



Inhalable Microplastics: A New Cause for Concern?

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1 Introduction

Microscopic plastic particles – microplastics – are a global issue for aquatic habitats. Recently, they have been reported in indoor and outdoor air raising concern for public health due to the potential for exposure via inhalation. However, very little is known about airborne microplastics, including their spatial and temporal concentrations; chemical composition; and, importantly, whether they occur in the inhalable size range [1].

Accurate estimation of the number of microplastic particles, films, and fragments is subject to significant methodological challenges. Correlating spectral data against plastic-only references may increase the chance of false-positive results, and visually discriminating between similar polymer spectra (such as polybutylene terephthalate and polyethylene terephthalate) could result in misclassification. Much work is still required in this area, and analytic techniques capable of detecting plastic particles at the nanoscale are needed.

Here we present data on the presence of microplastics in total atmospheric deposition sampled over one month at an urban background site in London, UK. Given the potential for microplastic misidentification due to overlapping aesthetic, morphological or fluorescent properties with non-plastic particulates, spectroscopic analysis is an analytical requirement. Raman Spectral Imaging (RSI) has been used as an alternative approach to combat operator bias and improve spectroscopic analysis of contiguous particulates.

2 Experimental

This study uses Raman spectral imaging for the identification of microplastics ($\geq 2 \mu\text{m}$) in ambient particulate matter, using different chemometric techniques. We show that Raman spectral images analysed using univariate and multi-variate statistical approaches is appropriate for the identification of both virgin and environmental microplastics $\geq 2 \mu\text{m}$ in size.

2.1 Materials

Ethanol (EtOH) was sourced from Fisher Scientific UK (Loughborough, UK). 1, 2, and 10 μm PS microspheres were sourced from Sigma Aldrich (UK). 4.16 μm PS microspheres were sourced from Spherotech Inc. (Illinois, US). PA, PE, PET, PS, PP, and polyvinyl chloride (PVC) particles were sourced from Goodfellows Ltd (UK) (otherwise referred to as virgin plastics). These were all stored as per the supplier recommendations. Environmental plastics collected opportunistically from a European Beach (Arenal d'en Castell, Menorca) were milled to reduce their finesse.

2.2 Methods

2.2.1 Preparation of Samples

To validate the proposed method against a representative background a 24-h PM₁₀ (Particulate matter <10 D) archived air sample collected from an urban road-side site (Marylebone Road, London, UK) was used. The sample was collected onto a Teflo filter (Polytetrafluoroethylene; PTFE) using a Partisol™ Plus 2025 Sequential Ambient Particulate Sampler (flow rate of 16.7 L/min). PM was extracted by submerging the filter in 5 mL of EtOH and agitating in a sonicating bath (5 min). The extracted PM was dried, weighed, and re-suspended in EtOH to a concentration of 312 $\mu\text{g/mL}$. Which corresponds to the mean daily sample weight collected by the desired method of sampling (MVCS) during a spring 2017 sampling campaign.

The PM was spiked with 2, 4, and 10 μm PS microspheres at a total concentration of 30,000 particles/mL (10,000 particles/mL for each size). The sample was diluted 1 in 10 and a 100 μL aliquot was dried dropwise on to an aluminium slide. A micrograph and SIs were acquired of the entire dried PM drop-cast. Due to the dried drop cast's area being relatively large and concerns over file size, the SI was acquired in 6 separate units at ~ 2.6 μm spatial resolution. Each individual SI unit once analysed was tiled together to generate a sample wide SI.

2.2.2 Analytical Techniques

To evaluate the chemometric protocols, microplastics identified in the SI were counted and compared to the expected concentrations. Image analysis (particle counts) was completed on all analysed SI in Icy and ImageJ. Image pre-processing of the SIs included a 64-bit raw grey scale image conversion, applying a gaussian blur filter with a sigmoid radius of 1.0, the application of a watershed transform [2], and a Huang thresholder to extract the objects (areas of Raman signal) from the background using Shannon's function [3]. Microplastics in the processed SI are counted using an Undecimated Discrete Wavelet Transform (UDWT) detector, which produces a multi-resolution representation of an image [4].

3 Results and Discussion

3.1 Results

From the SIs obtained of 1, 2, 4, and 10 μm PS microspheres dispensed onto a range of substrates, aluminium slides were found to be optimum for visualising PS microspheres and suppressing background fluorescence, while low-E slides performed worst due to optical contrast and substrate fluorescence (LOD: 10 μm PS microspheres at $\sim 2.6 \mu\text{m}$ spatial resolution) (Fig. 1).

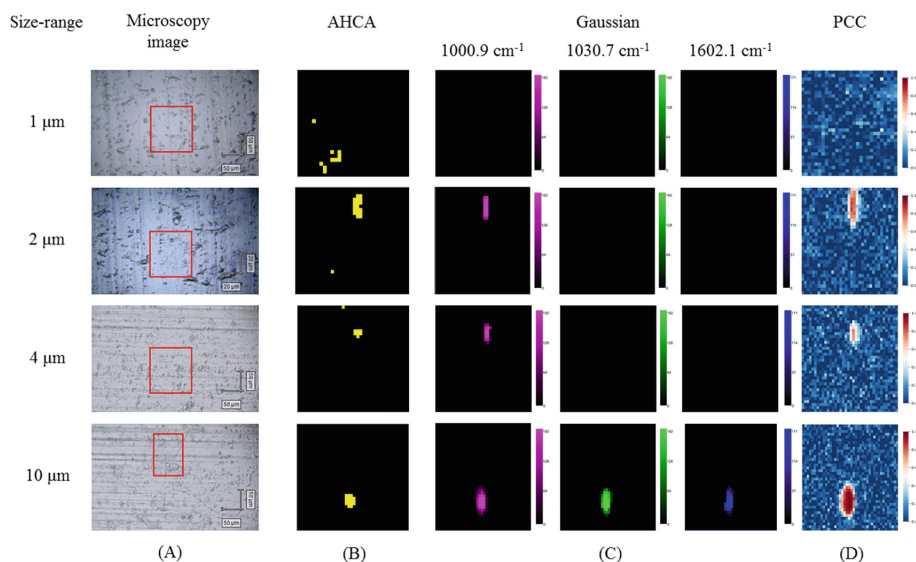


Fig. 1. Size dependent identification of polystyrene (PS) microspheres using Raman spectral imaging. Raman spectral images (SI) approximate area shown in Red and corresponding bright field micrographs (A) of PS microspheres were acquired. The SIs were analysed using AHCA (B), Gaussian (C), and PCC (D) to identify PS microspheres. 4 and 10 μm PS microspheres were obtained at 2.6 μm spatial resolution, and 1 and 2 μm PS SIs were acquired at 1.1 μm spatial resolution

Using Nile Red staining, bright field and fluorescence microscopy and Fourier-transform Infrared spectroscopy, ten times more fibrous microplastics were found than non-fibrous. This equated to an average deposition rate of 706 fibrous microplastics/ m^2/d , with polyacrylonitrile being the predominant polymer type.

The proposed chemometric methods were analysed for their speed at plastic classification in an SI of PS particles deposited on an aluminium slide (7875 spectra). The chemometric techniques identified the presence of PS in 13.3 s, 105 s and 2100 s for PCC, AHCA, and Gaussian analysis, respectively.

PS microsphere counts differ between the chemometric techniques, with PCC analysis detecting the greatest number of microspheres (particle count: 212), followed

by AHCA (particle count: 169), and lastly Gaussian (particle count: 111). Analysis of the mock ambient sample for the remaining plastics in the plastic spectral library resulted in the identification of PE ($n = 35$), PET ($n = 2$) and PP ($n = 5$) particles. The identified airborne microplastics ranged from 4.7 to 51.8 μm in size in their longest dimension. The total concentration of airborne microplastics in the 24 h urban roadside sample was calculated to be 174.6 microplastics/ m^3 .

3.2 Discussion

As global pressure to reduce road transport and fuel burning emissions increases, PM composition is likely to shift. In combination with a predicted increase in plastic use, especially in the textile sector (4%/year), the proportional concentration of airborne microplastics will become increasingly important. It is therefore timely to establish baseline knowledge of global airborne microplastic burdens and begin to understand what their potential role in PM-associated health effects might be.

The chemometric analysis methods utilised successfully identified 2, 4, and 10 μm PS microspheres dispensed on aluminium-, CaF₂-, gold-coated-, and stainless steel-Raman substrates in SIs. However, the intensity profile of Raman bands associated with PS differed among the test substrates. Aluminium-, CaF₂-, gold-coated-, and stainless steel- slides permitted the identification of ≥ 2 μm PS microspheres in SIs. As CaF₂-, gold-coated-, and stainless steel- substrates are relatively expensive, substrate reuse would be necessary to maximise economic viability. The low-cost of aluminium slides will permit sample archiving, future reanalysis and/or sample extraction.

RSI and the applied chemometric methods were capable of distinguishing virgin microplastics reflecting the most commonly observed plastic types in the environment, i.e. PA, PE, PET, PS, and PVC. However, in the environment, microplastics will undergo photo-, thermo-oxidative-, hydrolytic- and bio- degradation [5]. Depending on the plastic type, this has been found to impact or alter Raman spectra [6]. Whilst the duration and level of photodegradation is unknown, the identification of environmental microplastics in the current study (PE, CuPc, PP, and PS) indicates that this method is suitable for aged particles of a similar composition [6]. However, Lenz and colleagues [6] observed PVC's Raman spectrum is modified with UV-degradation and is completely altered after 1634 days of simulated noon-sunlight. Thus, it's important to continue to validate analytical methods on aged microplastics and recognise detection limits.

4 Conclusions

In conclusion, we have demonstrated that RSI analysed using Gaussian, AHCA, and PCC chemometric techniques can identify microplastics in the inhalable size-range. In this study, SIs were acquired at 1.1 μm and 2.6 μm spatial resolution, which enabled the direct identification of 2, 4, and 10 μm PS microspheres in ambient PM. In addition to ambient samples, the presented RSI method could be adapted to identify microplastics in marine and terrestrial samples, though adequate sample pre-processing

is recommended. Particulates shown to generate plastic signal in the SI should be further optically inspected to conduct morphological analysis (i.e. size, shape, colour).

RSI has a clear advantage of removing operator bias, while permitting the identification of particulates in the inhalable size range, enabling data on microplastic abundance, size distribution. Chemical composition and morphology to be attained. However, it suffers long acquisition and data processing times. For the field to progress towards microplastic monitoring in any environmental matrix, more streamlined instrumentation is required. Hence, RSI should be a complimentary technique alongside alternative methods, such as Py-GC-MS, to generate mass related datasets.

References

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