Quick detection of contaminants leaching from polypropylene centrifuge tubes with surface-enhanced Raman spectroscopy and ultraviolet absorption spectroscopy

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Anomalous surface-enhanced Raman scattering (SERS) peaks were identified for liquid sample stored in polypropylene (PP) centrifuge tubes for months. We observed unexpected Raman peaks during experiments with thiamine hydrochloride aqueous solutions stored in PP tubes for 2 months. In order to identify the contaminants, we have performed SERS experiments on deionized (DI) water stored in PP centrifuge tubes for 2 months and compared them with those from fresh DI water sample. We have also carried out ultraviolet (UV) absorption spectra for both fresh and contaminated water. We believe that the water is contaminated because of chemicals leaching from the PP tube. From the gas chromatography-mass spectrometry data, the main contaminants were found to be phthalic acid (PA) and its derivatives. Further SERS and UV absorption experiment for PA correlated well with the anomalous peaks identified earlier. We qualitatively confirmed the identification and quantitatively estimated the concentration of the suspect contaminants as between 1 and 10 μM with both SERS and UV absorption spectroscopy. With UV absorption spectroscopy, we precisely estimated the concentration as 2.1 μM. We have shown that the sample in PP tube can be contaminated by the leaching chemicals upon long-term storage, and suggest SERS and UV absorption spectroscopy as two quick and simple techniques to detect the contamination. Copyright © 2011 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: leaching; polypropylene centrifuge tube; surface-enhanced Raman scattering; UV absorption spectroscopy; mass spectroscopy

Introduction

It has been known for long that some plastic containers can contaminate their contents and may be harmful to health. For example, bottled water was found to be contaminated by antimony leaching from polyethylene terephthalate (PET).[1] The plasticizer bisphenol A (BPA), which is used in the production of polycarbonate, is widely found in drinking water and is reported to stimulate cell apoptosis.[2] A sulfoxide oxidative product along with a sulfone oxidative of didodecyl 3,3′-thiodipropionate (DDTDP) that is used to prevent oxidative degradation of synthetic polymers leaching from the polypropylene tubes has been reported and the chemicals were identified with mass spectroscopy.[3] Plasticizers outgasing from O-rings can lead to undesirable ion–molecule chemistry in an electrospray quadruple ion trap mass spectrometer.[4] Recently, it was reported that compounds such as di(2-hydroxyethyl)methyldodecylammonium (DiHEMDA) and 9-octadecenamide (oleamide) leaching from polypropylene tubes hamper the measurements in DNA and protein assays.[5]

Even though in most cases the leaching compounds from plasticware are in negligible amounts, which are unlikely to be toxic to humans, they may hamper experimental results, especially for those highly sensitive biochemical experiments. Because of the widespread use of plastic containers both in daily life and in the laboratory, it is imperative to detect the contaminants leaching from the plasticware in a simple and fast way. In most previous works, mass spectroscopy was used to identify the leaching compounds. Reliable though, mass spectroscopy has some limitations. Usually, the sample preparation to data acquisition processes in those instrument are time consuming and may take hours. Also, to get sufficient amounts of leaching compounds, relatively large amounts of sample solution need to be vaporized. The mass spectrometer setups tend to be complicated, huge and have special requirements for the operational environment and so they are generally not portable. To identify the structure of a compound, the molecules need to be broken into fragments with certain techniques such as high-energy electron bombardment.[6] Therefore the sample is not reusable.

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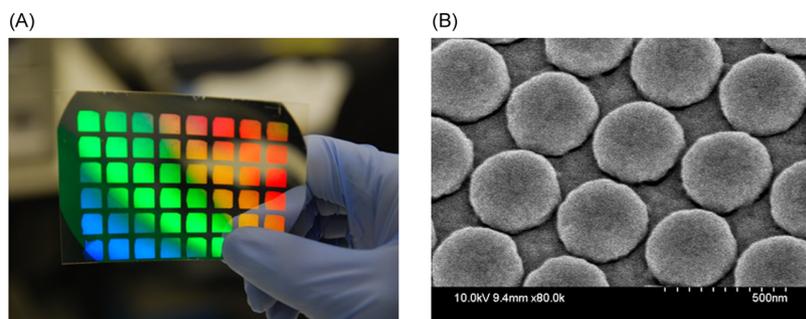c Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

(A)

(B)

Figure 1. (A) Photograph of the nanodome SERS substrate. (B) SEM image of the surface of the nanodome SERS substrate with the perspective angle of 25°.

Surface-enhanced Raman scattering (SERS) spectroscopy can overcome these limitations. As a label-free and noninvasive technique, Raman spectroscopy can provide information on the vibrational mode and symmetry of a molecule and thus can identify the chemical species.[7] Compared to normal Raman spectroscopy, SERS can enhance the Raman signal by several orders of magnitude, which allows this technique to be sensitive enough to detect single molecules.[8] Ultraviolet (UV) absorption spectrometry is another simple and fast quantitative technique for molecular detection and identification but it is not as sensitive as SERS and may damage the structures of organic compounds due to its high photon energy.[9]

Observations of anomalous Raman peaks in the SERS experiment of thiamine hydrochloride (TH) solution led us to examine the potential source of interferences from polypropylene (PP) centrifuge tubes, as the solution was stored for about 2 months before the experiment. We observed those anomalous peaks at fixed wavenumbers and with constant intensity irrespective of the concentration of the TH solution. For comparison, we also recorded the SERS spectra of freshly prepared TH solution with the same setup and configuration, but did not observe those peaks. In order to confirm the leaching of the PP tube, we performed an SERS experiment for fresh deionized (DI) water and DI water stored in a similar PP tube for about 2 months (we call it old DI water). Those same anomalous Raman peaks showed up in old DI water but not in fresh DI water. Thus we assert that the water was contaminated by the PP tube and those anomalous Raman peaks can be attributed to the chemicals leaching from the PP tube. In addition, we recorded the UV absorption spectra of old DI water and fresh DI water. We observed two distinct absorption peaks in the spectrum for the old DI water but not for fresh DI water. Furthermore, in order to identify the source of interference leached from PP tubes, the old DI water samples were analyzed by gas chromatography-mass spectrometry (GC-MS). The MS data revealed that the chemicals leached from PP tubes have low molecular weights ($m/z < 500$ Da; here m/z is the mass-to-charge ratio) and the major contaminant might be phthalic acid (PA). Finally, we performed SERS and UV absorption experiments on PA solutions. The characteristic peaks from PA correlated well with the earlier observed anomalous peaks from old DI water. The details of the experiments are described in the following sections.

Experimental

Nanodome SERS substrate

Figure 1(A) shows the photograph of the polymer-based SERS substrate made using the nano-replica molding process. The

details of the fabrication process are described elsewhere.[10] In brief, the SERS substrate used here is a two-dimensional periodic array of closely spaced plastic cylinders (nanodomes) coated by a thin layer of silver on the top. Figure 1(B) shows the scanning electron micrograph of the fabricated substrate after silver coating. The reproducibility and uniformity of this SERS substrate have already been demonstrated,[10] which allowed us to get repeatable and reliable data for quantitative analysis. The enhancement factor for the substrate was calculated using our earlier developed method[11] with Rhodamine 6G as the SERS probe, and was found to be 3.16×10^6.

Spectroscopy setup for data acquisition

The schematic of our SERS spectroscopy system is shown in Fig. S1 (Supporting Information). For excitation, a semiconductor laser beam with a wavelength of 785 nm and a power of 30 mW was focused on to the SERS substrate by a 10× objective lens after reflection from a dichroic mirror. The diameter of the laser spot on the substrate was about 20 μm as measured by the camera.

For UV absorption spectroscopy, we used an Evolution 60 UV–visible spectrophotometer (Thermo Fisher Scientific). For mass spectroscopy, we used an Agilent 6890N GC/5973 MS (GC/MS) system. It is equipped with electron impact ionization (EI) and chemical ionization units; it has flame ionization detector, thermal conductivity detector, and mass-selective detector.

Sample preparation

TH was dissolved in DI water to make solutions of different concentrations. The TH solutions and DI water sample were kept in PP tubes and stored at room temperature for 2 months. For new samples, TH solutions with the same concentrations were used and fresh DI water was prepared immediately before the measurements. Glass containers were used for the new samples.

For SERS spectral measurements, a sample of volume 3 μl was dropped onto the SERS substrate. Due to the hydrophobicity of the substrate surface, which is a result of the nanodome array structure,[12] the drops stand on the substrate with a contact angle of more than 90°. Then we waited for a few minutes until it dried. For TH solutions of high concentration, a stain due to crystallization with a diameter of about 200 μm was left after drying. For low-concentration TH solutions and DI water, no residue was visible to the naked eye after drying.
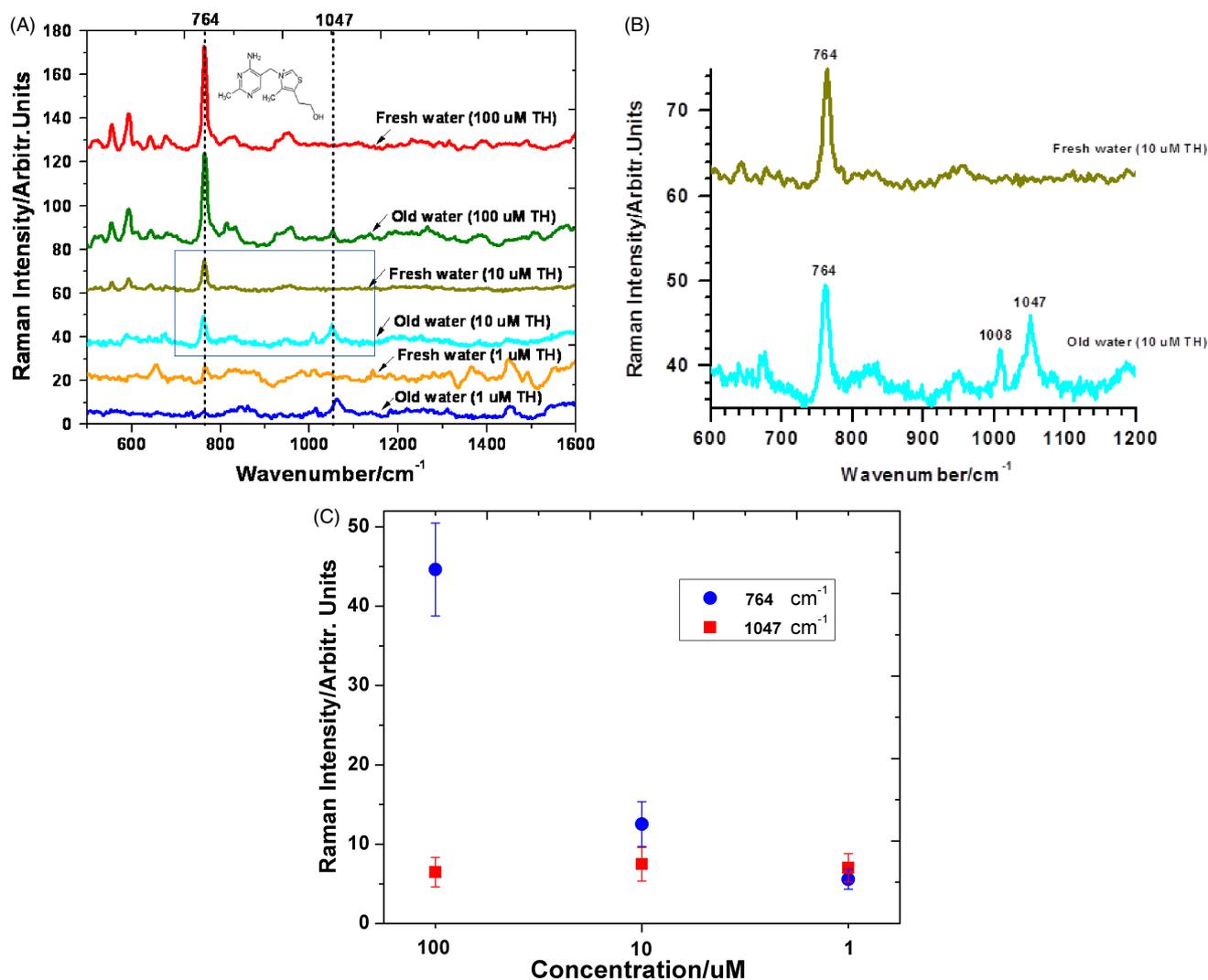
Figure 2. (A) SERS spectra of old and fresh thiamine hydrochloride (TH) solution of concentrations 1, 10, and 100 µM. (B) Comparison of the SERS spectra for TH solution with the concentration of 10 µM (zoomed in image for the cropped region on (A)). (C) Averaged intensities along with the standard deviations of the Raman peaks from old TH solution with three different concentrations for the wavenumbers 764 and 1047 cm^{-1}.

Results and Discussion

Interference of SERS spectra for old thiamine hydrochloride solution

As stated in the experimental section, the sample was prepared by dropping 3 µl of liquid on the SERS substrate. All the spectra presented here were collected with an integration time of 10 s. To facilitate the visualization and analysis of the Raman peaks, the autofluorescence background was removed with a modified multipolynomial fitting algorithm.[13]

Figure 2(A) shows the SERS spectra for both old and new TH solutions with concentrations of 1, 10, and 100 µM. The peak at 764 cm^{-1} showing up on all curves must be due to TH, since it varies with the TH concentration. We also confirmed this peak with regular Raman spectra of 10 mM TH solution (Fig. S2, Supporting Information). The strong peak at 764 cm^{-1} is generally attributed to the pyrimidine ring breathing mode of TH.[14] A closer comparison of 10 µM TH solution in old water revealed two anomalous peaks at 1008 and 1047 cm^{-1} which did not show up for TH solution prepared with fresh water (Fig. 2(B)). In fact, the peak at 1047 cm^{-1} consistently showed up with almost identical intensities for all old

TH solutions with different concentrations. Figure 2(C) shows the averaged peak intensities at 764 and 1047 cm^{-1} along with their standard deviations over 10–20 measurements of old TH solution at different locations on the substrates. It clearly shows that, as the concentration of TH goes down, the peak at 764 cm^{-1} also goes down (which is the characteristic peak for TH) while the intensity of peak at 1047 cm^{-1} remains almost the same. Also, because of the fact that the peak at 1047 cm^{-1} is absent in all TH solution prepared with fresh DI water, we can conclude that the old water must be contaminated by some chemicals giving a Raman peak at 1047 cm^{-1}.

As we already know that the old sample is contaminated by some chemical that can interfere with the SERS experiment, three questions remain to be answered here. What are those contaminants? Where do the contaminants come from? What is the concentration of the contaminants?

Identification of contaminants with mass spectrometry

To answer the first question, we performed mass spectroscopy to identify the contaminants. The GC-MS spectra showed nine
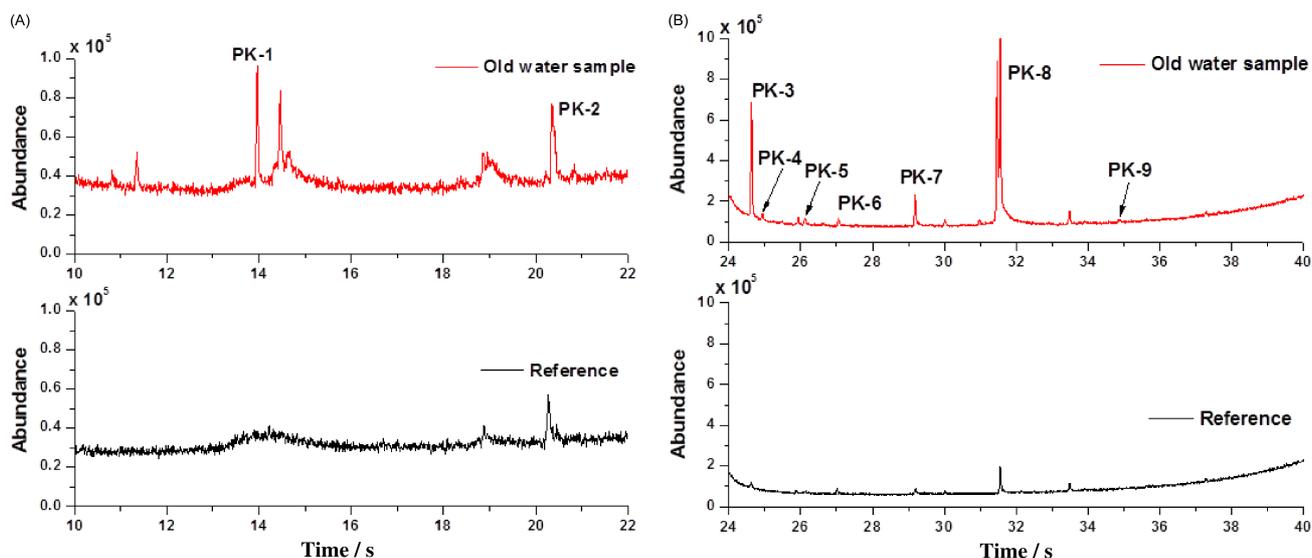
Figure 3. (A) GC-MS spectra of old water showing the two peaks corresponding to contaminants. The details of the contaminants are given in Supporting Information. (B) GC-MS spectra of old water showing more peaks at larger retention time corresponding to contaminants.
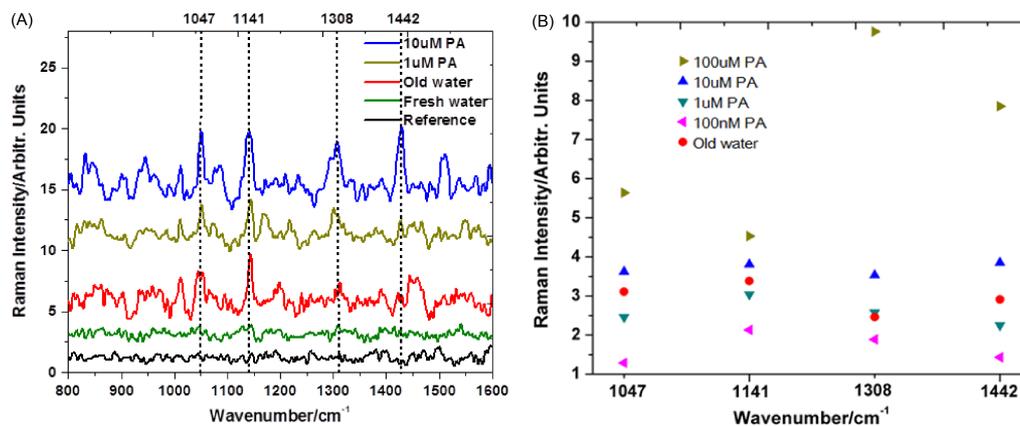

Figure 4. (A) SERS spectra of fresh water, old water, and phthalic acid (PA) solution with concentrations of 10 and 1 µM and the bare substrate as reference. (B) Averaged peak intensities of old water and PA with different concentrations at 1047, 1141, 1308, and 1442 cm^{-1}.

different peaks for the old water sample (Fig. 3). To interpret the mass spectra, the in-built Wiley and NIST libraries were used. The chemicals found are mostly of low molecular weight ($m/z < 500$ Da). The various peaks were predicted to correspond to benzaldehyde, 4-methyl (peak-1 or PK-1) with a mass of 91 Da; decane, 1-chloro (PK-2) with mass between 43 and 91 Da; 2-methyltetradecan (PK-3) with mass between 41 and 211 Da; phenol, 2,4- bis[1,1-dimethylethy-] (PK-8) with a mass of 191 Da; and benzoic acid, 4-methyl- (PK-9) with mass between 65 and 136 Da. Since GC-MS is a fragmenting technique, the subsequent analysis of major peaks revealed that water leachates are heterogeneous mixtures of small molecules and that PA may be the major chemical that is leaching into the water. It is also well known that PA is generally used as a plasticizer to make the plastic flexible.[15] In addition, PA along with its ester derivatives is widely used as additives in polymer synthesis.[16,17] The other chemicals found by GC-MS might have come from fragmentation of different biocides, slip agents, and/or oxidants used in the manufacturing of PP tubes (as concluded from the low molecular weights of those chemicals). We decided to continue our experiment with PA as a reference for our subsequent analysis.

Identification and concentration estimation of contaminants using SERS

In order to confirm the presence of PA in the leachants, we first performed the SERS of PA with different concentrations (Fig. S4 (supporting information)). The key vibrational signatures are situated between 700 and 1700 cm^{-1}, which agrees well with the literature information.[18–21] The Raman vibrational assignments of PA has been made independently by different authors (see the above references). Briefly, the strong peak at 1047 cm^{-1} is due to C–H wagging, the 1141 cm^{-1} band is attributed to C–H bending mode (9a in Wilson notation), the 1308 cm^{-1} band is the benzene ring torsional mode (3 in Wilson notation), and the 1450 cm^{-1} band is the ring-breathing vibrational mode (19a in Wilson notation). Further we compare the SERS spectra of fresh Millipore water and old Millipore water stored in the PP tube. Figure 4((A)) shows the comparison between the SERS spectra of fresh water, old water, and PA solution with concentrations of 1 and 10 µM. Clearly, two distinct peaks at 1047 and 1141 cm^{-1}, as well as two slightly weak peaks at 1308 and 1442 cm^{-1}, showed up in the SERS spectra for old water, whereas the SERS spectra of fresh water and that of reference background (substrate itself)
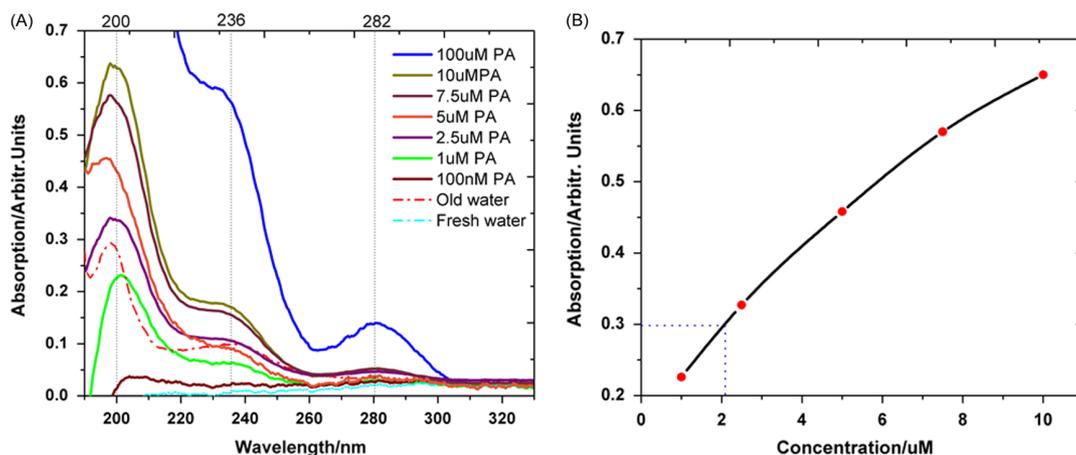
Figure 5. (A) UV absorption spectra for fresh water, old water, and phthalic acid (PA) solution with concentrations of 100 nM and 1, 2.5, 5, 7.5, 10, and 100 µM. (B) Curve-fitting to the absorbance at the wavelength peak around 200 nm on (A) for PA with concentrations of 1, 2.5, 5, 7.5, and 10 µM. The dotted line indicates the peak height for old water and its corresponding estimated concentration.

did not reveal any distinct peaks (Fig. 4(A)). From the similarity in SERS spectra of old water and that of the PA characteristic peaks, we believe that the contaminants in old water have a structure similar to that of PA. The SERS measurement also agrees well with the GC-MS prediction of PA as the suspected contaminant. To estimate the concentration of the contaminants, we compared the observed characteristic peak intensity of old water with that of PA with different concentrations. To be more reliable and statistically accurate, we took the average of the intensities from 15–20 measurements at different locations on the SERS substrate for the four characteristic peaks. As shown in Fig. 4(A) and (B), the Raman peak intensities of old water falls in between those of 1 and 10 µM PA solution. Hence, we conclude that the derivatives of PA in the old water are in the concentration range 1–10 µM.

Identification and concentration estimation with UV absorption spectroscopy

In addition to GC-MS and SERS measurement, UV absorption spectroscopy was also used to confirm and evaluate the suspect contaminants. Compared to SERS and GC-MS measurements, UV absorption works better for quantitative analysis though with lower sensitivity. In our experiment, a quartz cuvette containing the liquid sample was placed in UV–visible spectrophotometer for absorption measurement. For calibration of the instrumental error, another empty cuvette was used as reference. The scanning wavelength range was from 190 to 350 nm, the scanning step was 0.5 nm, and the integration time for each step was 1.5 s. We measured for fresh water, old water, and PA solution with different concentrations, and the results are shown in Fig. 5 and Fig. S5 (Supporting Information). The measured UV absorption spectra of the PA solution correspond to that in literature.[22] Comparing old water and fresh water, the absorption of old water is generally higher than fresh water over the whole wavelength range and shows several peaks. Fresh water gives nearly zero absorbance over the wavelength range. The strong absorbance for old water in the range between 190 and 350 nm points to the presence of contaminants that can absorb UV light. We compared the UV absorption spectra of old water and that of PA with different concentrations (Fig. 5(A)). The absorption of the PA solution increases with the concentration while the shape of the absorption spectra remains the same. The absorption spectrum for

PA showed three distinct peaks at the wavelength of 200, 236, and 282 nm. We found that the absorption spectrum of old water is very similar to that of PA, with distinct absorption peaks appearing at 200 and 236 nm. This shows that the contaminants in old water, which absorb UV light, have a similar structure as that of PA. Above all, the concentration of suspect contaminants in old water falls between 1 and 10 µM because the absorption curve for old water is placed between the curves for 1 µM PA solution and 10 µM PA solution, and from the interpolation of the peak intensity for old water, we estimate the concentration of the suspect contaminants as 2.1 µM. (Fig. 5(B)).

In conclusion, UV absorption spectroscopy confirms the chemicals identified by GC-MS and SERS measurements, and it is the derivatives of PA that contaminate the sample. SERS measurements give the quantitative estimation of the concentration of the suspect contaminants as between 1 and 10 µM.

Discussions and Future Outlook

Even though we have extensively studied the identity of the contaminants leached from PP tubes and managed to estimate their concentration, there are still some questions that need to be addressed for a more thorough understanding. Also we would like to share and discuss some interesting observations. First of all, the mechanism behind the leaching of chemicals from the tube and the dynamics such as how fast they leach are still unknown. The sample we used for testing was about 2 months old, but what happened during the first few weeks, days, or even hours? As the contaminants we identified are not a single compound, which one will leach first or faster? How do the temperature, pH, and polarity of the solvent affect the leaching process? Does the leaching ever reach a saturation so that the concentration of contaminants does not increase further? All these questions can be systematically investigated with SERS and UV absorption spectroscopy. But they are not covered in this article because of the limitations of time and space.

Secondly, we observed that some peaks of PA shifted with different concentrations. As shown in Fig. S4 (Supporting Information), the peak seen at 1075 cm^{-1} for the concentration of 1 mM and 100 µM is shifted to 1047 cm^{-1} for the lower

concentration of 10 µM, 1 µM, and 100 nM. The old water also shows the Raman peak at 1047 cm^{-1} corresponding to PA at lower concentrations, shown in Fig. 4(A). Although there is some evidence to show the SERS peaks of PA are shifted under different pH and concentrations,[23] the reason for the wavenumber shift for different concentration here, we believe, is the difference in types of adsorption. Since the PA molecule is adsorbed on the surface of silver substrate after the droplet is dry, the pH value does not make sense in this case. But the concentration does make a difference for adsorption. If the concentration is high, multilayers of molecules will cover the surface and so most molecules get physically adsorbed on the surface due to the weak van der Waals force. However, with low concentration, the molecules are more likely to form a monolayer on surface, and so most molecules will directly contact and form chemicals bonds with the silver surface, which is chemical adsorption (chemisorption). It is well known from charge transfer theory that chemisorption may result in peak shifts in the SERS spectra.[24]

Thirdly, even though SERS and UV absorption spectra for old water match well with those of the suspect contaminant PA, they are not identical. In Fig. 2(A), the Raman peaks at 1308 and 1422 cm^{-1} are not so prominent for old water as for PA. In Fig. 5(A) we can see that the absorption peak around 200 nm for old water is at a slightly lower wavelength than in the case of PA. Since we have identified the contaminants as derivatives of PA rather than the PA itself, there may be some differences in the structure of the contaminants and the used standard chemicals, which are reflected in the SERS and UV absorption spectra.

Finally, four major peaks at 1047, 1141, 1308, and 1442 cm^{-1} are observed for both old water and PA while only the one peak at 1047 cm^{-1} is significant for the thiamine hydrochloride solution in old water, as shown in Fig. 2(A). The explanation for this is that the Raman spectra of mixtures are basically not superpositions of the Raman spectrum of each individual compound, especially when the compounds are polar. For polar compounds, even though they do not react with each other, they are more likely to interact with each other at the molecular level and thus change their conformation, which can be reflected in the Raman spectra.[25] Neither PA nor TH seems to be nonpolar here.

Conclusion

We have found and confirmed that PP centrifuge tubes may contaminate their liquid contents by leaching chemicals as derivatives of PA. Even though low in concentration, the contaminants may interfere with the results of some highly sensitive analytical measurements such as SERS. GC-MS was used for the identification of the contaminants and, with the identity of the suspect contaminants known, SERS and UV absorption spectroscopy were used to confirm the contaminants and to estimate their concentration. The measurement results of the three spectrometric techniques agree with each other well. With UV absorption spectroscopy, we precisely measured the concentration of the derivatives of PA in water stored in PP tubes for 2 months as 2.1 µM. We propose SERS and UV absorption spectroscopy as two sensitive, simple, and quick techniques to detect the contaminants leached from PP tubes.

Supporting information

Supporting information may be found in the online version of this article.

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