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Optimizing the Workflow for FTIR Microspectroscopy of Microplastics Present in Environmental Samples

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It is estimated that there is in excess of 150 million tonnes of plastic materials in the World's oceans [1]. Much of this pollution is large items such as discarded drinks bottles or plastic carrier bags. However, there is increasing research into the amount of much smaller materials, termed microplastics, in the river and ocean systems as they present a different type of problem for marine life. Microplastics are generally particles that are less than 1mm in size and range typically down to a few micrometres.

Many analytical techniques can be used to identify and quantify microplastics, the primary technique adopted being Infrared (IR) microspectroscopy, since the infrared spectrum gives a unique fingerprint of the material present. However, microplastic samples can be obtained from many different origins within a wide range of matrices, such as salt water , sediments, wastewater effluents and even within living or dead river or marine species. Each of these matrices offer a significant challenge to infrared microspectroscopy since the matrices need to be removed in order to measure the "pure" spectrum of the microplastic alone. Hence, there are several stages required within the analysis workflow to allow the optimum sample analysis by IR microspectroscopy :

1. Sample collection. In order to perform meaningful studies it is important to develop a suitable strategy for sample collection.
2. Sample cleanup. Isolation of the microplastics from the matrix to enable filter separation. This can be physical or chemical separation, but is essential to allow efficient separation.
3. Filtration. The isolation of the microplastics from any matrix or interfering material.
4. Spectral data collection and analysis. Must be optimised to obtain the best results in the most speed efficient manner.

Many methods have been adopted for stages 1 and 2 of the sample workflow[2]. However, the methods adopted are often only suitable for visual detection of the microplastics and do not always consider the requirements for infrared analysis. The work presented here will consider the options for sample cleanup but will mainly focus on stages 3 and 4, the choice and optimisation of the filtration for infrared spectral analysis.

When considering filter materials for this type of analysis there are 3 important parameters; filter dimensions, pore size and substrate material(s). Transmission and reflectance measurements by infrared spectroscopy can measure samples down to about 5 micrometers in size, limited by the wavelength of the measuring radiation. However, this can be extended down to samples of about 2-3 micrometers with the use of Attenuated Total Reflectance (ATR) microimaging techniques [3]. Hence, the filter pore size should be optimised to collect samples from a few micrometers in size upwards. The filter dimensions should be restricted in order to allow a sensible IR microspectroscopic analysis time. IR imaging or mapping of a 25 or 37mm diameter filter could take several hours. Smaller filter dimensions, such as 13mm diameter, offer good filtration properties and allow faster analysis times. The choice of filter substrate material is extremely important when IR microspectroscopic analysis is to be performed. The

IR analysis is performed using either transmission, reflectance or ATR modes of operation. The filter substrate material should not have IR absorptions that will interfere with the spectra of the materials being measured. A range of substrate materials have been tested in both transmission and reflectance measurement modes to determine suitability, with a silicon substrate (Figure 1) being the most suitable when both measurement modes are required, but a gold-coated polycarbonate or silver membrane substrate offering the best sensitivity when only reflectance is performed. The choice of sampling mode; transmission, reflectance or ATR, is an important consideration. Transmission measurements would be the preferred mode since it will generate distortion-free spectra that can be compared against spectral libraries with in excess of 100 000 reference spectra. However, many of the particles can be in excess of 100 micrometers in thickness leading to very intense, over absorbing spectral features. Reflectance measurements are more easily performed, but the spectra have reflectance distortions and contain both reflected and "double transmitted" spectral components, leading to even more intense spectral intensities. ATR spectra are significantly less intense than transmission and reflectance and can be "ATR corrected" to enable library searching. However, single point ATR is a contact technique and, if measuring multiple particles, can be prone to ATR crystal contamination caused by particles sticking to the ATR crystal after contact. ATR imaging accessories are available with sampling areas up to 1100 x 1100 micrometers allowing fast ATR measurements, but over these limited dimensions. To summarise the IR microspectroscopy sampling methodology, there is not a single option that works for all samples, a system with multiple sampling modes gives the greatest versatility.

Data analysis is an important stage in the overall workflow. Numbers of particles, sizes and their identities are important results from the analysis. The IR spectra of inidividual particles can be searched against spectral libraries for identification, with color coding in the image representing different material types. Finally, Principal Components Analysis (PCA) can be applied to the complete IR image data to quickly and easily show the distribution of different chemical species throughout the image as shown in Figure 2.

References:
[1] https://www.oekosmos.de/artikel/details/wasserverschmutzung-durch-mikroplastikpartikel/
[2] M. Löder and G. Gerdts in "Marine Anthropogenic Litter", ed. M. Bergmann (Springer) p. 201.
[3] "Spatial Resolution in ATR FT-IR Imaging: Measurement and Interpretation", (Perkin Elmer).

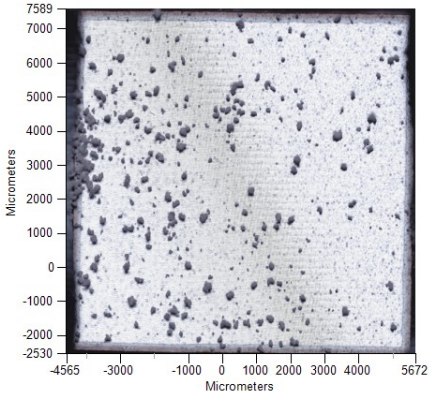

Figure 1. Visible image of microplastics collected on Silicon substrate

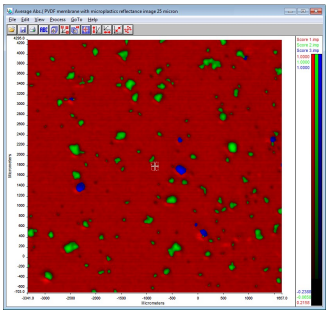

Figure 2. PCA analysis of IR image of microplastics on PVDF substrate.

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