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ARTICLE TYPE

SERS Optrode as a "fishing rod" to direct pre-concentrate analytes from superhydrophobic surfaces

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SERS optrodes were used to "fish" aqueous drops from superhydrophobic surfaces. The technique led to an improvement of 2-3 orders of magnitude in the lowest detectable amount of the Raman probe nile blue A, reaching 25 fg (34 attomoles). Further tests run on samples containing pesticide revealed that 20 pg of triazophos could be clearly detected from a single drop.

The development of methods capable of detecting minute amounts of either expensive or dangerous analytes from diluted samples is among the ultimate goals of analytical chemistry. Discovered in 1970s', surface-enhanced Raman scattering (SERS) has now become one of the most promising spectro-analytical methods, since it can provide both quantitative and qualitative information of trace amounts of analytes from a variety of matrices.[1] SERS substrates (metallic nanostructures that support the effect) fabricated by different top-down or bottom-up approaches have been widely reported.[1] Examples of SERS applications in several areas, including homeland security,[2] environmental monitoring,[3] biomedical,[4] and food safety,[5] are now common. Although very sensitive, SERS faces some challenges when dealing with highly diluted samples. A major problem is that there is no efficient mechanism to bring the target molecules to the SERS surface (more precisely, to the SERS hot spots, which are regions of strongly localized electromagnetic near-field that are required for the SERS effect.[6]). Highly-efficient SERS hot spots are rare and in the low concentration limit (meaning low surface coverage) the molecular transport to a hot spot is controlled by diffusion. As pointed out by Gentile et al,[6a] it could take 60 hours for a biomolecule to travel 1 cm in solution by diffusion. The probability for target analytes to find the hot spot in a short observation time window is then scarce. In fact, as the analyte concentration (and consequently the surface

coverage) decreases, the adsorption to a hot spot becomes stochastic, leading to strong fluctuations in SERS intensities.[7]

Different protocols based on local preconcentration effects have been proposed to minimize the diffusion-limit problem.[8] For example, Lin et al[9] utilized laser induced local heating to concentrate analytes into a small area. Bhandari et al[10] proposed a filter like SERS substrate, where analyte solution was forced through the substrate, inducing contact and adsorption. Cho,[11] Robinson[12] and Li et al[13] used electrokinetic effect and electrochemistry to preconcentrate analytes. In all these examples, however, sample volumes at mL level were still required.

Recently, hydrophobic[14] and superhydrophobic materials[6a, 15] have been successfully used for the pre-concentration of analytes in SERS analysis. It is well known that a process called self-cleaning can occur on the surface of superhydrophobic materials.[16] In that case, a drop of aqueous solution on the surface of superhydrophobic material concentrate the solute by solvent evaporation, and the sessile drop lead to a much smaller spot, compared with the size of the original droplet.[8, 15a] This phenomenon has been utilized to enhance the sensitivity of SERS analysis.[6a, 8, 14a, 15] In some of the approaches,[14a] the hydrophobic material only drives the analytes towards the SERS substrate surface (metallic NPs) to improve surface coverage density. Alternatively, the (super)hydrophobic material not only concentrates the analytes into a much smaller area, but also acts as SERS hot spots (superhydrophobic-SERS dual functional material).[8] Nevertheless, despite the extra sensitivity gained through those approaches, there are two drawbacks associated with most of the reported superhydrophobic-SERS dual functional substrates which may hinder their practical

applications: 1) some of the dual functional substrates require expensive advanced nanofabrication techniques; 2) it is reported that the dried spot measured on the superhydrophobic-SERS dual functional substrate is 50 μm or less in diameter.[8] Practically,
5 finding a colourless dot with a diameter smaller than a human hair on a centimetre sized substrate is problematic, even with the help of an optical microscope.

SERS optrode is a recently developed fiber optic SERS sensor,[17] in which both the excitation laser and the SERS signal travel
10 through the same fiber. In our previous work, it was shown that layer-by-layer self-assembly strategy could be applied to construct SERS optrodes with very high sensitivity.[17a, 17b] We demonstrated that Raman probes of different charges could all be detected at μM range,[17a] and identifying multi-analytes
15 simultaneously was possible in a microfluidic chip.[17b]

In this report, we will demonstrate that SERS optrodes combined with superhydrophobic materials can form a new system for trace analysis in aqueous solutions. A simple and inexpensive superhydrophobic material was constructed by coating sandpaper
20 with Teflon® NPs. The SERS optrode was fabricated following our previously reported procedure.[17a, 17b] The analysis protocol, summarized in Scheme 1, is simple: a drop of an aqueous sample solution is added on the superhydrophobic surface (Scheme 1a) and remains spherical to minimize its surface to volume ratio
25 (surface tension). Then the SERS optrode is positioned at the drop, as shown in Scheme 1b. As the solvent (water) evaporates, the drop size decreases (Scheme 1c). Since the tip of the SERS optrode is hydrophilic, the shrunk droplet will leave the superhydrophobic surface to stick to the optrode (Scheme 1d).
30 Thus, instead of random precipitation, as in the dual functional substrates,[8, 15a] in our configuration the solutes from the sample droplet will be directed towards the optrode during drying (Scheme 1e). In other words, the SERS optrode acts as both a "fishing rod" to capture the analyte molecules and a highly
35 sensitive sensing platform. The lowest experimentally detected amount of the Raman probe nile blue A (NBA) was 25 fg (34 attomoles). This lowest detected amount was 3 orders of magnitude lower than in our previous report.[17b] Triazophos, an organophosphate pesticide (OP) used in agriculture, was also
40 detected using this technique. We found that the actual detected amount of triazophos was as low as 20 pg (64 femtomoles).
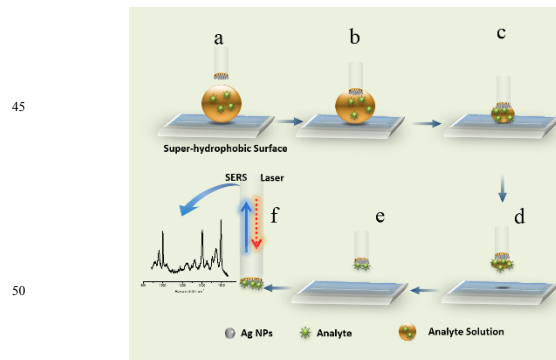
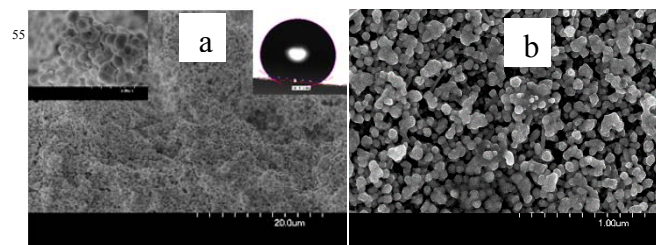Scheme 1 Schematic representation of the SERS fishing rod protocol

Fig. 1 SEM image of superhydrophobic Teflon coated sandpaper, insets show the high magnification SEM and an image of a droplet on its surface; b, SEM image of Ag NPs modified optical fiber tip.

65 Teflon nanoparticles has long been known as good material for preparation of superhydrophobic substrate.[18] Here, the procedure reported in the literature[18] was modified by using sandpaper as microscale-rough supporting material. Fig. 1a shows SEM images (low and high magnifications) of a sandpaper coated with
70 Teflon NPs, which is an example of the superhydrophobic platform used in this work. The whole surface of the sandpaper is homogeneously covered with Teflon nano-powder as shown in Figure 1a. The measured contact angle of a water droplet was 151.2º (inset of Fig. 1a), and the droplet freely slid on the surface
75 at a tilt angle as low as 7.5º. These values confirm the superhydrophobic property of the surface provided by the Teflon coating in combination with microscale roughness of the sandpaper.[19]

Following the procedure described in scheme 1, 10 μL of NBA
80 sample solutions were added to the superhydrophobic surface and then transferred to the optrode tip. SERS spectra were recorded from the SERS optrode after the evaporation of solvent. Special care was taken to make sure that the optrode was always inserted into the droplet during evaporation. Figure 2a shows a typical
85 SERS spectrum obtained using this procedure from a 3.4 pM (2.5 ppt) solution of NBA. Considering that all NBA from the 10 μL drop was transferred to the optrode, the NBA Raman band at 593

cm^{-1} is originated from only ~ 34 attomoles of NBA (2.0×10^7 molecules). Figure 2b shows a comparison SERS spectrum to an experiment where the preconcentration procedure with assistance of the superhydrophobic surface was not used. In that case, the spectrum was obtained by simply immersing the optrode tip in a 3.4 nM (2.5 ppb) solution of NBA for 30 s before taking the spectrum. Although the characteristic peak of NBA[20] in Fig. 2b is more dominant compared with that in Fig. 2a, the NBA concentration was 3 orders of magnitude higher. This indicates that the benefit from the superhydrophobic material is roughly 3 orders of magnitude enhancement in sensitivity. This enhancement is slightly lower than the approach reported in the literature.[8] However, the experimental procedure involving the optrode is simpler compared to the approaches suggested in previous reports. Further, no advanced nanofabrication technique was needed to prepare either the SERS or the superhydrophobic surfaces in our case.
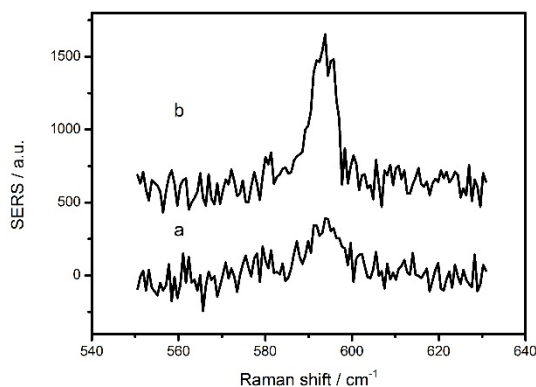
Fig. 2 Detection of NBA with (a) and without (b) the aid from superhydrophobic material. In a, the volume was 10 µL and the concentration of NBA is 3.4 pM (2.5 ppt); in b, the optrode was inserted in 2 mL of 3.4 nM (2.5 ppb) NBA for 30s.

Organophosphate pesticides (Ops), including triazophos, have been extensively used in agriculture due to their excellent insecticide activity.[21] As a result, they can sometimes be found in various environments, even in vegetable and fruits[22] and; hence, their presence might bring serious adverse effects not only to humans, but also to aquatic lives. The maximum residue limits (MRLs) of triazophos in fruits, vegetables, and cereals are reported to be 0.05–2.00 mg/kg in some countries.[23] The acceptable daily intake of triazophos is said to be 0–0.001 mg/kg body weight.[23] Currently, methods for the determination of triazophos are based on gas chromatography (GC),[23] high performance liquid chromatography (HPLC)[22] with and without

the combination of mass spectroscopy,[24] and ELISA-based techniques.[25] These methods have been used for the determination of triazophos in different samples, including water,[24, 26] fruit,[22] wheat and soil.[23] Among these methods, the limit of quantification (LOQ) reported are 50 ppb for GC,[23] 4 ppb for immunochromatography,[27] 1~1.5ppb for HPLC[21-22] and 1 ppb for HPLC-MS/MS[24].
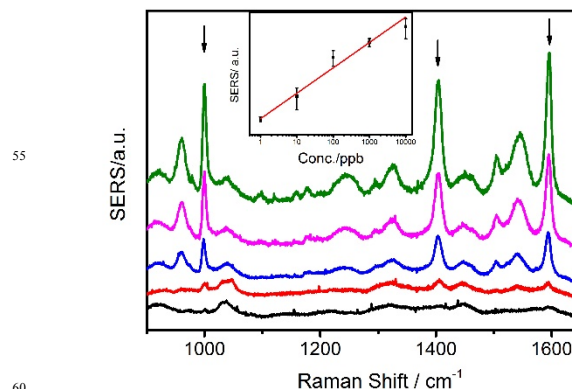
Fig. 3 SERS spectra of triazophos at different concentrations obtained by optrode with assistance of superhydrophobic surface. Top to bottom: 10 ppm, 1 ppm, 100 ppb, 10 ppb and 1 ppb. Arrows: the characteristic bands of triazophos. Inset: the log-log plot of SERS intensity at 999.9 cm^{-1} versus the concentration of triazophos. The error bar shows the deviation among 8 measurements.

In Fig. 3, the newly developed "SERS fishing rod" method was applied to the detection of pesticide triazophos from aqueous solutions of different concentrations. The main features for triazophos at 999, 1401, and 1594 cm^{-1} can be identified even at 1 ppb level from 20 µL solution, 2 orders of magnitude lower than that achieved on hydrogel SERS substrate.[28] The triazophos features at 1 ppb appear very weak in Fig. 3, but they are identifiable (see also Figures S1). This is further confirmed by using the first derivative of the spectra (shown in Figure S2). The 1ppb level of triazophos is one of the lowest experimentally detected amount reported in the literature.[24] Furthermore, since the total volume was only 20 µL, the absolute amount of triazophos in the droplet that was detected was only 20 picograms, or 64 femtomoles. In Fig. 3 inset, the log-log plot of SERS peak intensity at 999 cm^{-1} against the concentration of triazophos aqueous solution is presented. As a further proof of concept, triazophos spiked tap water samples were analyzed and the results are shown in Table 1. Even at ppb levels, triazophos were detected with a recovery rate ranging from 64.73~113.7%. The variations in recovery rate reflect the challenges of direct

quantification using SERS. In any case, the SERS method reported here can certainly be used as a screening probe to the presence of pesticides and other contaminants at trace level.

Table 1 Determination of triazophos in tap water by proposed SERS fishing strategy

Added(ppb)	Detected (ppb)	Recovery rate (%)	RSD%
50.00	32.37	64.73	25.3%
50.00	56.83	113.7	16.2%
50.00	45.08	90.16	22.7%

Conclusions

In this report, a novel strategy for trace amount analysis based on a SERS optrode in combination with superhydrophobic surface was demonstrated. In this approach, the SERS optrode is used as a "fishing rod" to preconcentrate minute amounts of analytes from small volumes. The drop is effectively "fished out" from the superhydrophobic surface, increasing the method sensitivity. This new method not only shows very high sensitivity, but also practical advantages relative to other pre-concentration approaches from superhydrophobic material. It is found that 3.4 pM of sample analyte NBA can be detected, a ~3 orders of magnitude improvement compared with the situation without superhydrophobic material. On the other hand, the detection of ~20 picograms of pesticide triazophos in aqueous solution was successfully achieved, 2 orders of magnitude more sensitive than our previous work using PVA hydrogel SERS substrate.[28] As a proof of concept, tap water samples spiked with triazophos were analyzed, and reasonable recovery rates were obtained.

Acknowledgments

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