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A simple filter-based approach to surface enhanced Raman spectroscopy for trace chemical detection†

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Abstract

We demonstrate an extremely simple and practical surface enhanced Raman spectroscopy (SERS) technique for trace chemical detection. Filter membranes first trap silver nanoparticles to form a SERS-active substrate and then concentrate analytes from a mL-scale sample into a mL-scale detection volume. We demonstrate a significant improvement in detection limit as compared to colloidal SERS for the pesticide malathion and the food contaminant melamine. The measured SERS intensity exhibits low variation relative to traditional SERS techniques, and the data can be closely fit with a Langmuir isotherm. Thus, due to the simple procedure, the low-cost of the substrates, the quantitative results, and the performance improvement due to analyte concentration, our technique enables SERS to be practical for a broad range of analytical applications, including field-based detection of toxins in large-volume samples.

Introduction

Detection of trace chemicals in solid and liquid samples is currently achieved by well-established methods that combine chromatography techniques with mass spectrometry. While sensitive, these approaches are labor intensive, time consuming, and costly. They also require expensive and bulky equipment, and hence are not portable. Surface enhanced Raman spectroscopy (SERS) offers an attractive alternative for chemical analysis. Typically, a SERS analysis involves spotting a μL-volume of sample onto a nanofabricated SERS substrate, allowing it to dry, and then detecting the Raman scattering. Unfortunately, the high cost and complications associated with the fabrication of SERS-active substrates have prevented the wide use of SERS. Furthermore, these substrates exhibit limited shelf life, with progressive reduction in SERS activity due to oxidation of the nanostructures.[1] To make SERS more applicable, microfluidic techniques have been combined with SERS;[2-8] however microfluidic SERS also introduces additional complexity to the fabrication of these devices and may decrease sensitivity due to difficulties in the optical coupling, decreased sample volume, and the inability to concentrate the analyte by drying.

To make SERS analysis simple and inexpensive, we have previously introduced inkjet-printed SERS substrates on paper.[9] To add to this, we report in this work the development of a new technique for trace chemical detection called filter SERS. Filter SERS completely avoids the high costs of nanofabricated SERS substrates and the complexity associated with conventional microfluidic-based SERS by leveraging the filtration process to create a SERS-active substrate and to concentrate the analyte. The technique requires only a filter

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membrane, a filter holder, and a syringe. The membrane traps and concentrates nanoparticles from a colloid solution and then serves as a SERS-active substrate through which a large volume of sample can be passed. Because the substrate is fabricated on-demand, shelf-life is no longer a concern. There are no complicated fabrication steps and no mechanical or electrical components needed, apart from a portable spectrometer. In addition, filter SERS allows large volumes of trace analyte samples to be processed very quickly, resulting in orders of magnitude increases in the number of analyte molecules that interact with the SERS-active surface as compared to current techniques of drying a sample onto a substrate or mixing a sample with a silver colloid solution. Consequently, filter SERS is extremely simple and inexpensive, but highly sensitive for trace chemical detection. The simplicity of the technique makes it particularly well suited for low resource, field based applications.

In this work, we demonstrate that the detection performance of the filter SERS technique is at least 1–2 orders of magnitude better than the typical approach of drying a sample in silver colloid onto a surface. We achieved a detection limit of 10 nM for the common model SERS analyte Rhodamine 6G (R6G) using a portable spectrometer and diode laser. Furthermore, we demonstrate the utility of the filter SERS technique for field based applications by detecting parts per billion (ppb) concentrations of melamine, a food contaminant, as well as ppb concentrations of malathion, a widely used pesticide, in aqueous solution. Importantly, our technique shows relatively low variability, and all acquired data sets could be fit well with a Langmuir isotherm. This indicates that our field based technique is not only simple and sensitive, but it can also be quantitative.

Methods

Materials and reagents

Nylon and Millipore PVDF filter membranes, 13 mm diameter, 0.22 μm pore sizes were purchased from Fisher Scientific (Pittsburgh, PA) and used with syringe filter holders from Cole-Palmer (Vernon Hills, IL) for performing the filter SERS assay. Silver nitrate, sodium citrate, sodium chloride and melamine were obtained from Sigma-Aldrich (St. Louis, MO). R6G was purchased from Exciton (Dayton, OH). Malathion was purchased from Cerilliant (Round Rock, Texas). Chemicals were used as received with safety precautions taken as according to their respective MSDS.

Nanoparticle synthesis

Silver nanoparticles were synthesized by the method of Lee and Meisel.[10] Briefly, 90 mg of silver nitrate was added to 500 mL of ultrapure water (18.2 ΩU), which was then brought to a boil in a flask under vigorous stirring. Sodium citrate (100 mg) was added, and the solution was left to boil for an additional 10 min. After the solution turned greenish brown, which indicated the formation of silver colloid, it was removed from heat. Silver nanoparticles are aggregated using 5 mM NaCl, as it was experimentally determined that this provided the optimal level of clustering.

Filter SERS assay

To perform the filter SERS assay, the filter membrane is first wetted by dipping it in a 50% ethanol-water mixture. The membrane is then placed into the filter holder, which can be attached to a typical syringe. A volume of silver colloid solution is loaded into the syringe. The filter holder is then attached to the syringe and silver colloid is passed through the filter membrane (Fig. 1, step 1). The membrane traps the silver nanoparticles, forming a SERS active substrate. The filter holder is removed, and the same syringe is then loaded with the sample. After re-attaching the filter holder, which now contains the nanoparticle-coated

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membrane, the sample is passed through the membrane (Fig. 1, step 2). Target analyte molecules become adsorbed onto the nanoparticles or to the filter membrane, effectively concentrating the analyte. The membrane is then removed from the holder, dried, and analyzed using a SERS detection setup consisting of a portable spectrometer (Ocean Optics QE65000), a 785 nm diode laser, and a fiber optic Raman probe. The filter SERS protocol and SERS detection setup is illustrated in Fig. 1. A silver-nanoparticle-coated filter membrane is shown in Fig. 2A. It is clearly visible in the SEM images that the silver nanoparticles are trapped within the pores of the filter, yielding clusters of nanoparticles that are highly SERS active.

SERS measurements were acquired from three randomly selected locations within each membrane and repeated over three membranes, giving a total of nine SERS measurements for each analyte concentration. The laser power used was 3 mW for R6G and 10 mW for melamine and malathion. A 1-second CCD exposure was used, with averaging of the SERS signal over 5 signal acquisitions. To determine the signal intensity, the height of the most prominent peak from the Raman bands was calculated (1509 cm^{-1} for R6G, 508 cm^{-1} for malathion and 690 cm^{-1} for melamine). Background contributions are removed from the signal by subtracting the spectrum measured for water from the spectrum measured for the analyte. To establish the SERS enhancement factor of the silver nanoparticle coated membranes, a 1 μL droplet of 20 mM R6G was spotted onto a plain filter membrane; the resulting signal was compared with the SERS signal from 1 μL of 1 μM R6G pipetted onto a filter membrane after loading silver nanoclusters.

Three analytes were used for analysis: R6G, melamine, and malathion. R6G and malathion were used in water, while 0.1% HCl was added to the melamine/water sample before loading to protonate the melamine. For all three analytes, the filter SERS technique was compared with a conventional SERS method. For the conventional method, 1 μL of silver colloid and 5 μL of sample analyte were spotted onto an aluminum foil surface. The Raman spectrum was acquired after the droplet dried onto the surface. Finally, to verify that the filter SERS technique generates quantitative data, all concentration curves were fit using the Hill equation with n = 1 (Langmuir isotherm) in Origin.

Results and discussion

The SEM image in Fig. 2A indicates that a high density of SERS-active hot spots exist across the membrane, which will enable a large enhancement as compared to conventional Raman spectroscopy. To quantify the enhancement of the silver-nanocluster-treated membrane, we measured the Raman signal for 1 μM R6G dried onto the membrane with silver nanoparticles and for 20 mM R6G dried onto a membrane with no silver. The two Raman signals are presented in Fig. 2B. The enhancement factor of the membrane is determined by:

$$EF = \left(\frac{I_{SERS}}{I_{Raman}}\right)\left(\frac{N_{Raman}}{N_{SERS}}\right) \quad (1)$$

where the intensity I is the height of the 1509 cm^{-1} R6G Raman peak and N represents the total number of R6G molecules deposited onto the substrate. Using the data in Fig. 2B, an enhancement factor of 3×10^5 is determined. However, according to SEM images, only approximately 20% of the membrane surface is covered by silver nanoclusters; thus, assuming that the analyte molecules are evenly distributed across the membrane, the enhancement of the silver nanoclusters is approximately 1.5×10^6.

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Intuitively, the amount of silver colloid passed through the membrane will affect the SERS activity. The aim is to load enough silver nanoclusters to create a high surface density; however, it is conceivable that loading too many silver nanoparticles will cause the membrane to become a relatively thick silver film instead of a plasmonic nanostructured surface. Fig. 3A shows the measured intensity of the 1509 cm^{-1} R6G Raman peak after first loading increasing volumes of silver colloid through the filter and then loading 10 μM R6G. As the data shows, the signal intensity decreases significantly when loading more than 0.5 mL of colloid through the filter. However, the variation in signal intensity is higher for 0.5 mL than for larger volumes. Therefore, for all subsequent experiments, we load 1 mL of silver colloid through the membrane before loading the sample through the filter.

We also explored the dependence of the Raman scattering intensity upon sample loading. Fig. 3B shows that the signal increased linearly for increasing sample volume when loading between 1 mL and 8 mL of R6G (1 μM). As would be expected, this implies that increasing the number of analyte molecules passed through the filter increases the Raman signal. For the experiments in this work, we limited the sample volume to 5 mL, which is a compromise between signal intensity and sample loading time.

Fig. 4 presents the detection performance of the filter SERS technique for R6G as the analyte. In Fig. 4A, the detection performance of filter SERS is compared with that of a typical SERS measurement, in which the sample of R6G is added to a silver colloid and dried onto a surface. The measured Raman signals in Fig. 4A show that the signal intensity for 1 μM R6G in colloid dried onto a surface is similar to the signal intensity for 100 nM R6G using the filter SERS technique. Thus, for the case of R6G, the filter SERS technique enables an order of magnitude improvement in detection performance as compared to typical SERS measurements.

To determine the detection limit for R6G using the filter SERS technique, we prepared dilutions of R6G in water from 100 μM down to 10 nM. Fig. 4B shows recorded Raman spectra for this range of R6G concentrations. Data points representing the peak height of the 1509 cm^{-1} R6G Raman peak are plotted against R6G concentration in Fig. 4C. Data for both the nylon and PVDF membranes are plotted and analyzed; very little difference is observed between the two. In the plot, the data points represent the mean value for three separate membranes, and the value associated with each membrane is the mean across three separate spots on the membrane (*i.e.*, nine data points are taken for each concentration). Error bars represent the standard deviation of the mean values for each membrane. Fig. 5 presents the variation of the signal intensity in more detail. Each bar represents the mean for a particular concentration and a particular membrane, while the error bar for each bar is the standard deviation of the signal intensity of three spots measured within the respective membrane.

Two important characteristics can be seen in the data plotted in Fig. 4C and in Fig. 5. First, the data in Fig. 4C was fitted with the Langmuir isotherm; the fit is excellent ($R^2 = 0.989$ for the nylon membrane, $R^2 = 0.985$ for the PVDF membrane). Second, the standard deviation of each data point is relatively low for SERS measurements. These two facts suggest that the filter SERS method is not only simple and practical, but it also has the potential to be a quantitative technique.

To demonstrate the use of this practical and portable technique for field-based applications, we performed detection of melamine, a toxic food contaminant, and malathion, a commonly used organophosphate pesticide. The detection performance for melamine is presented in Fig. 6. The signal obtained by filter SERS at 6.3 ppb melamine is compared with the signal measured for 1.26 ppm melamine in silver colloid dried onto a surface. It is evident that the

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filter SERS technique boosts the detection performance by a factor of 200 as compared to traditional SERS measurements.

Fig. 6B shows the measured SERS spectra for melamine for a concentration range between 12.6 ppm and 6.3 ppb (50 nM) when using the PVDF filter membrane, which proved to be more effective than nylon for melamine detection. The intensity of the 690 cm^{-1} Raman peak for melamine is plotted for each concentration in Fig. 6C. Similar to the case of R6G, the variability of the intensity values is relatively low, and the data can be fit well with a Langmuir isotherm. Even though a portable spectrometer is used for the measurements, the detection limit of 6.3 ppb is achieved, which is well below the currently accepted levels of 2.5 ppm for melamine in foods as established by the FDA,[11] making it possible to use filter SERS as a detection method for melamine in foods.

In a similar manner, we analyzed the performance of filter SERS for the detection of the organophosphate malathion in water. As shown in Fig. 7A, by drying a sample of malathion in silver colloid onto a surface, only 12.3 ppm malathion could be detected, while the filter SERS technique enabled the detection of 61.5 ppb, an improvement by a factor of 200. In this case, we used a nylon membrane, which proved to be more effective for malathion detection. The recorded SERS spectra for a malathion concentration range of 12.3 ppm to 61.5 ppb is presented in Fig. 7B. As proven in Fig. 7C, this data can also be fit well by a Langmuir isotherm and exhibits low variability, which suggests that quantitative detection of malathion in water can be performed using our low cost portable SERS detection technique.

Conclusions

We have developed an extremely simple and inexpensive, but highly sensitive method of performing SERS by filtration. The filter membranes create a SERS-active surface by trapping and concentrating nanoparticles from a colloid solution. They also concentrate analytes from relatively large sample volumes into the immobilized matrix of nanoparticles. The results presented here prove that the filter SERS technique is more sensitive than conventional SERS techniques and that it can be quantitative. Detection limits of 6.3 ppb for melamine and 61.5 ppb for malathion were achieved with this technique. Furthermore, quantitative performance was verified by Langmuir isotherm line fits. This work demonstrates that filter SERS is well suited for low resource settings, in particular, for on-site environmental monitoring and food analysis.

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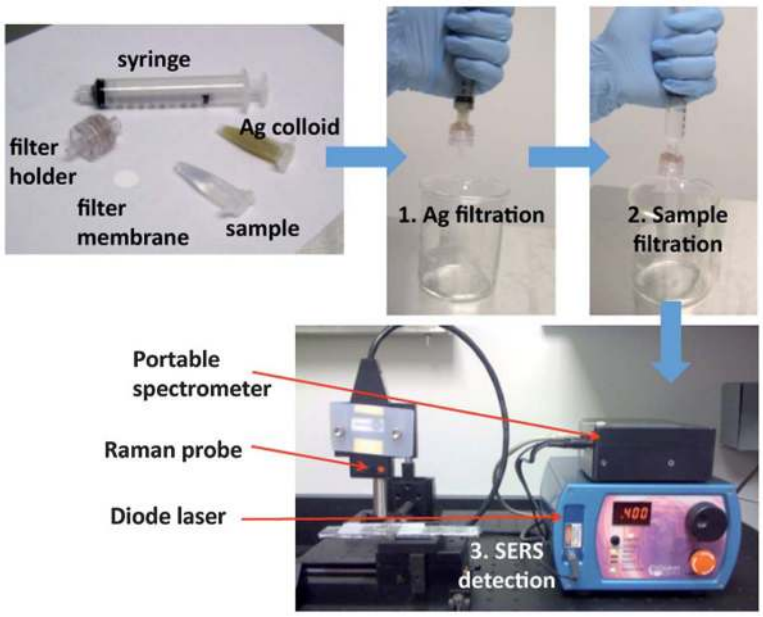

Fig. 1.

SERS-active substrates are created simply by passing a silver colloid solution through a filter membrane using a syringe. Analyte molecules are concentrated into the substrate from a large sample volume. The SERS signal is detected using a small and portable photonic setup.

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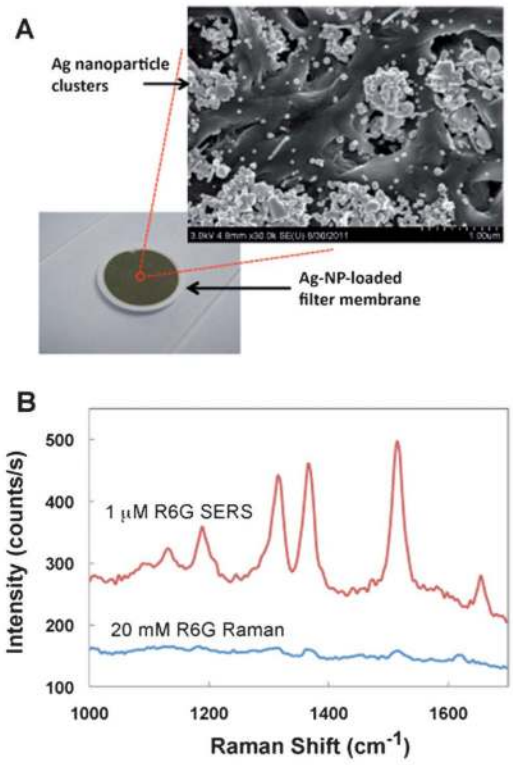

Fig. 2.
(A) Ag-NP-loaded filter membrane (bottom), and SEM image showing the clustering of silver nanoparticles in the pores of the filter membrane. (B) Measured Raman spectra for R6G with and without loading silver colloid through the membrane.

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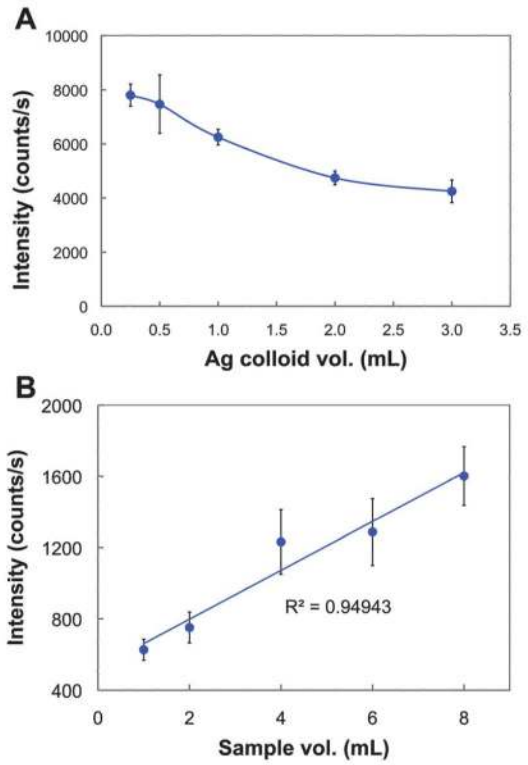

Fig. 3.

Measured intensity of the 1509 cm^{-1} R6G Raman peak for (A) increasing volumes of silver colloid loaded into the membrane filter, and (B) increasing volumes of sample loaded into the membrane filter.

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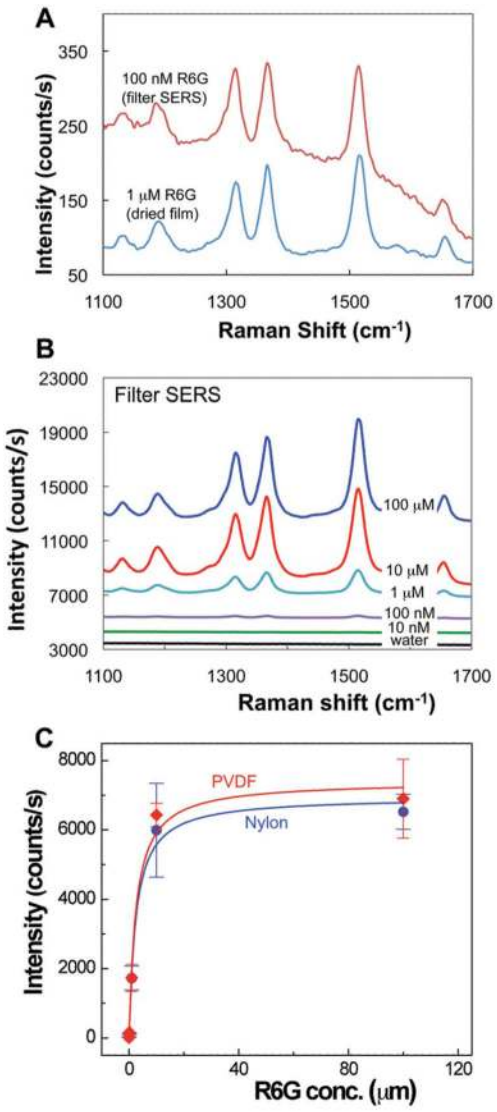

Fig. 4.

(A) Comparison of R6G detection performance of filter SERS with a sample in colloid dried onto a surface. (B) Signals of R6G detected using filter SERS. (C) Plot of the intensity of the 1509 cm^{-1} R6G Raman peak for various R6G concentrations using nylon and PVDF membranes. Each data set is fit with a Langmuir isotherm.

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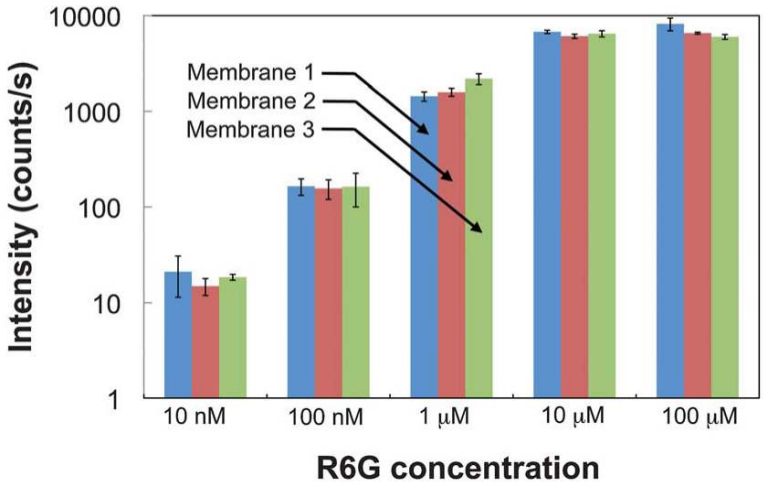

Fig. 5.

Variability of the SERS intensity of the 1509 cm^{-1} R6G Raman peak. Each bar represents the mean intensity from one membrane. The error bars show the standard deviation of the intensity measured at three random spots on the membrane.

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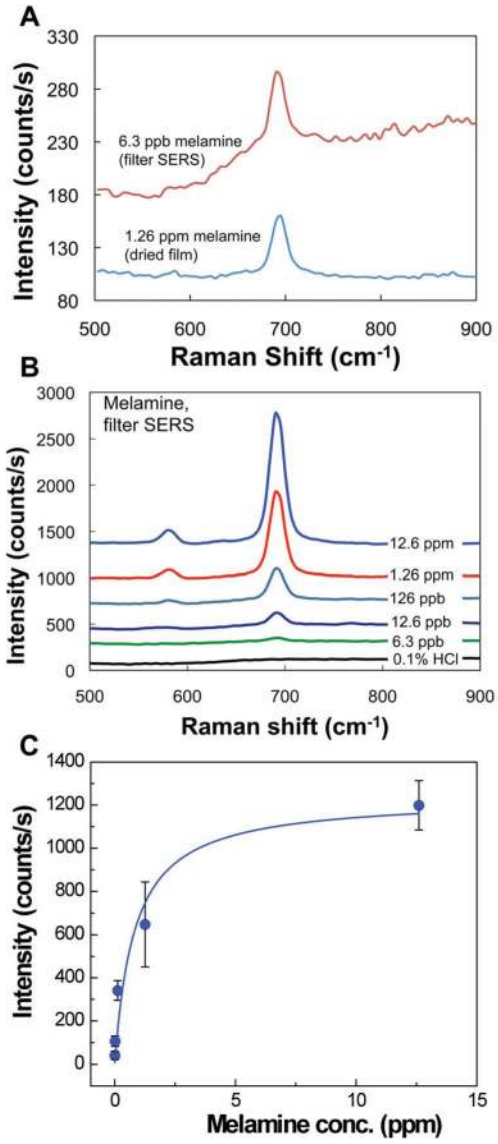

Fig. 6.

(A) Comparison of melamine detection performance of filter SERS with a sample in colloid dried onto a surface. (B) Signals of melamine detected using filter SERS. (C) Plot of the intensity of the 690 cm^{-1} melamine Raman peak for various melamine concentrations. The data is fit with a Langmuir isotherm.

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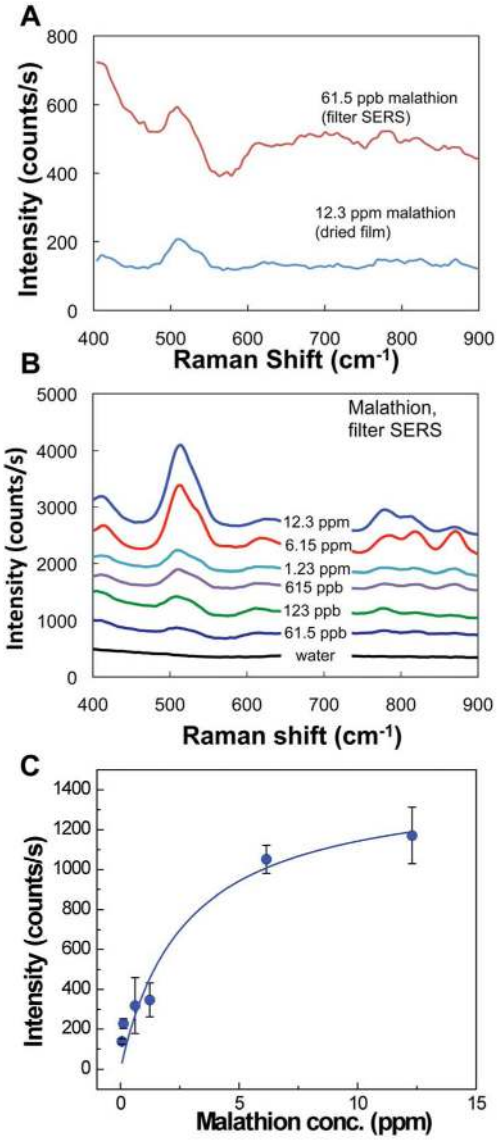

Fig. 7.

(A) Comparison of malathion detection performance of filter SERS with a sample in colloid dried onto a surface. (B) Signals of malathion detected using filter SERS. (C) Plot of the intensity of the 508 cm^{-1} malathion Raman peak for various malathion concentrations. The data is fit with a Langmuir isotherm.