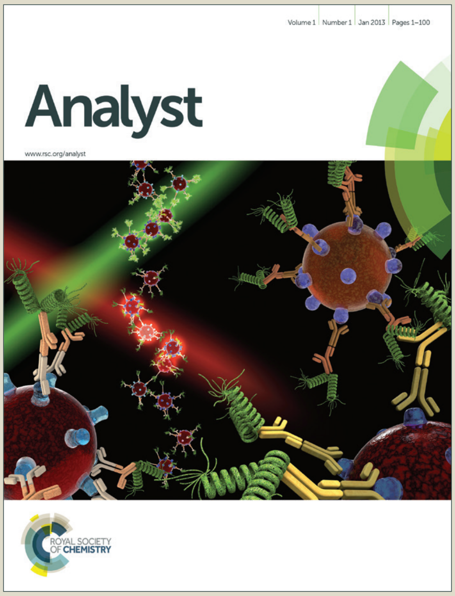
Analyst

Accepted Manuscript



ROYAL SOCIETY
OF CHEMISTRY

www.rsc.org/analyst

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Silver Nanoparticles Embedded Hydrogel as a Substrate for Surface Contamination Analysis by Surface-Enhanced Raman Scattering

Zhengjun Gong,[a] Canchen Wang,[a,b] Cong Wang,[b] Changyu Tang,[b] Fansheng Cheng,[b] Hongjie Du,[a,b] Meikun Fan,[a]* and Alexandre G. Brolo[c]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A surface enhanced Raman scattering (SERS) substrate, capable of extracting small amounts of organic species from surfaces of different types of materials with variable roughness, has been fabricated. The substrate consists of Ag NPs encapsulated in poly (vinyl alcohol) (PVA) hydrogels, commonly known as PVA "slime". Unlike traditional SERS substrates, such as colloidal suspensions, the resulted PVA slime SERS substrate presents good visco-elasticity, allowing it to conform to the surface of various materials of arbitrary roughness. Surfaces of different materials, including sandpapers, cotton, metal, and wood, previously contaminated with nile blue A (NBA) were analyzed with the PVA slime SERS substrate. Limits of detection (LOD) as low as 100 ppb (0.79 ng in total amount on an area of ~3 cm^2) were achieved for all surfaces tested. Pesticides and Sudan red III on glass surface have also been detected, with a LOD of 1.6 ng/~ 3 cm^2.

Introduction

In recent years, there has been an increasing demand for methods capable of performing fast, on-site detection and identification of various analytes on surfaces.[1-3] For example, safety concerns in aviation industry require fast screening methods for explosives detection;[4] real-time detection of traces of drugs and the identification of other forensic evidences are of high demand for criminal scene investigations;[4, 5] the quick verification of sanitary of raw materials and/or containers are important for the health and food industries; [2, 6, 7] and, finally, the identification of dyes and pigments in ancient artefacts are central in archaeology and art restoration.[8] Currently, ion mobility spectroscopy and mass spectroscopy are the main techniques used for surface contaminants analysis. However, the instrumentation size and cost associated to those techniques still need to be reduced.[9] Furthermore, the sampling efficiency is another major challenge encountered with the current state-of-the-art.[4]

Surface enhanced Raman scattering (SERS) is considered to be one of the most powerful spectroscopic analytical methods, due to its high sensitivity and capability of providing fingerprint-like information of analytes.[10-13] Most importantly, Raman instruments can be miniaturized without significantly sacrificing their analytical figures of merit.[14] There are then tremendous interests in taking advantage of the SERS technique for potential applications in different fields, such as biomedical,[15, 16] homeland security,[14] environmental monitoring,[17-19] and food quality assurance.[2, 20] As a result, enormous efforts have been directed into the developing of reproducible and-cost effective SERS substrates (nanostructured metallic platforms that support the SERS effect).[11, 21, 22]

The SERS community has already responded to the demand for new approaches in surface contamination analysis by developing the tip enhanced Raman scattering (TERS) technique,[23] the shell-isolated-nanoparticles enhanced-Raman spectroscopy (SHINERS) method,[2, 24] and by fabricating a new generation of soft and flexible SERS substrates.[25-27] In its current state, TERS is a complex and costly technique that is not ready for implementation in broad analytical applications.[2] The SHINERS technique, on the other hand, uses shell-isolated NPs, which may limit its sensitivity, since strong SERS hotspots arises from NPs aggregates.[2] The application of soft SERS substrates is, thus, very promising for the analysis of surface contaminants. Flexible SERS substrates can be further arbitrarily divided into two groups. The first group involves flexible SERS substrate that can perform direct swab sampling from a surface, such as metallic NPs immobilized on paper filter, [25] electrospun polymer mat,[28] and spin coated polymer films.[29] The other group focuses on soft and gel-like SERS substrates that are adaptable to surfaces of different shapes and roughness, such as NPs embedded in agarose,[30-34] poly(acrylic acid) (PAA) hydrogel,[35] and polydimethylsiloxane (PDMS).[27, 36]

The use of NPs-embedded soft materials as SERS substrates presents many advantages. For instance, they are non-destructive; can extract the analyte into the gel through a solid-state microphase extraction process;[37, 38] and it can even be potentially used for *in situ* chemical imaging.[27] Therefore, a combination of gel-like SERS substrates with miniaturized Raman spectrometers can provide fast, real time analysis of surface contaminants in the field. However, the gel-like SERS substrates reported in the

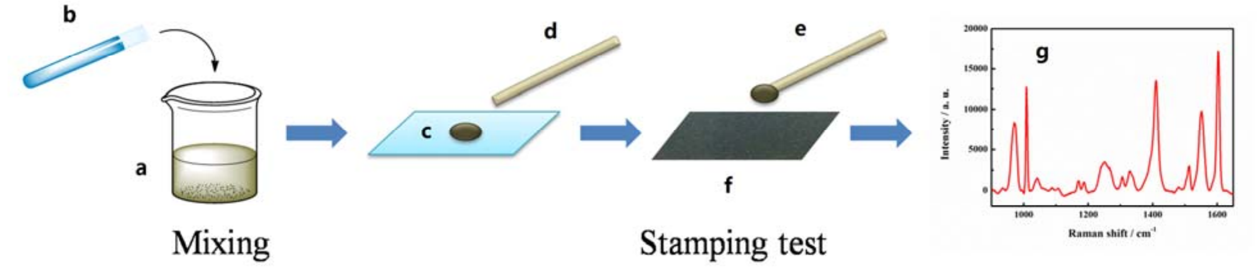
---

[a] *Faculty of Geosciences and Environmental Engineering, Southwest Jiaotong University, Chengdu, 610031, China.*
E-mail: meikunfan@gmail.com
[b] *Chengdu Development Center of Science and Technology, China Academy of Engineering Physics, Chengdu,610207, China.*
[c] *Department of Chemistry, University of Victoria, BC, V8W 3V6, Canada.*
E-mail: agbrolo@uvic.ca

literature have not yet fulfilled the performance requirements for widespread applications. For example, agarose is known to be brittle and the application of agarose-based SERS substrates to extract contaminants from rough surfaces has not yet been explored.[30, 34, 39] The preparation of Ag NPs loaded PAA hydrogel[35] requires complicated equipment and uses a dangerous chemical (HF) in the synthesis.

Poly-(vinyl alcohol) (PVA) has long been used as supporting polymer for the fabrication of flexible (SERS) substrate by either *ex-situ*,[26, 40, 41] or *in situ*,[40, 42-46] synthesis of gold/silver NPs. However, the resulting PVA substrates were either electrospun into nanofibers mats,[26, 40-42] or spin coated in a planar substrate to generate polymer films.[43, 44] Therefore, although those PVA-based platforms provided good SERS performance, they were not shape adaptable to be used for the extraction of contaminants from arbitrary surfaces. Furthermore, similar to the agarose hydrogel SERS substrate,[30] most of the reported PVA-based flexible SERS substrates used the *in situ* synthesis method,[40, 42] which reduced the possibilities of adjusting the size and the distribution of NPs in the polymer matrix.

Here, we report a simple process for the fabrication of a visco-elastic hydrogel SERS substrate based on the traditional preparation of PVA slime (slime SERS substrate). Ag colloidal suspension was first prepared and used as solvent for dissolving PVA (*ex situ* synthesis method). A centrifugation process was utilized to increase the Ag NPs density inside the polymer. Finally, sodium borate was added to the concentrated Ag NPs-PVA mixture to form the gel. The procedure did not require any toxic chemicals and specialized equipment, except for the centrifuge. The SERS slime showed a high viscosity (supporting video), but it could be easily manipulated and applied to different surfaces. The SERS slime reported here presents two key advantages compared to other NPs-embedded PVA SERS substrates previously reported in the literature:[40-45] 1) The SERS slime substrate is shape adaptable, which allow it to coat surfaces of different textures and extract contaminant species; 2) the synthetic protocol permits tuning of size, shape, and amount of nanomaterials inside the slime, leading to a variety of parameters that can be used to optimize the SERS performance. The Raman probe molecule nile blue A (NBA), the pesticides triazophos, isocarbophos, and methyl parathion, and illicit food additive Sudan red III were analyzed using the SERS slime substrate to extract them from different surfaces. The limit of detection (LOD) for NBA on glass was found to be 0.79 *pg* spread over an area of ~3 cm^2, and that of triazophos was 1.6 *ng*/3 cm^2. Moreover, various surfaces contaminated with the sample analyte NBA at sub-ppb level, including sandpaper and fabric, were analyzed and the probe molecule identified.



Scheme 1 Schematic representation of the preparation of the slime SERS substrate and the stamping analysis. a. Ag NPs/PVA mixture; b. Sodium borate; c. SERS slime; d. Wood stick; e. SERS slime on wood stick; f. Contaminated surface; g, SERS of the surface contaminant. See text for more detail.

Experimental section

Chemicals and reagents

The following reagents were used without further purification: Silver nitrate (99%), sodium citrate (99%), Poly (vinyl alcohol) (PVA, MW=89,000-98,000, 99+% hydrolyzed), nile blue a, Sudan red III, triazophos, isocarbophos, methyl parathion, sodium tetraborate (99%) and other chemicals were all supplied by Sigma-Aldrich. 18.2 MΩ.cm water (Nanopure, Thermo) was used throughout the experiment.

Instruments

SERS measurements were performed on a customized Raman microscope. The detector was a Pixis-100BR CCD (Princeton Instrument), the dispersion system was an Acton SP-2500i spectrograph, and the excitation source was a 20 mW He-Ne laser. Excitation wavelength of 632.8 and a 20× objective (N.A. = 0.45) were used throughout the experiment. The acquisition time was different for each spectrum, but the SERS intensity for all of them was adjusted and it is presented as counts-per-second (cps).

An UWave-1000 microwave (Sineo Micro-wave) was used to synthesize the Ag NPs. UV-Vis spectrophotometer (sp-756) and a scanning electron microscopy (SEM, FEI Inspect F) were used to characterize the Ag NPs and the SERS substrates. A Bohlin Gemini II Rheometer (Malvern Instruments, England) was used to characterize the rheologic performance of the slime substrates.

Ag NPs synthesis

Ag NPs (5 mM) was prepared by reducing silver nitrate with sodium citrate. Briefly, 0.5 g $AgNO_3$ was dissolved in 600 mL water and gradually heated to 98 °C with vigorous stirring and ultrasonic agitation in the UWave-1000 within 15 min. Then, 12 mL of 10% (w:w) sodium citrate solution was quickly added, and the solution was kept at 98 °C for another 30 min. Afterwards, the solution was cooled to room temperature with constant stirring. The above process was also followed for the preparation of lower concentrations of Ag NPs but 0.5, 1 and 2 mM $AgNO_3$, respectively, were used in that case.

SERS slime preparation (Scheme 1a-1c)

Firstly, 0.8 g PVA and 18.2 mL Ag NPs suspension were mixed in a beaker, and then heated to 90 °C in a water bath under vigorous stirring at the speed of 500 rotations/min for 2 h. After that the mixture was concentrated by centrifugation. For instance, a concentration factor of 5 was achieved by taking 10 mL of the PVA/Ag NPs mixture and submitting it to a 5 min centrifugation at 5000 r/min. After centrifugation, the Ag NPs formed a pellet at the bottom of the tube. 8.00 mL of the supernatant was removed and the NPs pellet was re-dispersed in the remaining solvent (2.00 mL) by sonication. PVA/Ag NPs with different "concentration factors" were obtained using a similar procedure. Finally, 10 parts of the concentrated PVA/Ag NPs mixture (Scheme 1a) was mixed with 3 parts of 4% (w:w) sodium tetraborate (V:V) (Scheme 1b) to yield the final slime SERS substrate(Scheme 1c). It was found that the final mixing procedure was the essential step to obtain reproducible SERS slime substrate. A glass rod was used to stir and knead the mixture. The mixing process took about 5 min.

SERS analysis

Two protocols were used in this work: 1) For characterization and optimization of the performance of the SERS slime substrate, a 5 μL 1 ppm NBA ethanolic solution was drop-dried on ~0.1 g of a SERS slime film (prepared by pressing with glass slide). The SERS spectra were collected after the solvent evaporation. 2) For stamping test on contaminated surfaces (Scheme 1c-1f), the appropriate amount of the probe solution was first dropped on different surfaces. After the solvent evaporated, 10 μL of EtOH was spread on the spot, followed by extraction with ~0.03 g of slime substrate attached to a wood stick (Scheme 1e). SERS spectra were recorded using the side that was in direct contact with the surface containing the analyte. Unless otherwise specified, all the spectra in this work were the average of 10 measurements.

Results and discussion

Optimization and characterization of the slime SERS substrate

Hydrogels are generally soft, viscoelastic, 3-D polymer networks. Their structural integrity is maintained during the process of deformation. The permeability is generally good since it contains large amount of water.[47] Thus, Ag NPs doped hydrogels would be an ideal SERS substrate for surface contamination analysis: it is conformable to surface curvatures, and water soluble (or soluble in equivalent solvents, such as methanol and ethanol) analytes can travel through the pore channels in the network to reach the Ag NPs (to produce SERS). However, the hydrogel can be flexible, brittle, soft or hard depending on the degree of cross-linking. Moreover, the SERS performance might also depend on the level of cross-linking. Therefore, a systematic investigation was first carried out to achieve the proper balance between the mechanical characteristics of the slime and its SERS performance.

The systematic optimization results are presented as supporting information. In summary, an optimized SERS performance was obtained in slimes containing 4~6% of PVA in PVA/Ag NPs mixture (Fig. s1). The mechanical characteristics of the SERS slime was probed by dynamic rheological measurements and it was found that slime synthesized by mixing 4% of PVA/Ag NPs mixture with 4% of the borax solution at 10:3 ratio (V:V) provided the best mechanical performance (Fig. s2). Slimes prepared with more than 6% PVA turned out to be brittle, while the 4% PVA SERS slime is a solid that can flow slowly with its own gravity (supplementary video).

After optimizing the ratio of PVA and borax, the concentration of Ag NPs in the final substrate was also examined based on the SE(R)RS[48] performance. Fig. 1(A) shows that the intensity of the SERS of NBA increased with the concentration of Ag NPs in the slime substrate. This is expected, since more Ag NPs should lead to more hot spots and a better SERS performance. Although higher concentration of Ag NPs in the hydrogel is correlated to a better SERS performance, it is not practical to synthesize Ag NPs using $AgNO_3$ solution higher than 5 mM (silver mirror will form on the glass surface instead of producing Ag NPs).

A centrifugation procedure was then used to further increase the concentration of Ag NPs in the hydrogel. PVA dissolved Ag NPs suspensions prepared using 5 mM $AgNO_3$ were first centrifuged. Then, the desired volume of the supernatant was removed to achieve a specific concentration factor (see a description in the experimental section). Finally, the remaining mixture was re-dispersed by ultrasound agitation. Fig. 1(B) suggests that the best SERS performance was achieved at the centrifugation concentration factor of 6 (confirmed by EDX mapping, Fig. s3). The SERS signal of NBA dropped at higher concentration factors (Fig. 1(B)). The abrupt drop of SERS signal of NBA is caused by inefficient dispersion of Ag NPs aggregates at higher concentration factors. This is partially explained in Fig. 1(C), where representative normalized UV-Vis spectra of Ag NPs embedded PVA hydrogels (SERS slime substrate) at different concentration factors are presented. Compared to the normalized extinction spectrum of Ag NPs suspension in water (Fig. 1(D)), the extinction spectra of SERS slime substrates present a shoulder around 690 nm which is a clear sign of Ag NPs aggregation inside the hydrogel.[21, 22] As the concentrating factor increases, the relative intensity of the 690 nm shoulder increases, a new shoulder appears around 780 nm which also rises with the concentration factor, and the 410 nm peak broadens. All of these changes indicate that increase in the concentration factor is accompanied by a higher degree of aggregation (i.e., larger aggregates). At concentration factor larger than 6, the NPs aggregation was so serious that it was not possible to get homogeneous re-distribution inside the hydrogel even after sonication. As a result, the probability of a probe molecule to meet the hotspot inside the gel decreases. Thus, the SERS signal of NBA drops at concentration factors higher than 6.

In conclusion, the optimized procedure to fabricate the slime SERS substate is as following: 4% (w:w) PVA/Ag NPs mixture was concentrated at the factor of 6 and mixed with 4% borax (w:w) at 10:3 ratio (V:V).

Performance of the slime SERS substrate

In order to test the reproducibility of the substrate, the optimized slime SERS substrate was compressed with a glass slide to form a flat surface (hereafter, called slime film). Then 5 μL of 1 ppm ethanolic solution of NBA was dropped onto the resulting slime film. This experiment was repeated with 13 slime films prepared in different batches. Ten SERS spectra were

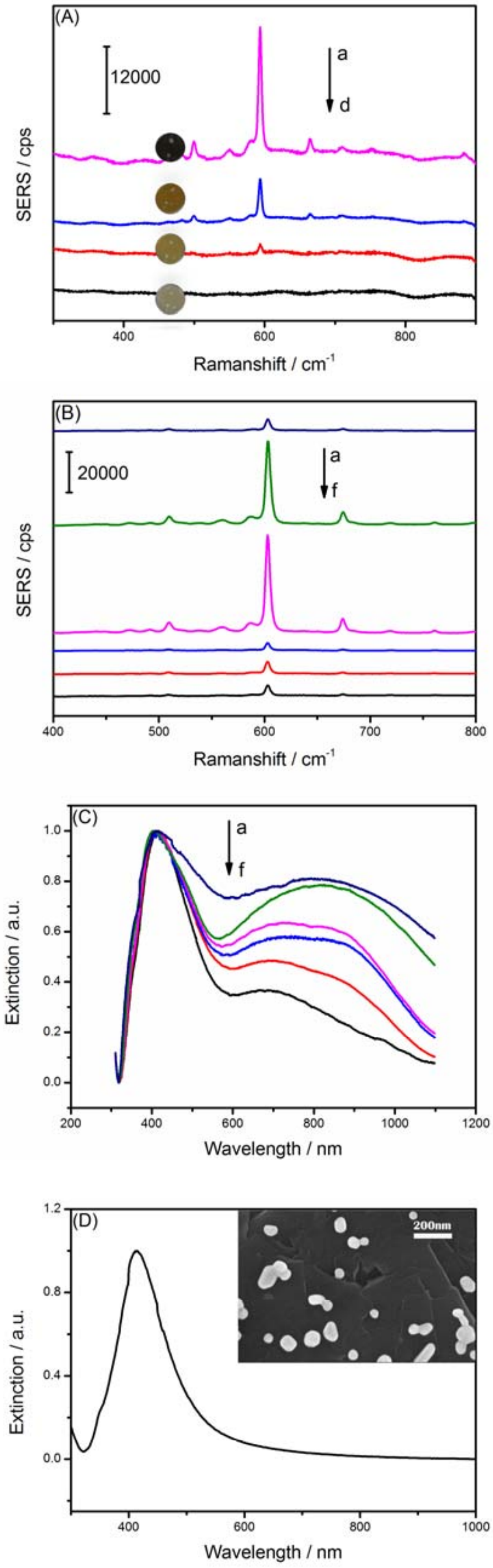


Fig. 1 (A) The effect of Ag NPs at different concentrations on the SERS of NBA. Ag NPs prepared from: (a), 5 mM (b), 2 mM (c), 1 mM (d), 0.5 mM $AgNO_3$. Inset are pictures of the slime SERS substrates prepared with different concentration of Ag NPs. Laser power, 600 µW. (B) The effect of the Ag NPs concentration factor: (a-f) concentration factors 10, 8, 6, 4, 2 and 0 -folds, respectively. See text for more information. Laser power, 300 µW. (C) Normalized UV-Vis extinction spectra of slime SERS substrates at different concentration factors. Top to bottom: concentration factors were (a-f): 10, 8, 6, 4, 2, 0, respectively (D) Normalized UV-Vis extinction of 5 mM Ag NPs suspension in water, inset is the SEM of this sample.

recorded from each film in different regions. Fig. 2(A) listed the statistics for these experiments. It was found that the percent relative standard deviation (RSD%) was 26% between the films, and the RSD% within a given slime film ranged from 13 to 32%. The higher RSD% within a film is probably caused by the non-uniform distribution of Ag NPs in the procedure of slime preparation. However, the sample to sample RSD% is comparable to our previous work[11, 21] and other reports.[35, 49] Naturally, this level of variation may have a negative impact in applications that require accurate quantification, for which methods such as internal reference calibration is needed.[50]

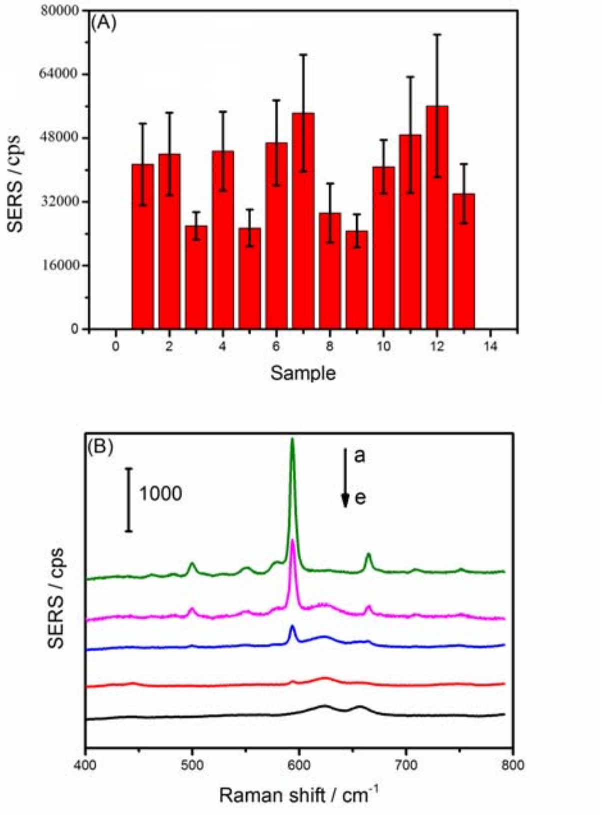


Fig. 2 (A) Statistics of 13 slime substrates obtained by using NBA as a Raman probe, see text for more information. (B) SERS spectra of NBA at concentrations of 1×10^{-6}, 1×10^{-7}, 1×10^{-9}, 1×10^{-10} and 0 g/g ethanol (a-e), respectively. Note that the spectrum *a* was divided by a factor of 10 for better comparison. Laser power, 300 µW.

The LOD was determined by extracting the pre-deposited organic probe molecule (NBA) on glass surfaces with the SERS slime, following the procedure described in scheme 1. Ten µL of various concentrations of NBA ethanolic solutions were dropped on glass slides. The contaminated areas were stamped with the

SERS slime, such that the organic contaminant from the surface could be extracted to the PVA matrix. SERS spectra of NBA were recorded on each substrate after extraction. Fig. 2(B) shows that the NBA SERS signature can still be identified even at 100 ppt, 0.79 pg (~1 femtomole) of analyte on the glass surface (~ 3 cm^2). The dye signature can be identified at low concentrations even in the presence of a broad background from the gel matrix (spectrum e in Figure 2(B)).

The shelf life of the substrate was also examined. One gram of slime SERS substrate was prepared, sealed in a centrifuge tube, and kept in a fridge. About 0.1 g of the slime substrate was taken out each day and its SERS performance was examined by adding 5 μL 1 ppm NBA ethanolic solution. It was found that the variation of SERS intensity of NBA was less than 20% during a course of a week.

In summary, these results indicate that the prepared SERS substrate is efficient. The reproducibility is on the average level of what has been reported in literature.

Stamping test of NBA on diverse surfaces

In order to confirm the flexibility of the slime SERS substrate in probing different surfaces, 10 μL of 0.1 ppm NBA alcoholic solution was dropped onto different materials and let dry in air. The procedure consisted of adding 10 μL of EtOH on the contaminated area followed by stamping with the slime substrate (as in scheme 1).

Real-time analysis of contaminants on rough surfaces using low cost instrumentation is a serious analytical challenge. One limitation is that it is difficult to collect the analyte from the surface, even by means of swabbing, in a reliable way. Since the slime SERS substrate presents some liquid characteristics, it can conform to surfaces of different roughness. This was first tested by using the slime SERS substrate to sample contaminants from sandpaper surfaces of different roughness (from 400 to 3000 mesh). As shown in Fig. 3(A), even from a surface as rough as 400 mesh sandpaper, the SERS signal of 1 picomole NBA can be detected satisfactorily. Fig. 3(B) shows the SERS spectra of 1 picomole of NBA extracted using the slime substrate from surfaces of different materials. The highest SERS signal was from the extraction from aluminium block, while the lowest was from cotton. This is not surprising, since cotton is very efficient in absorbing dyes. This makes it very difficult to extract the NBA out into the slime substrate. Thus, quantification of surface contaminants would be difficult since the extraction efficiency varies for different surfaces. Although calibrations curves could be obtained for a particular surface or other quantification procedures, such as the method of standard additions, could be pursued, those would significantly increase the analysis time. On the other hand, Fig. 3 clearly shows that the SERS slime substrate can detect surface contaminants above a certain level from different surfaces. This ability should be very useful in several rapid screening applications, where absolute quantification is not strictly required, such as in forensics, homeland security and food safety inspection.[51]

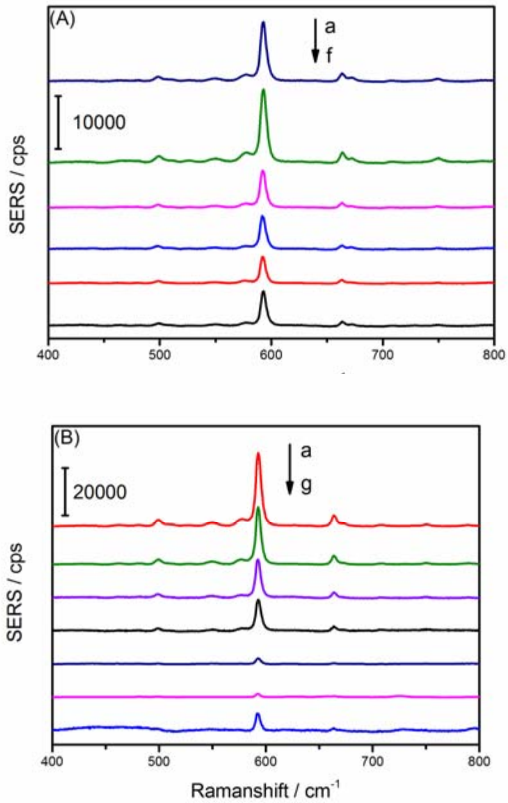


Fig. 3 (A) SERS spectra of NBA on sandpapers with various mesh numbers: (a-f) 3000, 2000, 1500, 1200, 800 and 400 mesh. (B) SERS spectra of NBA on different surfaces: (a-g) aluminum block, polystyrene foam, plastics, aluminum foil, lab bench, wood block, and cotton (×10 for comparison). All the results are the average of 5 tests. Laser power: 300 μW.

Stamping test of pesticides and illicit food additive on glass

To further illustrate the potential of the slime substrate for real world surface contamination analysis, the substrate was tested using 3 pesticides, triazophos, isocarbophos, and methyl parathion, and an illegal additive, Sudan III, found in adulated food. Triazophos are normally used for controlling insects on rice, fruits and vegetables. On the other hand, Sudan III is a banned azo dye, which has been reported to be illegally added to hot pepper[52] and navel oranges.[53] 20 μL sample alcoholic solutions of triazophos and Sudan III were applied to glass surfaces and then dried. After wetted with 10 μL of EtOH, the surface was stamped with the slime SERS substrate, as indicated in scheme 1. Even at 100 ppb (absolute amount ~1.6 ng), the characteristic bands of triazophos at 999 cm^{-1}, 1401 cm^{-1} and 1594 cm^{-1} are still visible (Fig. 4(A)). While the characteristic bands of Sudan III at 1133 cm^{-1}, 1416 cm^{-1} and 1593 cm^{-1} at the same concentration can still be identified in Fig. 4(B) as well. The background spectrum from the gel matrix also presented some SERS in this region (Figures 4(A) and 4(B)); however, the spectral features of the contaminants were still uniquely identified. Although this background might limit the applicability of the slime SERS substrate, techniques for spectral subtraction could be implemented to minimize this problem. We also explored the capability for differentiating two pesticides simultaneously in stamping test of the hydrogel substrate. 10 µL of 100 ppm isocarbophos and same amount of methyl parathion were dropped on the same spot on glass. Stamping test was performed and both pesticides could be clearly identified (Figure S4).

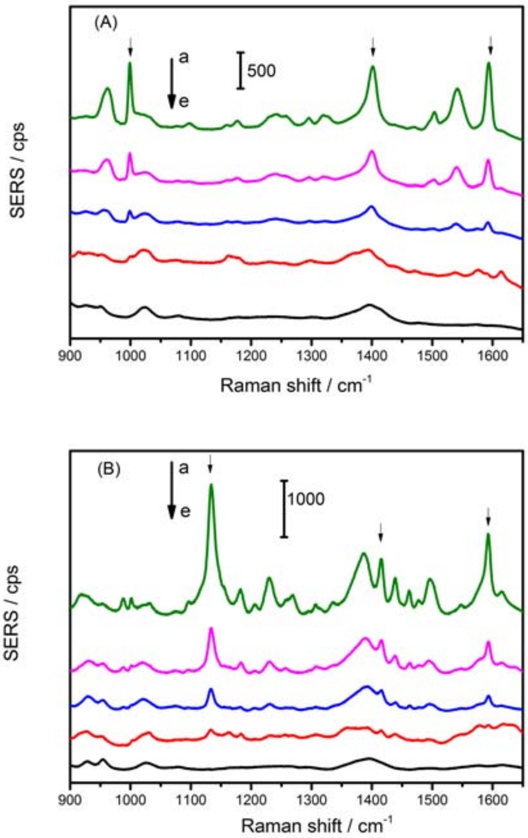


Fig. 4 (A) SERS spectra of triazophos extracted from a glass surface by stamping. The concentrations of triazophos were: (a-c) 10 ppm, 2 ppm, 1ppm, 0.1 ppm and 0 ppm, respectively (B) SERS spectra of Sudan III extracted from a glass surface by stamping. The concentrations of Sudan red III were: (a-c) 20 ppm, 8 ppm, 1 ppm, 0.1 ppm and 0 ppm, respectively. Arrows indicate the characteristic bands, respectively. Laser power: (A) 300 µW; (B) 500 µW.

Conclusion

In this paper, we report the fabrication of a new type of SERS substrate based on the traditional slime preparation. The fabrication process of the SERS slime is simple: PVA was dissolved in Ag colloidal solution before mixing with borax to form the slime. This new substrate can be used in various cases of surface contamination analysis due to its visco-elasticity and high SERS activity. Different factors that affect the performance of the slime SERS substrate have been examined, and the analytical characteristics of the slime substrate have been explored by applying a Raman probe (NBA) onto surfaces of materials of different roughness. It was found that the LOD for NBA is 0.79 *pg*/~ 3 cm^2 on glass. Furthermore, the pesticide triazophos and the illegal additive Sudan red III have been detected on surface at *ng* level. The characteristic of elasticity and viscosity of the slime SERS substrate, combined with a new generation of handheld Raman spectrometers, should allow it to be explored for surface contamination analysis in field applications.

Acknowledgments

This research is financially supported by the National Natural Science Foundation of China (Grant No. 21277131, 21105092, 51103141).

References:

1. C. H. Lee, M. E. Hankus, L. Tian, P. M. Pellegrino and S. Singamaneni, *Analytical chemistry*, 2011, 83, 8953-8958.
2. J. F. Li, Y. F. Huang, Y. Ding, Z. L. Yang, S. B. Li, X. S. Zhou, F. R. Fan, W. Zhang, Z. Y. Zhou, D. Y. Wu, B. Ren, Z. L. Wang and Z. Q. Tian, *Nature*, 2010, 464, 392-395.
3. L. Polavarapu and L. M. Liz-Marzan, *Physical chemistry chemical physics : PCCP*, 2013, 15, 5288-5300.
4. J. L. Staymates, J. Grandner and G. Gillen, *Analytical Methods*, 2011, 3, 2056-2060.
5. D. F. Rendle, *Chemical Society Reviews*, 2005, 34, 1021-1030.
6. C. Nerin, P. Alfaro, M. Aznar and C. Domeño, *Analytica chimica acta*, 2013, 775, 14-24.
7. M. A. Strege, *Analytical chemistry*, 2009, 81, 4576-4580.
8. A. Idone, M. Gulmini, A.-I. Henry, F. Casadio, L. Chang, L. Appolonia, R. P. Van Duyne and N. C. Shah, *Analyst*, 2013, 138, 5895-5903.
9. J. S. Caygill, F. Davis and S. P. Higson, *Talanta*, 2012, 88, 14-29.
10. Y.-K. Kim, S. W. Han and D.-H. Min, *ACS applied materials & interfaces*, 2012, 4, 6545-6551.
11. M. Fan, G. F. Andrade and A. G. Brolo, *Analytica Chimica Acta*, 2011, 693, 7-25.
12. R. Aroca, *Surface-enhanced vibrational spectroscopy*, Wiley. com, 2006.
13. E. Le Ru and P. Etchegoin, *Principles of Surface-Enhanced Raman Spectroscopy: and related plasmonic effects*, Access Online via Elsevier, 2008.
14. R. S. Golightly, W. E. Doering and M. J. Natan, *Acs Nano*, 2009, 3, 2859-2869.
15. X. Qian, X. H. Peng, D. O. Ansari, Q. Yin-Goen, G. Z. Chen, D. M. Shin, L. Yang, A. N. Young, M. D. Wang and S. Nie, *Nat Biotechnol*, 2008, 26, 83-90.
16. F. Gentile, M. L. Coluccio, N. Coppedè, F. Mecarini, G. Das, C. Liberale, L. Tirinato, M. Leoncini, G. Perozziello and P. Candeloro, *ACS Applied Materials & Interfaces*, 2012, 4, 3213-3224.
17. V. M. Zamarion, R. A. Timm, K. Araki and H. E. Toma, *Inorganic chemistry*, 2008, 47, 2934-2936.
18. D. Li, L. Qu, W. Zhai, J. Xue, J. S. Fossey and Y. Long, *Environmental science & technology*, 2011, 45, 4046-4052.
19. A. KumaráSingh and P. ChandraáRay, *Chemical Communications*, 2011, 47, 10326-10328.
20. C. Yao, F. Cheng, C. Wang, Y. Wang, X. Guo, Z. Gong, M. Fan and Z. Zhang, *Analytical Methods*, 2013, 5, 5560-5564.
21. M. Fan and A. G. Brolo, *ChemPhysChem*, 2008, 9, 1899-1907.
22. M. Fan and A. G. Brolo, *Physical Chemistry Chemical Physics*, 2009, 11, 7381-7389.
23. E. Bailo and V. Deckert, *Chemical Society reviews*, 2008, 37, 921-930.
24. J. F. Li, X. D. Tian, S. B. Li, J. R. Anema, Z. L. Yang, Y. Ding, Y. F. Wu, Y. M. Zeng, Q. Z. Chen, B. Ren, Z. L. Wang and Z. Q. Tian, *Nature protocols*, 2013, 8, 52-65.

25. C. H. Lee, L. Tian and S. Singamaneni, *ACS Applied Materials & Interfaces*, 2010, 2, 3429-3435.

26. X. Li, M. Cao, H. Zhang, L. Zhou, S. Cheng, J.-L. Yao and L.-J. Fan, *Journal of Colloid and Interface Science*, 2012, 382, 28-35.

27. G. Lu, H. Li and H. Zhang, *Small*, 2012, 8, 1336-1340.

28. C. H. Lee, L. Tian, A. Abbas, R. Kattumenu and S. Singamaneni, *Nanotechnology*, 2011, 22, 275311.

29. R. Liu, M. Si, Y. Kang, X. Zi, Z. Liu and D. Zhang, *Journal of Colloid and Interface Science*, 2010, 343, 52-57.

30. P. Aldeanueva-Potel, E. Faoucher, R. n. A. Alvarez-Puebla, L. M. Liz-Marzán and M. Brust, *Analytical chemistry*, 2009, 81, 9233-9238.

31. C. Lofrumento, M. Ricci, E. Platania, M. Becucci and E. Castellucci, *Journal of Raman Spectroscopy*, 2013, 44, 47-54.

32. T. Wilhelm, *Soft Matter*, 2011, 7, 1321-1325.

33. W. L. Zhai, D. W. Li, L. L. Qu, J. S. Fossey and Y. T. Long, *Nanoscale*, 2012, 4, 137-142.

34. A. Raza and B. Saha, *Forensic Science International*, 2013, 233, 21-27.

35. K. Shin, K. Ryu, H. Lee, K. Kim, H. Chung and D. Sohn, *Analyst*, 2013, 138, 932-938.

36. M. Gittings, L. Cipelletti, V. Trappe, D. Weitz, M. In and J. Lal, *The Journal of Physical Chemistry A*, 2001, 105, 9310-9315.

37. K. Shin and H. Chung, *Analyst*, 2013, 138, 3335-3346.

38. L. Liu, B. Wang, Y. Gao and T.-c. Bai, *Carbohydrate polymers*, 2013.

39. C. Lofrumento, M. Ricci, E. Platania, M. Becucci and E. Castellucci, *Journal of Raman Spectroscopy*, 2013, 44, 47-54.

40. D. He, B. Hu, Q.-F. Yao, K. Wang and S.-H. Yu, *Acs Nano*, 2009, 3, 3993-4002.

41. C.-L. Zhang, K.-P. Lv, H.-P. Cong and S.-H. Yu, *Small*, 2012, 8, 648-653.

42. M. H. Cao, S. Cheng, X. Z. Zhou, Z. X. Tao, J. L. Yao and L. J. Fan, *Journal of Polymer Research*, 2012, 19.

43. E. Hariprasad and T. P. Radhakrishnan, *Langmuir*, 2013, 29, 13050-13057.

44. Karab, S. ak, M. Kaya, T. Vo-Dinh, M. Volkan and rvet, *Journal of Nanoscience and Nanotechnology*, 2008, 8, 955-960.

45. D.-G. Yu, W.-C. Lin, C.-H. Lin, L.-M. Chang and M.-C. Yang, *Materials Chemistry and Physics*, 2007, 101, 93-98.

46. C. Sun, R. Qu, C. Ji, Y. Meng, C. Wang, Y. Sun and L. Qi, *Journal of Nanoparticle Research*, 2008, 11, 1005-1010.

47. J. Han, T. Lei and Q. Wu, *Carbohydrate Polymers*, 2014, 102, 306-316.

48. , The laser excitation used in this work (632.8 nm) falls within the absorption range of the molecular probe NBA. Thus, the obtained spectra of NBA carry also some contribution from the resonance Raman effect. In these cases, it is common in the literature to call the effect surface-enhanced resonance Raman scattering (SERRS). In order to keep the number of acronyms to a minimum and to avoid confusion, in this work we will always refer to the phenomenon as "SERS". Notice that the difference between SERS and SERRS is subtle and the use of the acronyms "SERS" here consistently does not have any effect on the interpretation.

49. M. Keating, Y. Chen, I. Larmour, K. Faulds and D. Graham, *Measurement Science and Technology*, 2012, 23, 084006.

50. K. Vangala, M. Yanney, C.-T. Hsiao, W. W. Wu, R.-F. Shen, S. Zou, A. Sygula and D. Zhang, *Analytical Chemistry*, 2010, 82, 10164-10171.

51. X. Wang, S. J. Wang and Z. W. Cai, *Trac-Trends in Analytical Chemistry*, 2013, 52, 170-185.

52. E. Ertaş, H. Özer and C. Alasalvar, *Food Chemistry*, 2007, 105, 756-760.

53. X. net, Navel oranges in Gannan were detected Sudan III which were red and bright not until harvest, http://news.xinhuanet.com/fortune/2013-11/04/c_117997773.htm, 2013.