

MICROPLASTICS DETERMINATION WITH μ -RAMAN: POTENTIAL AND PRACTICAL ASPECTS

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1 Introduction

Drinking water is the result of a chain of treatments aimed at purifying the water source from normed pollutants. However, thanks to the increasing analytical power of the available techniques, more pollutants of concern are being discovered both in the water source and in drinking water. In order to keep the drinking water as safe as possible, such pollutants need to be identified in terms of associated risk and fate in the drinking water treatment plants (DWTPs).

Microplastics (MPs) belong to those pollutants referred to as “of emerging concern”. MPs are broadly defined by the scientific community as plastic particles with main dimensions bigger than 100nm (or 1 μ m) and lower than 5mm [1].

This broad definition coupled with the MPs physical and chemical characteristics results in a challenging task for regulators worldwide for the establishment of acceptable limits and also of standardized analytical protocols.

μ -Raman is among the most used techniques for the analysis of particles as low as 1 μ m; however, many are the aspects to be taken into consideration for a reliable and meaningful analysis using this technique. Furthermore, when developing an analytical method, it has to be considered the different aims and goals of a water company, which is responsible for monitoring and managing the water cycle, and of the researchers interested in pure investigation. In the present work, the practical struggles of MPs analysis by μ -Raman are considered, underlining the main issues and proposing solutions to overcome such troubles. It focuses on the activities run in the laboratory, specifically MPs pre-treatments, separation, identification, quantification and characterization (IQC). Firstly, the main steps and issues to carry in the μ -Raman analysis are presented and described; then, the results obtained and the possible solutions are reported.

2 Experimental

2.1 Materials and methods

Drinking water for MPs analysis was sourced from private house taps, municipal water towers and DWTPs. Samples were collected in glass containers (volumetric flasks) with volume of 250-1000mL.

Hydrochloric acid (HCl), acetic acid (AA) and ethanol were of analytical grade and supplied by Carlo Erba, EDTA powder was purchased from VWR.

Silicon filters with porosity of 1 and 5 μ m were used for vacuum filtration, in all glass set-ups. All filtration procedures were run under a laminar hood.

μ -Raman DXR3 by Thermo Fisher coupled with Omnic Spectra and Omnic Atlas softwares were used for the characterization and identification of MPs.

2.2 Pre-treatments

Pre-treatments phase is crucial to a proper IQC. Indeed, without pre-treatments the time needed for the analysis increases, as the particles agglomeration and the filter crowding can occur.

Dealing with drinking water, pre-treatments are mostly needed to reduce the presence of Calcium and Magnesium carbonates. At pH of 3, most carbonates salts are supposed to dissolve, being the carbonates mostly present in the form of carbonic acid. Therefore, HCl was used to reach a pH of 3 or lower, whereas trials were also run with acetic acid (AA) reaching a pH of 4.

Another option to dissolve salts is the use of a chelating agent. In the present case, EDTA was selected: it was first dissolved in MilliQ water and then used in an amount equimolar to the carbonates supposedly present in the water analysed.

The experimental conditions tested are shown in table 1

Table 1 Experimental conditions for pre-treatment evaluation

| Chemical | Value/Dosage | Time |
|----------|-------------------------|-------------------|
| HCl | pH 1-4 | Few minutes – 12h |
| AA | pH 4 | Few minutes – 12h |
| EDTA | Equimolar to carbonates | Few minutes – 12h |

2.3 Filtration

Filtration was the chosen method for MPs separation. The vacuum filtration system was all glass made, except for the rubber gasket. The silicon filter was squared with a length of 10mm, whereas the gasket internal diameter was of 9mm. This introduced a first challenge: optimal positioning of the gasket to avoid water dragging and loss of particles. Issues related to filtration are:

- time needed;
- formation of bubbles due to the high concentration of carbonic acid, its equilibrium with dissolved CO₂ and the local difference in pressure at the pores. The formation of bubbles causes a non-homogeneous distribution of the particles on the filters that strongly hinders the reliability of the ICQ phase;
- the choice of an appropriate operative volume to avoid a too crowded filter.

2.4 IQC

The Identification Quantification and Characterization phase begins with the image acquisition. Indeed, to automate the process as much as possible, to guarantee a lower relevance of the operator and to lower the time requirements for the analysis, the particles to be analysed are selected on the filter through image analysis, selecting a threshold either by an algorithm or by the operator. The first choice to make was the magnification to use: this affects the resolution of the image, the stability of the focus, the opportunity to use the autofocus, the time of the image acquisition. It has to be noted the relevance of the use of proper focus distance to obtain quality spectra. The presence of particles of significantly different dimension from average is to be avoided as they can both confuse the image analysis algorithm and anyway end up out of focus. Another aspect often overseen is the possible movement of the filter on stage as that of the particles on the filter; these aspects gain relevance with the decrease of the particle size analysed and might define the lowest particle size analytical limit, as the stitching error for the tiles (pictures taken by the microscope) that composes the mosaic of the whole image to be analysed.

Once the image is acquired and the coordinates of the particle of interest defined, the laser is directed on those. The parameters of relevance are: laser wavelength, laser power, time of acquisition and repetition of acquisitions. It is well known that the main issue of the Raman technique is the high risk of fluorescence, which can be slightly decreased by the use of different lasers. However, the time requirements linked to this option, make it not suitable for routine MPs analysis. Therefore, one wavelength has to be chosen, and the other parameters decided as best.

The acquired spectra are then compared to reference libraries, and the outcome of the identification depends both on the library used (the reference spectra present in terms of pure polymers, blends, pristine and aged MPs) and on the comparison method used. The method “correlation” is the mostly used in literature, with an identification threshold of 60-70%.

2.5 QA/QC

Due to MPs ubiquity, it is of utmost relevance to carry on negative controls to check for contamination. It is also of utmost relevance to carry on “positive controls” as recovery rates with reference material. If the numbers reported as results for MPs content are corrected by blanks results, the way and reasoning behind it should be carefully described, considering the full MPs spectrum: size, polymer, shape.

Blanks were evaluated with ultrapure water type 1 from Arius VF pro by Sartorius. Recovery rates were initially evaluated thanks to the reference material provided by Quasimeme and using green Polyethylene microspheres acquired from Cospheric with size $>75\mu\text{m}$.

3 Results and discussion

3.1 Pre-treatments

The tested pre-treatments to analyse particles $>30\mu\text{m}$ (using hence a 10x magnification) showed all comparable results. The pre-treatment choice is hence dictated by practical observations. The use of AA was hindered by the sporadic formation of a film on the $1\mu\text{m}$ filter due to the interaction between AA and substances present in water or possibly with the ethanol used to speed up the filtration. The use of AA is hence still deemed of potential interest, but was discarded due to practical aspect to be yet clarified. EDTA also showed a similar effect as HCl in reducing the particles on the filter; however, it might also interact with the ethanol used to speed up the filtration, precipitating on the filter therefore losing efficiency. The beneficial effect on the particles present on the filters thanks to the use of HCl at a pH of 3 for 15min is shown in Figure 1.

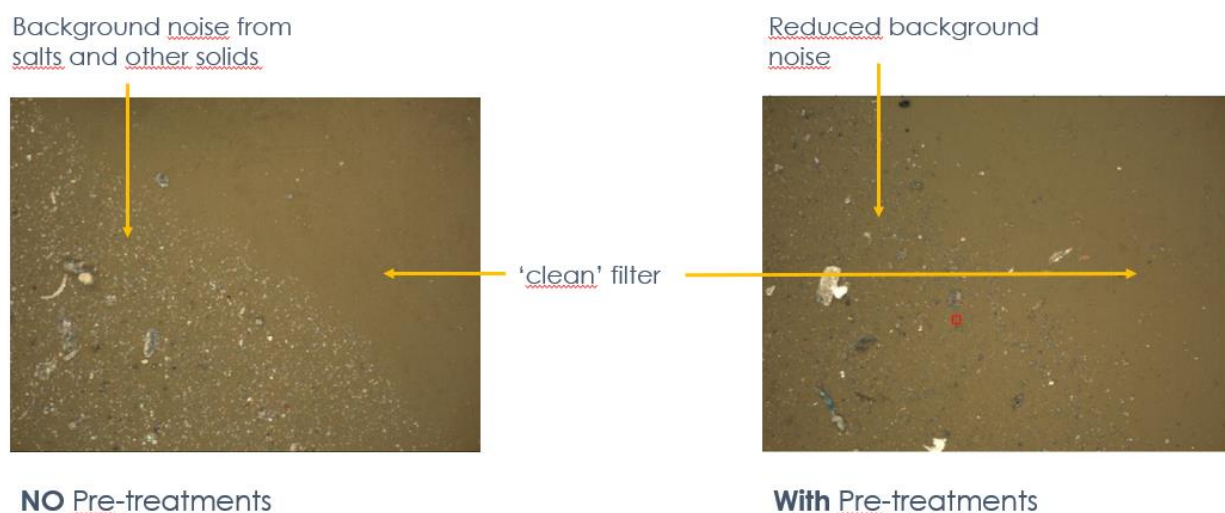


Figure 1 Effect of pre-treatments by HCl

Regarding the choice of pre-treatments for MPs <math><20\mu\text{m}</math>, preliminary trials showed better efficiencies based on duplicates for HCl compared to EDTA, with the latter showing about 25% particles more than the former. The particles were counted by the automatic algorithm implemented in the image analysis software of the DXR3, with a 50% magnification, on 25 windows of size 4*FOV (Field of View), chosen randomly on the filter by an algorithm designed on the software R studio. The choice of the windows is dictated by the time available for the analysis. However, these data might be hindered by the high presence of small particles that were later on discovered being released from the glassware.

Therefore, also for lower particles size, the pre-treatment of choice is HCl, although trials are ongoing to test the effect of such pretreatment on the MPs of smaller size range.

3.2 Filtration

Regarding the filtration phase, the time was significantly reduced by filtering a small amount of ethanol before the sample. This procedure was instead not required when using filters with nominal porosity of $5\mu\text{m}$.

The presence of bubbles was reduced by mixing or gently shaking the sample before filtration, allowing the extra gas produced during the pre-treatments to be released. Bubbles were instead not formed when using the $5\mu\text{m}$ filters. The optimal volume to be filtrated on one filter in the present case was observed to be not exceeding 500mL, to avoid filter overcrowding.

3.3 IQC

It was noted how the automatic algorithm implemented in the software got confused in the presence of a clean filter (mostly when using the 10x magnification), or in the presence of relatively large particles. Therefore, it was decided to use an operator selected threshold, based on two factors: the position of the inflection point of the image intensity histogram (image intensity is the parameter used by the algorithm for thresholding) and the image intensity value that allows the non-selection of background filter points. The comparison of particle numbers was in this case highly hindered, but it allowed for a consistent analysis methodology.

The relative movement of the particles on the filter and the stitching error linked to the filter stage minimum step size, did not allow for the automatic analysis of MPs under $5\mu\text{m}$. This obstacle can

be overcome by using a very precise motorized stage (figure 2). The analysis was anyway possible with an “point and shoot” approach, where the point of interest was chosen one by one manually by the operator. However, this procedure may be considered acceptable for research purposes, not for routine goals.

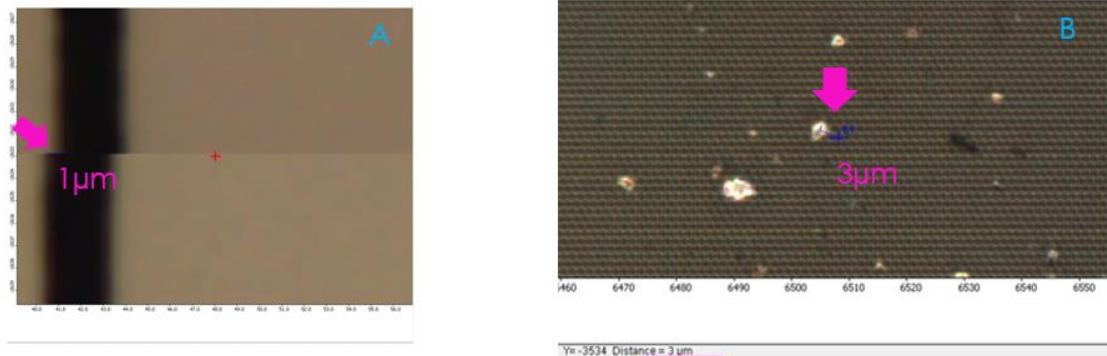


Figure 2 A stitching error, B relative movement of particle. 50x magnification

An acceptable focus must be set on average for all the particles analysed. The use of autofocus is not recommended due to its sensibility to the presence of slightly out of size particles and the time required to acquire the image.

The parameters for proper spectra acquisition are to be set as average for routine goals, whereas the chance of using optimizing tools based on the Signal to Noise ratio can be of high advantage for research purposes. The performance of an average set of parameters compared to optimal ones for each particle is still to be assessed.

3.4 QA/QC

Blank results showed random presence of Polyethylene and Polypropylene particles, in an amount comparable to that of the preliminary results obtained with drinking water; therefore, for the presentation of the results to come, it was decided not to apply blank correction.

Regarding recovery rates, the results initially obtained with material acquired by Quasimeme were not considered satisfying, being the recovery rates lower than 60%; therefore, a rinsing stage after filtration was included. More trials with Cospheric microspheres are ongoing.

4 Conclusions

MPs analysis by μ -Raman proved to be challenging due to the many sources of errors and uncertainties that must be necessarily addressed in establishing the optimal protocol. Some errors might be reduced by carefully improving the practical aspects of the analysis; however, it is of utmost relevance to quantify the uncertainties of the number given as a result of the analysis, coupled with a complete description of the protocol used. In this context, it is highly recommended to take as a reference the proposed check index by Cowger et al [2].

Considering the issues reported in this report, for routine monitoring, a set of three different protocols have been conceived and are going to be developed:

- 1) 10X magnification, screening routine ($> 30 \mu\text{m}$)
- 2) 20X magnification, specialistic ($10\text{-}30\mu\text{m}$)
- 3) 50X magnification, highly specialistic ($5\text{-}10\mu\text{m}$)

For practical reasons, the use of 5µm silicon filters appears more convenient considering the need of an automated analysis for routine monitoring, as the use of HCl pre-treatments. Next research activities will include the evaluation of the effect of the chosen pretreatment on MPs integrity for smaller size ranges, and the screening of DWTPs with the developed method.

References

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- [2] W. Cowger *et al.*, *Reporting Guidelines to Increase the Reproducibility and Comparability of Research on Microplastics*, vol. 74, no. 9. 2020.