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**CHEM 4400**

**Method Development for the Determination of Microplastics in Freshwater.**

**Date of experiment:** October 15th, 2024

**Date of submission:** October 29th, 2024

**Abstract**

A method was developed to detect microplastics in freshwater using Raman spectroscopy. Freshwater samples were filtered twice to target microplastics in the 0.45-25 μm range. The 0.45 μm filter paper was analyzed by Raman spectroscopy to detect microplastics. Spiked water samples were also run through the method. The Raman spectrometer failed to detect microplastics in any of the real water samples or the spiked water samples. Microplastics were detected by Raman spectroscopy in a standard solution of 0.01% polystyrene beads. This demonstrates that the method may have some validity, but still needs work to adequately detect microplastics in freshwater.

**Introduction**

Microplastics are becoming an increasing topic of concern. They are defined as polymers less than or equal to 5 mm in size and have been identified as environmental contaminants.1 Sources of microplastics include textiles, personal care products, tires, and other plastic products.2 As more research is done, microplastic particles are being found nearly everywhere. Microplastic particles have been found in remote areas such as glaciers and mountains.3 The particles have even been found in human lung tissue, blood, placenta, and other organs.4 The impacts of microplastics on human health and the environment are not fully understood. The mechanisms of transport for microplastic particles are also not entirely clear. The effects of microplastics on marine environments is another topic that needs further research. Many methods have been developed to analyze microplastics in water samples, often using Raman spectroscopy. This project seeks to develop a method to easily identify microplastics in freshwater.

**Experimental**

Six water samples were taken from freshwater bodies around the Kamloops area. The samples are summarized in table 1.

|  |  |
| --- | --- |
| **Sample** | **Location** |
| J1 | Jacko Lake boat launch |
| J2 | Jacko Lake west |
| E1 | Edith Lake south dock |
| E2 | Edith Lake north dock |
| R1 | Thompson River, Riverside Park |
| R2 | South Thompson River, Valley View boat launch. |

**Table 1:** Samples and sampling locations.

Water samples were collected manually in 1L glass jugs. Sample containers were covered with aluminum foil and then sealed with the container lid. Samples were transported to the lab and set aside for filtration.

|  |  |
| --- | --- |
| **Stock solution** | 10% 1.02 μm polystyrene bead solution |
| **Instrument** | Anton Paar Cora 5001 Raman Spectrometer |
| **Acquisition Wavelength** | λ = 785 nm |

**Table 2:** Experimental data

A 0.01% polystyrene standard was prepared from polystyrene stock solution. The polystyrene beads were observed under a microscope. Real water samples were also observed under microscope, but no microplastics were found. Microscope analysis was eventually omitted from the method. Real water samples were filtered with 25 μm filter paper and the filtrate was filtered again with a 0.45 μm membrane filter. The 0.45 μm filter papers were analyzed by Raman spectroscopy to detect any microplastic particles. The 0.01% polystyrene solution was put through the filtering process and then analyzed by Raman spectroscopy. Two real water samples were spiked with the 0.01% polystyrene solution to concentrations of 0.0001% and 0.0004%. The spiked samples were filtered and analyzed by Raman spectroscopy. Data from Raman spectroscopy was analyzed to identify microplastics.

**Data and Results**

In the initial water sample runs a match was found in the spectral library, it appeared that polyethylene terephthalate, a common microplastic, had been identified by Raman spectroscopy. However, it was later realized that the nylon 0.45 μm filter paper was being detected instead of microplastic beads. After subtracting the nylon filter paper from the spectra, no microplastics were identified in any of the real samples. This is illustrated in figures 1 and 2.

A graph of a normalized blood pressure

Description automatically generated with medium confidence

**Figure 1:** Spectral data from first run of R1 0.45 μm filter paper. PET was identified, but this was later proven to be the nylon filter paper.

**A graph of a normalized pulse

Description automatically generated with medium confidence**

**Figure 2:** Spectral data from second run of R1 0.45 μm filter paper. The nylon filter paper was subtracted from the spectra, and no microplastics were identified.

The 0.45 μm filter paper from the 0.01% polystyrene solution was analyzed by Raman spectroscopy and peaks characteristic of polystyrene were identified. The 0.45 μm filter paper from the two spiked samples were then analyzed and compared to the 0.01% polystyrene spectra. No polystyrene peaks were identified on the spiked samples spectra.

A graph of a graph

Description automatically generated

**Figure 3:** Spectral data from the 0.01% polystyrene run. A characteristic polystyrene peak was identified around 1050 cm-1.

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**Figure 4:** Spectral data from the 0.0001% spiked sample run. There are no peaks characteristic of polystyrene present.

**Discussion**

A method for the detection of microplastics in fresh water was developed and tested. The initial method plan was to filter each water sample with 25 μm filter paper and then a 0.45 μm membrane filter. The filtering steps were designed to target microplastic particles in the range of 0.45-25 μm. The 0.45 μm filter paper would then be analyzed by microscope and Raman spectroscopy to identify microplastics. Analysis of 0.45 μm filter paper by microscopy did not work. It was far too difficult to discern microplastic particles from other particles.

The method also failed to detect microplastics in the real water samples. This was not surprising, as microplastic concentrations in water tend to be very low. One study found that freshwater microplastic concentrations were around 0.4 particles per litre.5 After failing to detect microplastics in the freshwater samples, spiked samples were filtered and analyzed in the same way. A 0.01% polystyrene solution was also analyzed and compared to the spiked samples. The added polystyrene was not detected in the spiked samples. This was likely due to the very small concentration of polystyrene in the spiked samples, 0.0001% and 0.0004%, that was meant to represent a real sample. The only sample that the method successfully detected microplastic particles in was the 0.01% polystyrene sample.

Sources of error in this experiment could have been interference from other particulate on the 0.45 μm filter paper. Some of the samples had a lot of sediment/dirt particles that could have covered microplastic particles and prevented them from being detected by the Raman spectrometer. A way to minimize this interference could be including some sort of separatory step, centrifuging for example. Another very probable source of error is the low sample volume. With microplastic concentrations being extremely low in freshwater, a large sample volume is needed to detect any microplastics. A way to address this would be to filter the water on-site, rather than in the lab. Filtering on site using a pump, would allow very large quantities of water to be sampled, without needing to transport large quantities of water to a lab.

**Future work**

If I were to continue this project, I would include a way to separate microplastics from other particles and I would do on-site filtration. I would then like to increase the number of sampling locations and the type of sampling locations. For example, comparing microplastic concentrations in ocean samples and freshwater samples. Examining a different sample matrix for microplastics, such as food, tissue, or air and comparing them would also be very interesting.

**Conclusions**

The developed method failed to detect microplastics in freshwater samples and spiked freshwater samples. Microplastics were detected in a 0.01% polystyrene bead solution using the method. The detection of microplastics in the 0.01% solution shows that this method can be appropriate for detection of microplastics, but additional steps are needed to detect microplastics in real samples.

**References**

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**Appendix**



**Figure 5:** Initial R1 run without subtracting the nylon filter paper.



**Figure 6:** Second R1 run, after subtracting nylon filter paper.



**Figure 7:** R2 spectrum.



**Figure 8:** J1 spectrum.



**Figure 9:** J2 spectrum.



**Figure 10:** E1 spectrum.



**Figure 11:** E2 spectrum.



**Figure 12:** Nylon filter paper spectrum.



**Figure 13:** 0.0001% polystyrene spiked sample spectrum.



**Figure 14:** 0.0004% polystyrene spiked sample spectrum.



**Figure 15:** 0.01% polystyrene standard spectrum.