great prospective method toward rapid, reliable, and on-spot cocaine detection for public safety.

Key words: Surface-enhanced Raman spectroscopy; Cocaine; Urine; Liquid-liquid microextraction; Self-assembly

1. Introduction

The high sensitivity and selectivity of surface enhanced Raman spectroscopy (SERS) make it an ideal method for the detection and characterization of low-concentration analytes in a complex biological environment.[1-3] SERS can provide fingerprint information of target molecules and has been considered to be an ultrasensitive label-free detection method for a variety of applications. [4-6] Nowadays, the superiority of expanding the SERS technique to surface analysis, including drugs in urine, saliva, or blood has been a strong driving force. [7, 8] In our previous work, Dong etc. developed a novel method to detect and direct readout of methamphetamine (MAMP) and 4-methylenedioxy methamphetamine (MDMA) in human urine without any sample pretreatment, using dynamic surface-enhanced Raman spectroscopy (D-SERS) combined with portable Raman spectrometer on assembled gold nanorods (GNRs). The classification algorithm with support vector machines realized the visual online detection and fast accurate identification of drugs in body fluid in 2 min, which makes it highly practical for field applications.[9] Another example was Han etc. established a rapid separation and purification of MAMP and MDMA in real human urine samples for multiplex SERS detection by the use of three-dimensional (3D) hotspots in spherical colloidal silver superstructure, which demonstrated excellent stability and reproducibility as well as capability for the quantitative examination of drugs in both aqueous and organic phases. [10] Recently, a portable kit has been established for practical on-spot SERS detection of MAMP and MDMA in real human urine, which included a SERS substrate of highly reproducible GNR two dimensional (2D) arrays, a rapid urine sample pretreatment, as well as a hand-held Raman device for SERS analysis, which own a great promising application as a rapid, reliable, and on-spot analyzer.[11] Although many efforts have been made to explore SERS technique for detection of illicit drug, there are pervasive hurdles in extensive of SERS for drug detection: (1) the historic

view that both SERS signals and SERS-active surfaces exhibit poor reproducibility, (2) lower SERS active of drug molecule, (3) trace concentration of analytes in complicate biological system, (4) analytes must be close to ($\sim 1-2$ nm) or adsorbed on a roughened metal surface. Therefore, in consideration of gaining a reliable substrate of drugs detection, designing the uniform, highly sensitive, stable and ordered nanostructure has been drawn our attention.

At present, the research on self-assembly of nanostructures has gained wide attention due to the unique characteristics of the regular arrangement.[12-16] For purpose of obtaining excellent capacity of self-assembly SERS nanostructure for drug detection, the quality (size and size distribution) and shape is vital to generate uniform “hot spots” on the nanosubstrate. As we know, surfactant molecules can self-assemble into micelles in aqueous solution above the critical micelle concentration and certain cationic surfactants such as alkyltrimethylammonium halide, which can form rodlike or globular micelles at high concentrations, thus causing the solution viscoelastic. [17, 18]Moreover, as the metal nanoparticle is modified with different capping agents, the gold nanoparticles (GNPs) can form large-area ordered assemblies and produce a high yield of sub-10-nm gaps upon slow evaporation of GNPs solution on solid substrates.[19] Notably, GNPs prepared in cetyltrimethylammonium bromide (CTAB) are expected to provide useful materials for potential advantages:(1) CTAB can form self-assembled bilayers on gold nanostructures, which leads to a net positive charge on the nanoparticles surfaces, providing a net repulsive interaction between the nanoparticles to prevent random disordered aggregation during solvent evaporation; (2) CTAB-capped GNPs are stabled, which are further suitable to on-site detection.(3) CTAB-capped GNPs are present close-packed and ordered 2D GNPs nanostructure,[19] which has excellent sensitivity and reproducibility for drug detection. Because of the low concentrate cocaine in human urine, [20, 21] ordered 2D GNPs film as SERS substrate will play an importance role in cocaine

detection with the advantages of sensitivity and reproducibility.

Cocaine abuse is regarded as a global challenge, which has increased dramatically during the past three decades, causing serious social and public security problems. [22] Mass spectrometry is the state of the art technique to detect cocaine in a human saliva sample, but this detection method is not portable and brings along several laborious sample preparation steps.[23] By virtue of the high sensitivity and unique spectroscopic fingerprint of SERS platform to develop simple, rapid and cost-effective tools for the sensitive detection of cocaine is urgently needed. Nowadays, urine has been become common biological remains for it being easily-accessible bodily fluid that provides metabolic information.[24, 25] However, the complicated composition of urine would have serve effect on SERS platform for drugs detection in real human urine. Therefore, the strategy to rapidly separate and collect the drugs in human urine is effective method for purifying and concentrating analytes in complicate system.

Herein, we developed a rapid separation and purification of cocaine in real human urine samples for SERS detection using uniform and ordered 2D GNPs film functionalized with CTAB. The bilayer capping of CTAB on GNPs leads to a net positive charge on the nanoparticles surfaces, which fabricate of sub-10-nm gap of ordered 2D GNPs film. Due to nonpolar nature of cocaine, it is easier to extract with organic solvents in alkaline medium in human urine. In view of rapid separation collection cocaine in human urine, we select hexane acted as the extraction solvent examined extraction rates by ultraperformance liquid chromatography (UPLC). The outstanding sensitivity and reproducibility of ordered 2D GNPs film can provide consistent signals on SERS analysis. The purification and detection progress only needs 5 min, which makes it highly practical applications on spot detection.

2. Experiment Section

2.1 Chemicals and Materials

Hydrogen tetrachloroaurate trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), trisodium citrate dihydrate, cetyltrimethylammonium bromide (CTAB), hexane, and sodium hydroxide (NaOH) were analytical grade and obtained from Shanghai Chemical Reagent Company without further treatment. 4-aminothiophenol (4-ATP) was purchased from Aladdin Company. Cocaine hydrochloride was got from the Academy of Military Medical Sciences (AMMS), Beijing, China. Human urine was randomly collected from ordinary people in hospital. Ultrapure water ($18.2 \text{ M}\Omega \cdot \text{cm}$) was produced by Millipore Milli-Q gradient system. All experiment was carried out under room temperature (25°C).

2.2 Characterizations

Transmission electron microscopy (TEM) images were recorded by a FEI Tecnai G2 F20 S-TWIN high resolution transmission electron microscopy. The scanning electron microscopy (SEM) images were taken on a field-emission scanning electron microscope (Quanta 200FEG). Ultraviolet–visible (UV–vis) absorption spectra were obtained by a Shimadzu UV-2550 spectrophotometer (Japan). Raman spectra were performed on LabRAM HR800 confocal microscope Raman system (Horiba Jobin Yvon) with excitation wavelength of 633 nm from a He–Ne laser. The lasers were focused by a LWD 50/0.5 NA objective lens and the laser spots had a diameter of about $1 \mu\text{m}$. The laser powers on the sample location were approximately 1 mW and the exposure time for each spectrum was 3 s. Meanwhile, handheld Raman spectrometer was used with Tactic ID (B&W Tek, USA), and a laser power of 270 mW using 785 nm laser excitation with 10 s integration time. The laser spots had a diameter of is about $10 \mu\text{m}$. UPLC assays were recorded by ACQUITY UPLC H-Class instrument (Waters Company). The HPLC system consisted of an

Agilent 1100 system equipped with standard micro auto sampler, quaternary pump, series multiple wavelength TUV detector, micro vacuum degasser and ACQUITYUPLC@BEHC8 (1.7 μm 2.1 $\times$ 50mm Column). The mobile phase was 0.1% methane acid in HPLC-grade water. The eluent buffer was a mixture of solvent A (20 mmol ammonium acetate and 0.1% formic acid in water, v/v) and solvent B (methanol) with isocratic elution of 40% solvent A and 60% solvent B with flow rate was 0.3 mL min⁻¹. The UV detection of drugs was operated at the wavelength of 235 nm. The injection volume was 10 μL for quantitative measurements and room temperature of column temperature.

2.3 Purification of human urine samples

0.5 mL collected human urine and 0.5 mL cocaine solution with different concentration were added to 1.5 mL EP tube. Then, 30 μL of 2 w% NaOH was added to adjust pH to mild alkaline (>9) and 100 μL of hexane as the extraction solvent was added to extract the cocaine molecules from human urine. Eventually, the mixture was shaken evenly and stranded for liquid layers to separate. After 1.5 min, the 5 μL organic layer was carefully transferred for the following SERS experiments. This whole preparation process could be finished in 3 min.

2.4 Seeded growth synthesis of citrate-stabilized uniform gold nanoparticles

The synthesis of citrate-stabilized uniform GNPs was described by a seed-growth method developed by Neus G. Bastus.[26]

Briefly, a solution of 2.2 mM 150 mL sodium citrate was heated in a 250 mL three-necked round-bottomed flask. After boiling, 1 mL 25 mM of HAuCl₄ was injected and the color changed to soft pink in 10 min. This solution was used as a seed solution, in the same reaction vessel, then reaction temperature of solution was set to 90°C and 1 mL 25 mM of a HAuCl₄ solution was

injected. After 20-30 min, the reaction was finished. This process was repeated twice. After that, the sample was diluted by extracting 55 mL of sample and adding 53 mL of ultrapure water and 2 mL of 60 mM sodium citrate. This solution was then used as a seed solution, and the process was repeated again for different generations of GNPs of progressively larger sizes were grown.

2.5 Self-assembly of ordered 2D GNPs film [19]

A solution of 40 mM CTAB in deionized water was first prepared, and then added to GNPs solution. The sample was kept for about 1 h at 30 °C in water bath, and then was subjected to another centrifugation step and resuspended in ultrapure water with particle concentration of approximately 10^{12} particles mL^{-1} . Finally, the solution was assembled onto a clean substrate under ambient conditions and close-packed structures of ordered 2D GNPs film could be easily obtained.

3. Results and discussion

Characterization of the assembly uniform 2D GNPs film

Uniform GNPs were successfully synthesized following a kinetically controlled seeded growth strategy via the reduction of HAuCl_4 by sodium citrate. After seed synthesized, the temperature of the solution was decreased to 90 °C, which allows both the inhibition of the new nucleation of GNPs and the slowing down of the reaction as well as is beneficial to form uniform GNPs. The optical properties of gold colloidal solutions from different growth steps are depicted in Figure S1. Figure S1A is shown the corresponding UV-vis spectra of Au colloidal solution in different growth stages. The surface plasmon resonance (SPR) bands are slight red shifts as a consequence of particle growth, ranging from 518 to 531 nm, respectively (Figure S1B), and optical pictures are showed in Figure S1C. Furthermore, from Figure S1D one can observe the surfactant CTAB molecules have no effect on GNPs morphology in process of self-assembly.

Figure 1

The highly ordered 2D GNPs film was fabricated with sub-10-nm spacing between adjacent nanoparticles by a simple and cost-effective method. Scheme S1 (A) illustrates the fabrication procedure for ordered 2D GNPs film. The uniform citrate-stabilized GNPs were dispersed in aqueous solution (scheme S1A (I)) and then functionalized with capping surfactant of CTAB, which can form self-assembled bilayers on Au nanostructures (scheme S1A (II)). Then, drying a 10- μ L droplet of CTAB-capped Au colloid solution on silicon wafer under ambient conditions resulted in the formation of hexagonally close-packed and ordered GNPs film (scheme S1 (III)). An average diameter of 31.7 nm of ordered 2D GNPs film was clearly evidenced by a SEM image in Figure 1A and TEM image in Figure 1C (SEM for different growth steps can be seen in Figure S2, and larger field of view in average diameter of 31.7 nm GNPs are showed in Figure S3) as well as the particle size distribution in Figure 1C. Figure 1A and C are present quasi-spherical, close-packed, uniform GNPs film capped by CTAB. Besides, one can observe the gap distance distribution of ordered 2D GNPs film (Figure 1D, insets illustrate the specific measurements of GNPs size and gap distance distribution of interparticle separations), are expected to generate the ordered hotspots for SERS detection (scheme S1B). It is worth mentioning that there were well-defined gaps with a narrow size distribution between ordered 2D GNPs film, which would be very beneficial for forming hot spots and achieving large near-field enhancement effects, and being advantageous for SERS activity.[27]

SERS performance of ordered 2D GNPs film

Figure 2

In this work, an average diameter of 31.7 nm of close-packed and ordered 2D GNPs film was used as SERS substrates. To evaluate the potential application of as-obtained GNPs film as SERS substrate, 4-ATP was firstly chosen as the reporter molecule. We first examined the SERS sensitivity of ordered 2D GNPs film, and we measured the SERS spectra of 4-ATP molecules with

different concentrations. Figure 2A shows results of SERS spectra with different 4-ATP concentrations from 10^{-6} to 10^{-8} M, which show that the determination capability of our SERS probe was below 5×10^{-8} M, indicating the 2D GNPs film exhibits good SERS activity and sensitivity as SERS substrate. The primary vibrations of 4-ATP are confirmed according to our previous work and the literature.[27, 28] To further assess possible fluctuations in every location of each array, $20\ \mu\text{m} \times 20\ \mu\text{m}$ area scanning mapping of the SERS spectra based on the intensity of the spectral band at $1076\ \text{cm}^{-1}$ of 10^{-6} M 4-ATP was shown in Figure 2B, where different colors indicates different Raman intensities of the particular peak. On the basis of above experiments in Figure 2B, the 2D GNPs film indicates the remarkable uniformity for SERS analysis as substrate.

Figure 3

In order to verify the excellent capability of our 2D GNPs film, we firstly detected 10 ppm cocaine by use of GNPs without of capping by CTAB and ordered 2D GNPs film with CTAB-capped. As shown in Figure 3, the intensity of the $1002\ \text{cm}^{-1}$ peak attributed to aromatic ring breathing produced by the GNPs was only about 550 (a.u.), and the standard deviation (σ) value was 34.3%. In comparison with above circumstance, the corresponding SERS intensity of the $1002\ \text{cm}^{-1}$ peak of cocaine adsorbed on ordered 2D GNPs film was about 1446 (a.u.) and the standard deviation (σ) value was 9.4%, as shown in Figure 3, indicating the excellent capability of 2D GNPs film. With excellent capability of ordered 2D GNPs film, we reason that CTAB-capped GNPs are present close-packed and ordered nanoparticles film, which has remarkable advantage for SERS detection.

Figure 4

In term of the sensitive of ordered 2D GNPs film, the cocaine at different concentrations ranging from 50 ppm to 100 ppb was measured in Figure 4A, which demonstrate the feasibility of aqueous

phase detection. Cocaine peaks are found at 617 cm^{-1} assigned to ring deformation, 888 cm^{-1} to $\nu(\text{C}-\text{C})$ of pyrrolidine ring, 1002 cm^{-1} to aromatic ring breathing, 1029 cm^{-1} to C-H deformation, 1273 cm^{-1} to $\nu(\text{O}-\text{C}-\text{O})$ of ester, 1598 cm^{-1} to C-C stretch of the benzoic acid moiety, and 1718 cm^{-1} to $\nu(\text{C}=\text{O})$ of benzoate ester. Besides, the vibrations between 800 and 900 cm^{-1} are tentatively assigned to C-C stretches.[8, 29] The problem reproducibility has always been a critical issue in SERS, which has restricted the development of SERS technology. Considering the reproducibility of ordered 2D GNPs film, a series of SERS spectra of 20 ppm cocaine in aqueous solution were carried out from randomly collected 50 spots in 2D presentation (Figure 4A). The intensity of the main vibration of cocaine from 50 spots of SERS data was shown to suggest good reproducibility in Figure 4A. Moreover, to obtain statistically significant effects, the relative standard deviations (RSD) of the intensities of the main vibrations were calculated in Figure 4 C–D, respectively. From Figure 4C–D, the values of the RSD for the vibrations at 1002 cm^{-1} and 1598 cm^{-1} are 8.4, and 8.6%, respectively, which are consistently less than 20%, further indicating the uniformity of the substrate. In addition, different batches of SERS substrate also illustrated excellent reproducibility (Figure S4).

The pretreatment procedure of cocaine in human urine

Figure 5

Table 1

As we know, it is a great challenge for SERS direct detection of drugs in complex biological environment. The main reason is lower concentration of analytes and the signal interference of SERS-active molecule in urine, for example: uric acid, creatinine, urea and so on. Therefore, a method of rapid purification and concentration cocaine in human urine is an effective way for improving SERS detection of drugs. Cocaine is easier to extract with organic solvents in alkaline

medium due to its nonpolar nature.[30] In this work, hexane was selected as extracted solvents, due to conveniently sampling at separation and purification by organic solvents and higher extraction recovery of drugs.[31, 32] The performance of this purification process was examination by UPLC analysis. Figure 5 shows the comparison of UPLC spectra obtained from the standard solution (red line) and the extracted phase from human urine with 1 ppm cocaine (black line), and the calculated extraction efficiency with the added cocaine concentrate in human urine from 0.1 ppm to 10 ppm was listed in Table 1. From Table 1, the extraction rate of are larger than 75%, indicating hexane as extracted solvents in alkaline medium has excellent separation and purification capability. Besides, the whole pretreatment processes only need 3 min without other instrument, and the concentration factor reaches 7.5, due to 100 μ L extraction phase for 1 mL sample volume $((1\text{ mL} \times 75\%)/100\text{ }\mu\text{L})$, which are remarkable advantage for rapid detecting low concentration drugs in human urine combining with SERS platform. Considering the uniformity and sensitivity of our 2D GNPs film, efficient purification of cocaine molecules from human urine would has outstanding performance in the following rapid SERS analysis.

Detecting hexane-extracted drug from human urine by use of ordered 2D GNPs film

Figure 6

To verify the practicability of the proposed SERS platform for the identification and detection of cocaine in practical applications, the detection of cocaine in urine samples was demonstrated here by using our purification procedures and SERS platform. In this experiment, 5 μ L of the supernatant hexane including the drugs from urine was add to dry GNPs film in silicon wafer, SERS detection can conduct after 3 s because of effumability of hexane, which greatly reduces the detection time. Figure 6 shows the SERS characteristic spectra of cocaine with a concentration ranging from 50 ppm to 100 ppb in human urine, and each set of the Raman spectrum is composed

of ten quantitative data values from ten random spots on each 2D GNPs film. The blank sample for the extraction from urine sample without drug additives was also measured as the background SERS signal and the peaks are found at 750 ,1440 cm^{-1} assigned to (CH_3) rock mode and asymmetric and symmetric C–H scissoring vibrations from $\text{N}+(\text{CH}_3)_3$ group. [33, 34] The SERS characteristic peaks of cocaine are clearly distinguished (617, 888, 1002, 1273, 1598, 1718 cm^{-1}) by comparing to Figure 4A. In Figure 6, each data set contains ten Raman spectra collected from ten random spots and produced an σ value of the peak intensity at 1002 cm^{-1} , which were calculated as the indicator of signal reproducibility. We find the results show a positive relationship between the σ value and the cocaine concentration. As the added concentration of cocaine decreased from 50 ppm to 500 ppb in human urine, the σ value increased from 5.0% to 27.0%. Although the σ value was increased to 27.0% as the cocaine concentration decreased to 500 ppb, it could be acceptable for signal recognition. Therefore, the ordered 2D GNPs film have good reproducibility of SERS signals and limit of detection (LOD) of cocaine in urine samples evidenced the purification of cocaine in human urine are simple and useful approach for cocaine analysis. Here, the LOD of cocaine in aqueous solution (100 ppb) is lower than the LOD of human urine sample (500 ppb) of SERS analysis, which most possibly originated from the impurities in human urine of hexane extraction. These impurities in extractant adsorbed onto the metal substrate surface and produced the background signals of the SERS spectrum, meanwhile, which indicated the difficulty of SERS analysis on complex biological samples. Hence, the successful detection of cocaine in hexane extraction from urine explores a new path for practical cocaine detection by the SERS technique.

Practicability of on-spot cocaine detection by virtue of portable Raman spectrometer

Figure 7

The miniaturization, portability, as well as price reductions of portable and handheld Raman

spectrometers have been designed for food safety[35], medicines quality[36], and drug abuse[11, 37], due to the advantage of detecting its signal nondestructively, as well as analyzing a sample rapidly. Here, a handheld Raman spectrometer was used for reliable SERS detection of cocaine in real human urine to reach real-time analyzer. Figure 7 presents the SERS characteristic spectra of cocaine with a concentration range from 50 ppm to 1 ppm in human urine, and each set of the Raman spectrum is composed of ten quantitative data values from ten different runs on each 2D GNPs film. 50 ppm of cocaine in urine produced an σ value of 8.5%, indicating an excellent reproducibility of handheld Raman spectrometer, and the concentration of cocaine decreased to 1ppm, the σ value increased to 25.7%, which show a positive relationship between the σ value and the cocaine concentration. Moreover, the LOD of cocaine in human urine can reach 1ppm, which is good sensitive of the handheld Raman spectrometer. For combining the rapid, reliable, economical way of pretreatment of human urine with portable Raman spectrometer, our SERS platform opens vast possibilities of on-spot detection of cocaine.

4. Conclusions

We demonstrated an ordered 2D GNPs film with controlled sub-10-nm gaps with advantages of high sensitivity, uniformity and reproducibility as SERS substrate for the detection of cocaine in aqueous solution and human urine sample. On the other hand, considering the lower concentration of drugs and interferences of other biological molecules, a rapid separation and concentration of cocaine molecules in human urine have been developed by the use of hexane as organic extraction solvents in alkaline medium. The UPLC examination indicates the larger (75%) extraction rate in our pretreatment strategy with cocaine concentration from 10 ppm to 0.1 ppm, which strongly exhibits the efficiency of pretreatment and improve the sensitivity of drugs. Moreover, the ordered 2D GNPs film with good reproducibility and high sensitivity of SERS signals evidenced the

purification of cocaine in human urine are practicable and useful approach for cocaine analysis, so that it and could resistance to false positives, which is a vital problem for law enforcement applications. Most importantly, economic and on-site detection for cocaine detection in human urine also have been realized using a handheld Raman device. The established method presents significant advantages in terms of simplicity and rapidity for the sensitive analysis of cocaine in human urine. It is hoped that this explored pretreatment strategy combined with SERS platform opens vast possibilities for the rapid, portable, and on-spot ultratrace detection in various fields, especially for public safety and antiterrorism.

Acknowledgements

This work was supported by the National Science Foundation of China (21571180 and 21505138), Special Financial Grant from the China Postdoctoral Science Foundation (2016T90590) and the China Postdoctoral Science Foundation (2015M571950).

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Figure captions

Figure 1 Characterization of ordered 2D GNPs film with average diameter of 31.7 nm. Typical SEM (A) and TEM (C) images of 2D GNPs film; (B) the statistical size distribution of GNPs film; (D) Gap distance distribution of GNPs film and the specific measurements of GNPs interparticle separations (inset, the scale bar is 10 nm)

Figure 2 (A) SERS detection by the use of uniform 2D GNPs film of 4-ATP in aqueous solution with concentrations of 1×10^{-6} , 1×10^{-7} , 5×10^{-8} , 1×10^{-8} M, respectively. (B) SERS area mapping on a specific GNPs film of 10^{-6} M 4-ATP with area of $20 \mu\text{m} \times 20 \mu\text{m}$ and a step size of $1 \mu\text{m}$.

Figure 3 SERS detection of cocaine in aqueous solution with the concentration of 10 ppm by the use citrate-capped GNPs (line b) and CTAB-capped self-assembly of ordered 2D GNPs film (line a). Each spectrum set contains ten spectra from different runs, which illustrate the standard deviation (σ).

Figure 4 (A) SERS detection of cocaine with different concentrations in aqueous solution by the use of ordered 2D GNPs film. (B) 2D presentation of 50 spectra collected randomly spots from 50 spots with 20 ppm of cocaine. (C and D) Histogram of SERS intensity at 1002 and 1599 cm^{-1} of the 50 spectra in B, respectively.

Figure 5 The examination on extraction efficiency of 1 ppm cocaine in human urine (black line) and standard solution (red line) by UPLC.

Figure 6 SERS detection cocaine of in human urine by the use of ordered 2D GNPs film. The added concentration of cocaine from 0 to 50 ppm, each data contains ten spectra from different runs and illustrates the standard deviation (σ) of peak intensity at 1002 cm^{-1} .

Figure 7 SERS detection cocaine of in human urine by the use of ordered 2D GNPs film by handheld Raman device. The added concentration of cocaine from 0 to 50 ppm, each data contains

ten spectra from different runs and illustrates the standard deviation (σ) of peak intensity at 1002 cm^{-1} .

Table 1 UPLC analysis extraction efficiency of cocaine in human urine

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Table 1:

Added concentration in urine(ppm)	Standard solution (10 ⁶ uv·s)	Extracted phase from urine (10 ⁶ uv·s)	Extracted Efficiency (%)	RSD (%)
10	1.708	1.353	79.2	4.8
5	0.728	0.598	82.1	5.2
1	0.146	0.111	76.0	4.3
0.1	0.010	0.0089	85.0	5.6