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Raman Spectroscopy

Analysis of microplastics in hand sanitizers using ParticleFinder™

Application Note

Pharmaceuticals
Cosmetics
RA86

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Abstract: Due to the corona crisis, hand sanitizer has become part of our daily routine. However, their use is not without potential risks because of their microplastics content. HORIBA offers all the tools necessary to analyze and characterize the presence of microplastics in hand sanitizers: High-performance Raman microscopes, filtration kit, and especially the powerful particle detection software ParticleFinder™. We analyzed 3 hand sanitizer samples, from different countries, and we were able to identify the different plastic content of each.

Keywords: Raman Microscopy, ParticleFinder™, Microplastics, Hand sanitizer.

Introduction

At a time of global pandemics, the use and production of hand sanitizers and disinfectants has grown profusely. According to Statista Research Department(1), sales of hand sanitizers in multi-outlet and convenience stores grew by about 58% in 2020 compared to the previous year. Hand sanitizers became an essential item that people carried with them as soon as they stepped outside their homes. Yet, these products are not as harmless as one may think. Similarly, to any over-the-counter pharmaceutical product, serious attention must be paid to verify their inoffensiveness and wholesomeness to ensure safe, worry-free use.

Hand sanitizer consists of roughly 60% alcohol along with other ingredients such as emulsifiers, scent, dyes and what a lot of people are not aware of: Microplastics. According to the 2-minute team(2), 99% of the gel-based products are likely to contain plastics or ingredients that harm the environment. It is not a secret to anyone that plastics represent a big threat for the environment because of their very slow degradation process. On top of that, microplastics are becoming a concern for the human health as well, since they could contain substances recognized as toxic such as: • Persistent Organic Pollutants (POPs), Polychlorinated Biphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs), Phthalates etc… (3)

Microplastics can absorb and be an aggregation center for these substances particularly all when dissolved in water, due to their higher chemical affinity with respect to water increasing their load and potential toxicity. Moreover, some studies(4) suggest that the particle uptake by the human body is strongly linked to the size of the particles, as smaller particles may penetrate organs easier.

Considering this, it is crucial to focus on the analytical techniques which allow the identification and characterization of the smallest particles, such as Raman Microscopy.

Raman microscopy is a non-destructive, non-contact technique that provides:

- Full morphological information for each particle through the analysis of the optical image (diameter, ellipse ratio, area…);
- Quantitative analysis (number of particles);
- Chemical identification of each particle (by exploiting a dedicated microplastic database library).

Hand sanitizers are usually presented as a gel. Therefore, a sample filtration is required. For this reason, HORIBA developed an easy-to-use filtration kit specifically for this application. Allying this with the powerful particle analysis tool ParticleFinder™, and with the comprehensive spectral identification library KnowItAll®, we get a complete and simple procedure to study and diffrentiate the components of hand sanitizers.

Instrument and methods

Figure 1: Filtration apparatus: glass funnel, glass support base, silicone stopper, glass flask, and vacuum pump.

Samples preparation

We selected 3 hand sanitizer samples coming from different countries. We have called them Sample 1, 2 and 3. They were diluted in Ethanol, then filtered using HORIBA's filtration kit. We used Silicon filters of various pore sizes as they are the most suitable for the Raman analysis of microplastics.

Table 1: Filtration conditions for each sample.

Sample	1	2	3
Volume of sample in mL	50	25	50
Volume of Ethanol in mL	50	75	50
Filter pore diameter in µm	5	10	10

Raman platform

The Raman acquisition was made using LabRAM Soleil. It is the latest model of the LabRAM microscope series, specifically designed for wide spectral range UV-VIS-NIR confocal imaging, thanks to its ultimate optical design. Its multiple innovative hardware and software features enable us to obtain precise optical images and high-resolution spectra, with advanced automation and at an unbelievable pace.

Figure 2: LabRAM Soleil Raman microscope

LabRAM Soleil offers a great variety of optical viewing modes: reflected or transmitted illumination, bright-field/dark-field, phase contrast, etc. We used cross-polarization illumination to reduce the brightness of the Silicon filter and to facilitate the location of particles with ParticleFinder™.

ParticleFinder™

The ParticleFinder™ application for LabSpec6™ allows automated location of particles, analysis of key particle parameters, such as size and shape, and subsequent chemical characterization with Raman. It is especially beneficial in cases where the number of particles is large, like microplastics, where manually locating and marking each particle would be time-consuming, outweighing the benefits that Raman can offer.

ParticleFinder™ is an integrated part of the LabSpec6™ software, linked to related modules for data acquisition, processing, analysis and display. Its workflow is simple and intuitive.

First, we start with the image acquisition. We used the 50x objective to have an appropriate spatial resolution considering the size of particles (diameter < 80 µm). To analyze the entire 1 cm x 1 cm filter by first imaging it all by montaging together multiple image tiles and then returning to each particle requires an exceptionally high positional accuracy sample stage. However, ParticleFinder™ overcomes this unnecessary constraint using its Dynamic mode. This acquires each image tile in turn but before moving to the next it is analyzed and each particle is analyzed spectrally. This enhances positional accuracy (as XY travel distances remain small) and also minimizes the time between particle location and identification, reducing the chance of the analysis being impacted by a particle moving due to environmental or thermal drift. Since this mode requires no additional user interaction it is highly recommended for ultimate performance and precision for the characterization of both large samples and small size particles.

Then, we can select the particles. An automatic threshold and multiple morphological filters are used to accurately identify the particles. Using pre-filters allows some particles to be excluded from the analysis based on parameters like area, brightness, circularity, etc. This is very useful not only to select particles of interest only, but also to exclude background features like the regular square holes in the silicon filters. We selected only particles whose diameters were between 20 µm and 80 µm to focus on the smallest particles, and whose ellipse ratio exceeded 0.3 in order to avoid the detection of the fibers contained in the gels.

Finally, after the Raman acquisition, the data was analyzed. Besides a baseline correction, no processing was done to the spectra. We identified the different components of our samples using the KnowItAll® spectral library. We then used families to segregate the particles into groups allowing statistics and interpretation of the results based on a higher-level segmentation.

Results

The first type of comparison is the image of each filter after the filtration:

- In the table below, the coloured particles are the ones that were selected for the analysis. They are the smallest particles (between 20 µm and 80 µm diameter) since this is the focus of our study.
- The composition of the samples is different from one another. While the filter corresponding to sample 1 is packed with particles, sample 2 contains many fibers, and the sample 3 one is the least dense.
- Particles in sample 1 are brighter and more opaque than the other samples where the particles are quasi-transparent. Indeed, the mean brightness (in a grey scale of 0 to 255) of the detected particles in each sample is: 108.599 for sample 1, 60.7182 for sample 2, and 43.4436 for sample 3. The opacity of the particles has an influence on the Raman signal, as we see globally better spectra in sample 1.

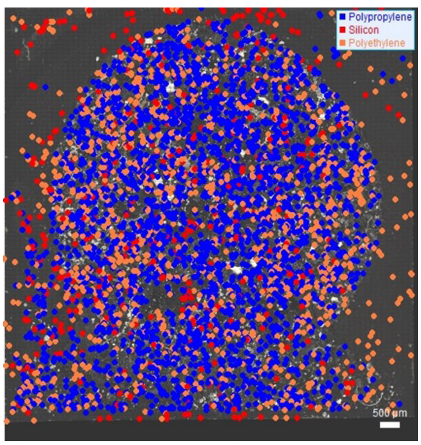
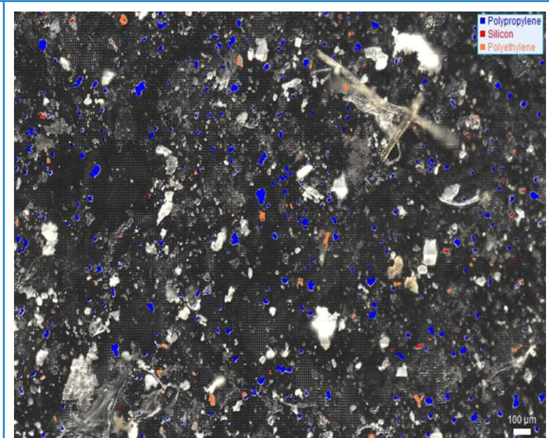
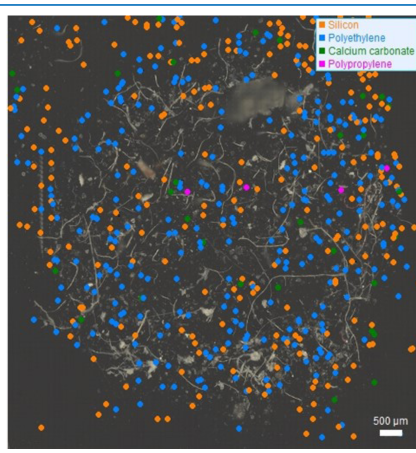
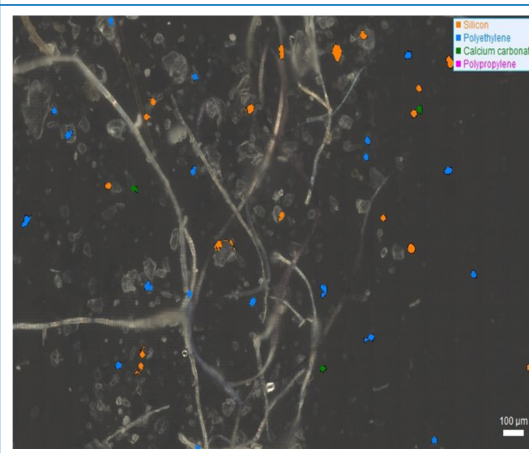
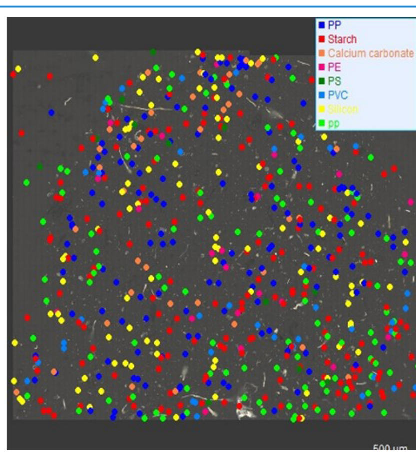
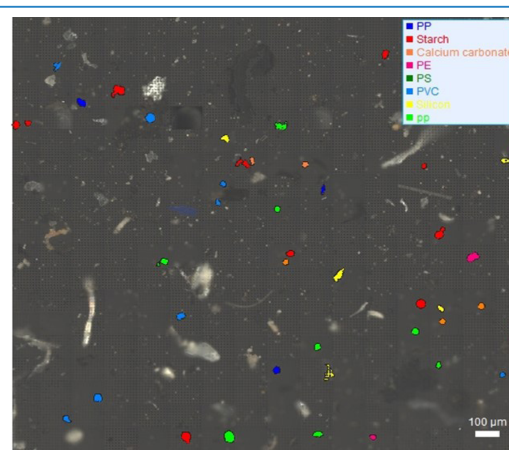
Sample	Full filter image (scale 500µm)	Zoom on a small area (scale 100µm)
1		
2		
3		

Table 2: Table showing the images of the full filter (left), and a zoom on a small area of the filter (right). The coloured surfaces correspond to the detected particles

The second type of comparison is the total number of particles and the number of plastic particles in each sample.

To examine the results, we chose to compare the number of plastic particles per milliliter.

Sample 1 has the biggest number of plastic particles per milliliter with 3733 particles for 50 milliliters. Sample 2 comes in second place with 207 particles for 25 milliliters, and finally Sample 3 with 353 particles for 50 milliliters. Figure 3 illustrates this result.

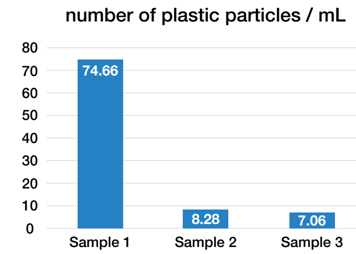
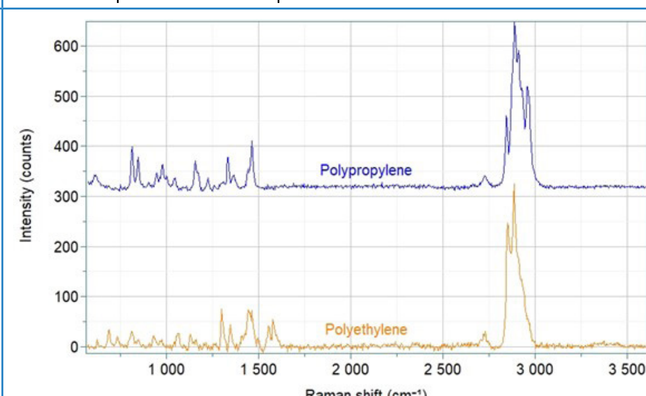


Figure 3: Bar diagram presenting the number of plastic particles per milliliter for each sample.

The third and final type of comparison is the chemical composition of the samples. We found many components in each sample. The most prominent ones are plastics, but we also found other elements like: Calcium carbonate, which is usually used as a filler in plastics(5), and Starch, which is a biopolymer(6).

To sum up, the three samples differ by the morphological properties of their particles, the number of plastic particles per milliliter (ranging from 7,06 to 74,66), and the types of plastic they contain (Polypropylene and Polyethylene being the most common ones).

Table 3: Table presenting the composition of each sample (right) with the corresponding spectra (left).

<table>
<tr><th>Sample</th><th>Raman spectra of components</th><th>Distribution of components</th></tr>
<tr><td>1</td><td>


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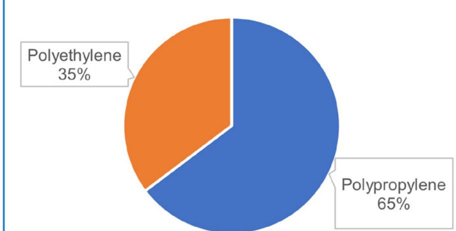

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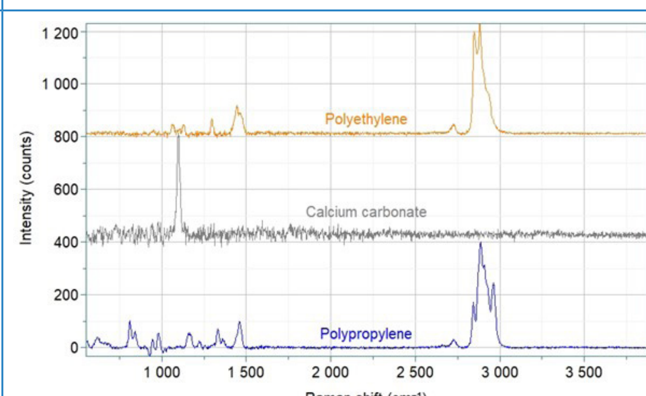
<tr><td>2</td><td>


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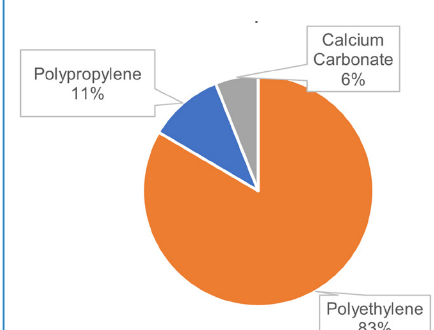

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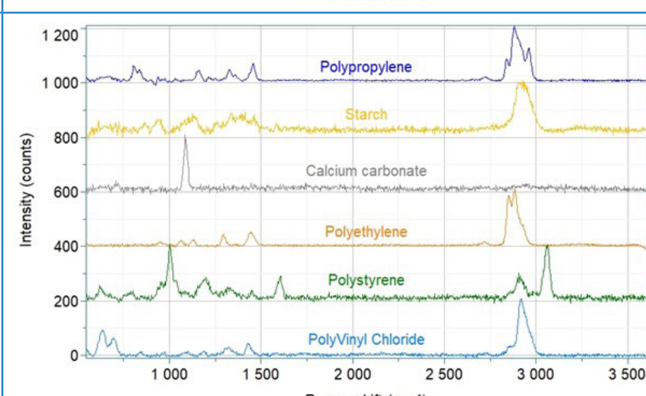
<tr><td>3</td><td>


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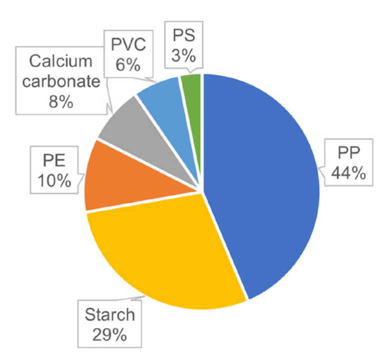

</td></tr>
</table>

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Conclusion

In this application note, we described the intuitive and automated method of ParticleFinder™, allying the robustness of Raman microscopy with the sophistication of HORIBA's software to allow the screening of a large quantity of microplastics in a precise way. Thanks to ParticleFinder™, we demonstrated the presence of microplastics in three samples of hand sanitizers, we were able to distinguish the different types of plastics each sample contains, and study their statistical distribution.

References

1. https://www.statista.com/aboutus/our-research-commitment
2. https://2minutebeachcleanblog.wordpress.com/2020/07/07/the-hidden-truth-behind-hand-sanitiser/
3. https://www.horiba.com/int/scientific/applications/environment/pages/microplastics/#what-are-microplastics
4. Lusher et al., FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, Italy. 2017
5. https://en.wikipedia.org/wiki/Calcium_carbonate
6. http://polymerdatabase.com/polymer%20classes/Polysaccharide%20type.html

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