Self-assembled Nanocube-based Plasmene Nanosheets as Soft SERS Substrates towards Direct Quantitative Drug Identification on Surfaces

Kae Jye Si,¹,² Pengzhen Guo,¹,² Qianqian Shi,¹,² and Wenlong Cheng¹,²*

¹Department of Chemical Engineering, Faculty of Engineering, Monash University, Clayton 3800, Victoria, Australia

²The Melbourne Centre for Nanofabrication, 151 Wellington Road, Clayton 3168, Victoria, Australia

Correspondence and requests for materials should be addressed to W. L. Cheng (wenlong.cheng@monash.edu)
ABSTRACT

We report on self-assembled nanocube-based plasmene nanosheets as new surface-enhanced Raman scattering (SERS) substrates towards direct identification of trace amount of drugs sitting on topologically complex real-world surfaces. The uniform nanocube arrays (superlattices) led to a low spatial SERS signal variances (~2%). Unlike conventional SERS substrates which are based on rigid nanostructured metals, our plasmene nanosheets are mechanically soft and optically semitransparent, enabling conformal attachment to real-world solid surfaces such as banknotes for direct SERS identification of drugs. Our plasmene nanosheets were able to detect benzocaine overdose down to parts-per-billion (ppb) level with an excellent linear relationship ($R^2 > 0.99$) between characteristic peak intensity and concentration. On banknote surfaces, a detection limit of $\sim 0.9 \times 10^{-6}$ g/cm$^2$ benzocaine could be achieved. Furthermore, a few other drugs could also be identified, even in their binary mixtures with our plasmene nanosheets. Our experimental results clearly show our plasmene sheets represent a new class of unique SERS substrates, potentially serving as a versatile platform for real-world forensic drug identification.
The global increase in drug abuse problems such as illegal drug consumption and trafficking have been a major concern for many countries because they contribute greatly to international tensions, high crime rates, social disruptions and even deaths. Recently, it was estimated that about 183,000 cases of drug-related deaths worldwide were originated from drug overdose.\(^1\) Current drug identification methods relies on commercial colorimetric immunoassay kits such as enzyme-linked immunosorbent assay for screening, followed by verification and quantification in clinical laboratories \textit{via} techniques such as gas or liquid chromatography coupled with mass spectrometry.\(^2,3\) These methods are time consuming, which require complex instrumentation, highly-qualified personnel and tedious sample preparation.\(^3-6\)

A potential alternative analytical technique is Surface-Enhanced Raman Scattering (SERS), which is a highly sensitive optical analytical technique providing unique finger-print vibrational information of specific molecules.\(^7-9\) It is a non-destructive technique and offers detection limit down to single molecule level.\(^2,3,10-17\) SERS substrates play a critical role in enhancing inelastic scattering signals to realise high sensitivity. As far as drug identification is concerned, it is ideal to detect directly drugs sitting on banknote or other topologically complex solid surfaces. However, conventional SERS substrates are usually based on rigid roughened metallic surfaces, thus preventing their direct conformal attachment to various real-world topologically complex solid surfaces such as such coins and banknotes. Even in some cases when good attachment could be achieved, the optically opaque nature of these conventional SERS substrates prevents direct acquisition of SERS signal. In addition, signal reproducibility is also an issue due to poor structural homogeneity of SERS substrates from batch to batch.

Here, we report on a new drug identification approach using self-assembled plasmene nanosheets as a soft and flexible SERS substrate. We have previously reported fabrication
and characterisation of such a new class of plasmonic nanosheets,¹⁸,¹⁹ and now apply them for quantitative identification of illicit and pharmaceutical drugs on real-world solid surfaces. In this report, nanocubes were chosen as the building block for the nanosheets over simple spherical particles due to its sharp edges which offers additional SERS enhancement as a result of the optical antenna effect.²⁰ The structural homogeneity of our plasmene nanosheets provided a concentrated region of SERS active ‘hotspots’ that are evenly distributed throughout the substrate. We could achieve a signal variance of ~2% for the detection of benzocaine. This general platform can also be used for reliable identification of other illicit and pharmaceutical drugs, even in their mixtures. Elastic nature of our nanocube-based plasmene sheets enabled establishment of their conformal attachment to banknote surfaces. This attribute in conjunction with their optical transparency enabled direct SERS acquisition of drugs sitting on banknote surfaces without additional sample-processing steps. The method established here may also be extended to homeland security applications such as detection for the presence of drugs and explosives on hand wipes at airport safe checks.

EXPERIMENTAL SECTION

Materials

Gold (III) chloride trihydrate (HAuCl₄·3H₂O, ≥99.9%), hexadecyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride solution (CTAC, 25 wt. % in H₂O), silver nitrate (AgNO₃), sodium borohydride (NaBH₄), L-ascorbic acid (AA), 4-aminothiophenol (4-ATP), benzocaine, acetaminophen, ibuprofen and aspirin were purchased from Sigma Aldrich. Tetrahydrofuran (THF) and chloroform were obtained from Merck KGaA. Thiol-functionalized polystyrene (ₘ = 50,000 g/mol, ₘ/ₘ = 1.09) was purchased from Polymer Source Inc. Poly(dimethylsiloxane) (PDMS) Sylgard (184) silicon elastomer, curing agent, and precursor were purchased from Dow Corning, USA. All
chemicals were used as-received unless otherwise indicated. Deionized water was used in all aqueous solutions, which were further purified with a Milli-Q system (Millipore). All glasswares used in the following procedures were cleaned in a bath of freshly prepared aqua regia and were rinsed thoroughly in H₂O prior to use.

Gilder extra fine bar grids (2000 mesh with 7 × 7 µm² square holes) were purchased from Ted Pella.

**Plasmene Fabrication:**

The synthesis of Au@Ag nanocubes and subsequent assembly into plasmene sheets were achieved by adopting a recently reported publication with minor modifications. In a standard synthesis, a seed solution was first prepared via a two-step procedure: 1) reducing 10 ml HAuCl₄ (0.25 mM) and CTAB (0.1 M) mixture with 0.6 ml NaBH₄ (0.01 M) to get ~3nm seeds, followed by 2) growing 0.3 ml of the seeds in 6 ml CTAC (0.2 M), 6 ml HauCl₄ (0.5 mM) and 4.5 ml AA (0.1 M) to yield ~11nm seeds. To grow Au@Ag nanocubes, 25 ml of AgNO₃ (2 mM) and AA-CTAC solution (50 mM AA, 40 mM CTAC) were injected simultaneously at a flowrate of 1 ml/min into a heated aqueous mixture of 22.5 ml CTAC (0.02 M) and 2.5 ml Au seeds.

The nanocubes were further functionalized in a homogenous ligand exchange process with thiolated-polystyrene (PS). The as-prepared nanocubes were dispersed in a PS-THF solution (4 mg/ml) overnight, followed by repeated purification with chloroform to remove any excess PS ligands. Using a water droplet deposited on a holey carbon grid as a subphase, a concentrated droplet (1 µl) of PS-NCs was spread on the water/air interface. Subsequent solvent evaporation leads to the formation of a monolayered plasmene sheet.

**Plasma Treatment:**
For plasma treatment, plasmene sheets assembled on copper grid were placed in a UV ozone chamber at an oxygen flow rate of 0.5 L/min. The duration of treatment was varied at 3 min, 5 min and 10 min.

**Characterization of plasmene**

Electron imaging was carried out using a Hitachi H-7500 field emission TEM operating at 80 kV.

The optical extinction spectra of the nanosheets were obtained using a J&M MSP210 microscope spectrometry system. Optical micrographs were captured using a Nikon industrial bright field microscope (ECLIPSE LV 100D) under transmission mode.

**Raman experiments**

For the swipe-based drug detection, the treated plasmene sheets were first transferred onto silicon wafers using a PDMS stamping method that have been reported previously. The drug analyte, which was dissolved in ethanol to get the desired concentration, was then swiped onto the plasmene surface using a cotton swab and allowed to dry prior to SERS measurements.

For trace detection of drug on banknotes, a droplet of drug solution was doped onto the banknote surface and allowed to dry, before stamping of treated plasmene sheet onto the doped circular region. SERS spectra were acquired immediately after stamping.

SERS spectra were recorded by using a Renishaw RM 2000 Confocal micro-Raman System equipped with an excitation laser wavelength of 514 nm (laser spot size of 1 µm, laser power of 0.1 mW). All Raman spectra were recorded by fine-focusing a 50× microscope objective under data acquisition time of 10 s, and corrected by cubic spline baseline subtraction to exclude the fluorescence contribution. For all quantitative analysis, 10 SERS
measurement were acquired on different locations of a specific plasmene nanosheet. The dominant peak exhibited by each drug analyte was selected to produce the calibration plot.

RESULTS AND DISCUSSION

Plasmene nanosheets comprising of core-shell Au@Ag Nanocube (NC) particles (~35 nm edge lengths) were fabricated via a polystyrene (PS)-based evaporation-mediated self-assembly at air-water interface as described in our previous paper.\textsuperscript{18,19} TEM images of the as-fabricated plasmene sheets revealed the assembly of NC particles into close-packed monolayered arrays with high degree of pattern regularity and average interparticle spacing of $10 \pm 0.8$ nm (Figure 1a). The assembled plasmene sheets were then exposed to short time intervals of oxygen plasma treatment, which aimed to increase detection sensitivity by stripping away the surface polystyrene ligands that represents a barrier for analyte adsorption. As the primary function of the polystyrene ligands is to act as a spacer molecule which controls the interparticle spacing, the duration of the plasma treatment needs to be properly optimized to ensure controlled ligand removal without causing any structural disordering.\textsuperscript{21,22}

The structural changes of the treated plasmene sheets were characterized by TEM images as shown in Figure S1. With the short treatment time (3 minutes), the NC plasmene sheets retained their particle ordering with reduced interparticle spacing of $3.2 \pm 0.7$ nm (Figure S2). This is attributed by the fact that the assembly process is governed primarily by the balance of core-to-core van der Waals (vdW) attractive forces and PS ligand-ligand repulsive forces.\textsuperscript{23} Controlled removal of the surface PS ligands resulted in imbalance between steric hindrance and vdW forces, and strong vdW forces brought NCs closer together. Excessive plasma treatment ($\geq 5$ minutes) resulted in vdWs attractive forces being dominant, thus resulting in nanoparticle aggregation, loss of particle ordering and formation of cracks. This was further verified by a study of the extinction spectra evolution. The
optimized plasmene sheet initially exhibited a red shift in plasmonic resonance from 474 nm to 514 nm due to stronger plasmonic coupling, but the peak shape and intensity eventually diminished and broadened excessively as treatment duration was increased (Figure 1b).

As the SERS enhancement is known to scale to the fourth power of the local electromagnetic field,\(^6\) the optimized plasmene with decreased interparticle spacing is expected to possess intensified electromagnetic ‘hotspots’ due to stronger plasmonic coupling. Intrinsically, this will contribute to a significant enhancement of the SERS response. As a further proof, we investigated the SERS performance of the plasma treated sheets by using 4-aminothiophenol as a model Raman analyte. As observed in Figure 1c, the optimized plasma-treated plasmene sheet (red spectrum) exhibited enhanced response in comparison to untreated plasmene (black spectrum). However, excessive plasma treatment might cause oxidation of the silver surface, with weakening local electromagnetic fields leading to weaker SERS response (blue and pink spectra).

To test the ability of our NC plasmene nanosheets in SERS identification towards illegal drugs, we have swabbed plasmene sheets stamped onto silicon wafers with aqueous drug solution followed by direct SERS signal acquisition (Figure 2a). Our primary drug model was chosen to be benzocaine, which is a common drug marker with similar physicochemical properties to cocaine. Figure 2b shows the recorded SERS spectra of benzocaine, which were dominated by a set of peaks located at 860, 1173, 1280, 1604 and 1682 cm\(^{-1}\). The vibrational assignments of these peaks were summarized in Table S1. Our system demonstrated a better sensitivity as compared to current chromatography-based methods (Supporting information, Table S2), with a limit of detection (LOD) down to 10 ppb. Such sensitivity is sufficient for detection of a typical saliva overdose concentration, which is generally at parts-per-million (ppm) level for most drugs.\(^{2,4}\) Another critical aspect for application of SERS for routine applications is to ensure reliable and excellent signal
reproducibility. Owing to the nanostructurally homogenous nature of the plasmene sheets, the SERS ‘hotspots’ are distributed uniformly and endow high signal reproducibility across the whole substrate. This was investigated by obtaining and superimposing the SERS signal at 10 random spots on the plasmene sheet, demonstrating a spot to spot variance of < 2% for the peak intensity at 1604 cm\(^{-1}\) (Figure S3) as a proof of excellent signal reproducibility.

Beyond the high sensitivity and reproducibility, we further show that it is possible to quantify the amount of drug present in a single cotton swab swipe. A plot of the dominant peak intensity corresponding to C=O peak at 1604 cm\(^{-1}\) against the drug concentration revealed a simple adsorption–saturation Langmuir relationship (Figure 2c). At the low concentration regime (0.1 ~ 5 ppm), a linear relationships between the peak intensity and benzocaine concentration was observed (Figure 2d). This was because the SERS intensity is directly proportional to the number of molecules adsorbed on the SERS ‘hotspots’.\(^\text{13}\) The linear regression gave the following empirical ruler equation:

\[
I_{\text{SERS}} = 1188.5 \text{ [Benzocaine]} + 1469.1 \text{ with } r^2 = 0.998
\]

where \(I_{\text{SERS}}\) and [Benzocaine] is the intensity of the 1604 cm\(^{-1}\) peak and concentration of the adsorbed benzocaine, respectively. The limit of quantification (LOQ) could then be determined to be \(\sim 6.05 \times 10^{-7} \text{ M}\). In addition to benzocaine, we also demonstrated detection of over-the-counter (OTC) pharmaceutical drugs such as acetaminophen, aspirin and ibuprofen that commonly resulted in accidental overdose emergency visits. Each drug compound could be specifically identified by its characteristic vibrational bands (Figure S4, see Table S1 for mode assignments), and similarly exhibited a linear quantitative behaviour within the low concentration range and the maximum physiologically allowable intake limit (Table 1, Figure S5). These excellent quantitative behaviours highlight the effectiveness of our plasmene nanosheets for accurate SERS quantitative analysis of drug overdose.
Since the drugs we investigated showed narrow bandwidth and fingerprint characteristics in their respective SERS spectra, we could then barcode them for a simple readout. We converted the SERS spectra into barcodes based on the wavenumber shift and the integrated SERS intensity around the central vibrational peaks (see Supporting Information Section VI), following the approach in the literature.\textsuperscript{15,24} The resulting barcodes are shown in Figure 3. To demonstrate the ability in identifying drugs in a mixture, drugs were mixed in 1:1 mole ratio and swiped onto the plasmene surface prior to SERS spectral acquisition. The SERS spectra were then converted into barcode and compared with the reference barcodes generated by combination of the standard isolated drug barcodes (Figure 3). Remarkably, for each mixture investigated, the specific drugs could be positively identified (Figure 4), highlighting the potential of our plasmene sheets for creation of an enormous searchable library of drug codes for SERS identification in mixtures.

In the drug trade, the trace amounts of illicit drugs are often present on seized banknotes, which can serve as evidence in suspected drug trafficking cases. However, it is challenging to directly detect the trace amount of drugs without any additional sample processing steps. Our nanocube-based plasmene nanosheets could overcome this challenge in that they are soft and optically transparent, enabling conformal attachment to suspected banknotes and direct SERS signal acquisition, respectively (Figure 5a). This is not possible to achieve with conventional rigid and opaque SERS substrates. As a proof of concept, we deposited benzocaine solution onto a United States one-dollar bill. After drying, our plasmene nanosheets were stamped onto the stained area, followed by direct SERS signal acquisition. Figure 5b shows that trace amounts of benzocaine could be detected only when the plasmene nanosheet was attached to the banknote surface (blue curve). Examination of the peak intensity for quantitative analysis revealed a linear dependence for a concentration range from 0.5 to 10 $\mu$g/cm$^2$ (Figure 5c). Operating at the laser power of 0.1 mW with a laser
spot area of 1 µm², the lowest detectable benzocaine mass, which is influenced by the volume (5 µl) and circular area (1.14 cm²) in which the drug droplet spreads into, was estimated to be ~0.9 x 10⁻⁶ g/cm². Remarkably, our plasmene-based methodology of direct SERS identification of drugs could be extended to many other types of banknotes, regardless of the material (paper or plastic) and quality (new or old) of the banknotes (see Supporting Information Section VII).

CONCLUSION

In summary, we have demonstrated that our plasmene nanosheets can be utilized as a unique SERS substrate for analytical analysis of illicit and pharmaceutical drugs. Optimized plasmene sheet with interparticle spacing of ~3 nm was achieved after optimized plasma treatment duration. We implemented these plasmene sheets to demonstrate a unique and powerful analytical method for the detection of drug overdose as well as trace detection of drugs on banknotes. In comparison to current practical drug analytical methods, this offers distinct advantages such as low-cost, simplicity, high sensitivity, direct qualitative and quantitative analysis without additional sample processing steps, and potential real-time and in-situ detection with a handheld Raman instrument. In addition, plasmene nanosheets could be tailor-designed with other ‘meta-atoms’ in the plasmonic periodic table, meeting specific requirements such as the excitation laser wavelengths. Based on these unique attributes, we believe our soft plasmene sheets represent a new class of SERS platform for direct chemical identifications on real-world solid surfaces.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION
Corresponding Author

* To whom correspondence should be addressed. Email: wenlong.cheng@monash.edu

Author Contributions

W.C. and K.J.S. conceived and planned the experiments, which were carried out by K.J.S., P.G., and Q.S; W.L.C. and K.J.S. analyzed the experimental data, discussed the results and co-wrote the manuscript.

ACKNOWLEDGMENT

This research was financially supported under the Australian Research Council’s Discovery projects funding schemes DP120100170 and DP140100052. This work was performed in part at the Melbourne Centre for Nanofabrication (MCN) in the Victorian Node of the Australian National Fabrication Facility (ANFF). The authors also gratefully acknowledge the use of facilities at Monash Micro Imaging (MMI) Centre.

REFERENCES


Table 1: Analytical Parameters for Quantitative Characterization of Drug Overdose

<table>
<thead>
<tr>
<th>Entry</th>
<th>Benzocaine</th>
<th>Ibuprofen</th>
<th>Acetaminophen</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak position (cm⁻¹)</td>
<td>1604</td>
<td>1608</td>
<td>1325</td>
<td>1606</td>
</tr>
<tr>
<td>Peak assignment</td>
<td>ν(C=C)</td>
<td>ν(C=C)</td>
<td>δ(C-H)</td>
<td>ν(C=C)</td>
</tr>
<tr>
<td>Linearity</td>
<td>0.998</td>
<td>0.996</td>
<td>0.998</td>
<td>0.996</td>
</tr>
<tr>
<td>Slope</td>
<td>1188.5</td>
<td>6.6</td>
<td>5.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Intercept</td>
<td>1469.1</td>
<td>234.7</td>
<td>390.6</td>
<td>310.7</td>
</tr>
<tr>
<td>LOQ (M)</td>
<td>6.05 × 10⁻⁷</td>
<td>4.85 × 10⁻⁵</td>
<td>6.62 × 10⁻⁵</td>
<td>5.55 × 10⁻⁵</td>
</tr>
</tbody>
</table>
Figure 1. Nanocube (NC) plasmene for optimized SERS performance. (a) TEM images of NC plasmene, showing NC building blocks in an ordered arrangement. (b) Spectral evolution of plasma treated plasmene sheets. (c) Comparison of 4-Aminothiophenol SERS spectra at 1 µM concentration recorded from untreated and treated plasmene nanosheets.
Figure 2. Swipe sampling for drug overdose identification. (a) Scheme depicting the swipe sampling procedure for drug detection. (b) SERS spectra of solid benzocaine and aqueous benzocaine at various concentrations. (c) Adsorption-saturation Langmuir plot between intensity of the dominant 1604 cm\(^{-1}\) peak and the corresponding benzocaine concentration. (d) Linear calibration plot at low drug concentration for quantitative analysis. Dotted line shows the current allowable benzocaine intake limit.
Figure 3. SERS spectra and barcode representation for multiple real world drugs. SERS spectra and corresponding barcodes for (a) benzocaine, (b) acetaminophen, (c) ibuprofen and (d) aspirin. The stars for each SERS spectra represent the selected relevant peaks for barcode conversion.
Figure 4. Spectral barcode multiplexing for identification of dual drug mixtures. The dual drug mixtures prepared for this study are (a) benzocaine + aspirin, (b) aspirin + acetaminophen, (c) aspirin + ibuprofen, and (d) benzocaine + acetaminophen. The spectra from the dual drug mixture is converted into barcodes (orange colour) and compared with the reference drug barcode (multiple colours). For each comparison, the black, blue, green, red and orange dotted arrows indicate spectra present in acetaminophen, aspirin, benzocaine, ibuprofen and both drugs, respectively.
Figure 5. Direct plasmene stamping for trace drug detection on new United States banknote.

(a) Scheme depicting the procedure to stamp plasmene sheet onto doped banknote surface.

(b) Comparison of SERS spectra of benzocaine on banknote surface, showing signal enhancement capability of the plasmene sheet.

(c) Calibration plot showing the linear relationship between the intensity of the dominant 1604 cm$^{-1}$ peak and the corresponding benzocaine concentration.
Table of Contents

- Benzocaine
- Acetaminophen
- Ibuprofen
- Aspirin