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METHODOLOGY **Open Access**

A rapid method to quantify sub-micrometer polystyrene particles in aqueous model systems by TOC analysis

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Abstract

For several laboratory experiments with microplastics, a simple and fast quantification method is advantageous. At the same time, the requirements are often lower compared to microplastic detection from environmental samples. We determined the concentration of non-purgable organic carbon of polystyrene (PS) particles (diameter 0.5, 1, 2, 6 µm) in suspension with known concentrations. Commercially available PS particles were used to test the Total Organic Carbon (TOC) analyzer method for quantifying microplastics in the lower micrometer range under absence of other organic compounds. Addition of iron or aluminum hydroxide to the samples prior to the measurement increased the recovery from 52.9 to 89.7% relative to measurements in the absence of metal hydroxides. With increasing particle size, the recovery in the presence of iron hydroxides decreased from 95.1% at 0.5 µm to 67.1% at 6 µm PS particles and in the presence of aluminum hydroxides from 92.6% at 0.5 µm to 88.9% at 6 µm PS particles. We conclude that metal hydroxides have a catalytic effect on the thermocatalytic oxidation of PS particles and allow a complete conversion to CO_2 for a successful quantification of PS particles using a TOC analyzer. Especially for particles larger than 0.5 µm, in the absence of metal hydroxides, the TOC device is not able to fully oxidize the PS particle to CO_2 and subsequently detect its concentration. Thus, TOC analysis of PS particles in the presence of metal hydroxides provides a cheap and simple alternative for quantifying microplastic particles in the lower micrometer range for laboratory experiments (e.g. sedimentation studies) where no other organic substances are present.

Keywords Microplastic quantification, Catalytic effect, Metal hydroxide, Iron, Aluminum, Thermocatalytic oxidation

Introduction

More than 20 million tons of plastics ended up in the environment in the year 2019 [1]. By now, plastic particles have been found in nearly every environmental compartment [2–4], even at most remote places such as Antarctica and the deep sea [5–7]. Microplastics (MP), plastic particles < 5 mm, are of particular concern as they can be taken up by organisms and cells [8–10]. Quantification of MP particles, especially in the sub-micrometer range, remains a challenge. To estimate the amount of microplastic pollution in the environment, several identification techniques are used, such as Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, and gas chromatography - mass spectroscopy (GC-MS) [11]. Each of these techniques has advantages but also limitations (such as detection limit, lower-size limit of detection, complex sample preparation, analysis time,

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accuracy, high measurement costs, availability of equipment) [11].

Here, we are presenting a rapid method for quantification of MP in model systems based on the determination of the organic carbon content that allows to generate experimental data within a short time (approx. 10 min per sample with two measurement repetitions). Under well-defined laboratory conditions, the analytical demands for a method are often lower, e.g. if type and size of plastic as well as other substances present in the sample are known. An example of such experiments are sedimentation studies of colloidal MP after interaction with inorganic environmental particles [12]. In samples where MP particles represent the only organic compound, their concentration can be determined by the carbon content of the sample. Total Organic Carbon (TOC) Analyzers are well established for the quantification of dissolved (DOC) and particulate (POC) organic carbon in aquatic samples [14–18]. Yet, application of a TOC Analyzer for quantification of MP particles is rare [12, 19] even though it allows quantification of MP particles in colloidal size range as no lower-size limit exist. Hong et al. [19] used a TOC method with solid sample combustion at 900 °C to quantify MP (45–500 µm) in sewage samples after removing other natural organic matter (NOM) by digestion with Fenton's reagent. The recovery for six different types of plastic particles ranged between 76% and 98% [19]. In contrast, recovery of the determination of 1 µm polystyrene (PS) beads used as a POC source in an interlaboratory study on the determination of POC were unsatisfactory with an average of 43.3% [14].

In a previous work, we used a TOC method (680 °C high temperature catalytic oxidation with Pt catalyst) to determine the PS concentration in aquatic samples from sedimentation experiments [12]. Addition of ferrihydrite, a naturally occurring iron (oxy)hydroxide, enhanced recovery of TOC measurements to 92.7% relative to samples without ferrihydrite with a recovery of 34.7%. These findings suggest that addition of ferrihydrite has a catalytic effect on the conversion of PS to CO_2 [12] and that the poor recoveries observed for PS particles in absence of such a catalytically active metal (oxy)hydroxide [12, 14] reflect incomplete oxidation of PS particles to CO_2 during the TOC measurement.

In this study, we have therefore systematically studied the effect of iron (Fe) and aluminum (Al) hydroxides on the recovery of differently sized PS beads (0.5-6 µm) using a TOC detection method. We selected PS as model polymer material as it is commercially available in many different sizes in the sub-micrometer range.

Methods

Material

For our experiments, we used spherical PS particles with diameters of 0.5, 1, 2, and 6 µm (Polysciences Europe GmbH). For each particle size stock solutions of 100 mg/L were prepared by diluting the original dispersion with ultrapure water (18.2 MΩ cm).

Synthesis of Fe hydroxide (6-line ferrihydrite) was done following the procedure described by Cornell and Schwertmann [20]. Two liters of ultrapure water were heated to 75 °C and 20 g of unhydrolyzed $Fe(NO_3)_3 * 9\ H_2O$ crystals were added while rapidly stirring the solution. The mixture was placed in an oven at 75 °C for 10 min and then quickly cooled by plunging it into ice water. After 30 min, the solution was transferred to dialysis bags and left in distilled water for several days with the water being exchanged multiple times until the conductivity was less than 5 µS/cm. Once the conductivity reached this level, the reaction product in the dialysis bags was transferred to a storage container and kept at 4 °C.

For the synthesis of Al hydroxide, 40 g $AlCl_3 * 6\ H_2O$ were diluted in 0.5 L ultrapure water. By adding 30% NaOH, the initial pH value of 2.6 was adjusted to 6.5. Then the mixture was stirred for 1 h. The pH value was checked during this time, but it remained stable without further addition of NaOH. Afterwards the mixture was transferred to dialysis bags and left in distilled water for several days. Analogous to the synthesis of ferrihydrite, the water was exchanged multiple times until the conductivity was smaller than 5 µS/cm. Once the conductivity reached this level, the reaction product in the dialysis bags was transferred to a storage container and kept at 4 °C.

TOC measurements

To determine the concentration of PS, we analyzed the samples with a TOC-L-Analyzer (Shimadzu). By measuring the non-purgeable organic carbon (NPOC) of each sample, the PS concentration can be calculated. As all carbon (C) in PS is present as organic C and non-purgable, this method is suitable to determine the entire C content of PS.

In brief, during NPOC measurement, the TOC-device and its tubes are firstly washed with the sample solution. Afterwards the actual sample (1 mL) is taken, acidified, and purged with synthetic air (O_2:N_2=20:80) for 1 min at 80 mL/min. Originally this step is done to remove any inorganic C from the sample. In our case however, this is also used to re-suspend the sample directly before 50 µL of the sample are injected to the oven. With the carrier gas (O_2:N_2=20:80), the sample passes through the oven (680 °C) where a Pt/Al_2O_3 catalyst is present to convert all C to CO_2 which is subsequently measured with a CO_2 specific non-dispersive infrared (NDIR) detector. The entire procedure from sample uptake, purging, and measurement is repeated. In case the relative standard deviation of the first two measurement results is larger than 2%, a third sample is taken.

Sample preparation

The differently sized PS particles were analyzed at varying concentrations between 0 and 15 mg/L in the absence and presence of 10 mg/L Fe or Al hydroxides. The concentration of 10 mg/L Fe or Al hydroxides was chosen based on additional experiments that showed that this concentration is sufficient for successful TOC measurements (Supporting Information). All samples were diluted with ultrapure water (18.2 MΩ cm) to a volume of 10 mL.

Approx. 10 min before the measurement, we acidified the samples with 2 M HCl. Furthermore, the samples were pre-treated in an ultrasonic bath for 5 min to dissolve any formed aggregates (which may form especially in samples containing Fe or Al hydroxides). To make sure that any sedimentation of particles does not affect the measurement, the samples were placed on a magnetic stirrer during analysis instead of using the auto-sampler of the instrument. For all samples, duplicates were prepared and measured.

Data analysis

For each analyzed set of PS samples, a linear regression between initial PS concentration and measured C concentration was calculated and compared to a reference value β_{ref} accounting for the total C concentration to be expected based on the C content of the PS. The mean stoichiometry of PS is $(C_8H_8)_n$ so that $\beta_{ref}=0.923$.

Furthermore, the recovery in percent was calculated. This value represents how much of the expected C ($NPOC_{ref}$) of a PS sample was actually measured with the TOC Analyzer ($NPOC_{measured}$). For one specific sample, it was calculated using the equations (1)– (2). The units of NPOC and PS concentrations are mg/L. For one complete dataset (e.g. 1 µm PS particles without addition of metal hydroxide) the total recovery was calculated by dividing the factor of the linear regression (β) of the corresponding dataset by $\beta_{ref}=0.923$ (equation (3)).

$$c(NPOC_{ref}) = 0.923 * c(PS) \tag{1}$$

$$recovery\ \% = \frac{c(NPOC_{measured})}{c(NPOC_{ref})} * 100 \tag{2}$$

$$total\ recovery\ \% = \frac{\beta}{\beta_{ref}} * 100 \tag{3}$$

Data was analyzed and figures were created using R 3.6.3 / RStudio 2022.12.0.

Results and discussion

NPOC concentrations of PS samples measured with the TOC Analyzer in the absence of metal hydroxides vary between the differently sized PS particles. For all particle sizes, the measured C concentration is below the expected one for PS concentrations larger than 0.1 mg/L (Fig. 1a). However, depending on the diameter of PS particles, the difference between measured and expected C concentration varies highly. While this difference is rather low for the smallest PS particles (total recovery = 89%), it increases as the particle size increases from 0.5 to 6 µm. For 1 and 2 µm PS particles, the total recovery is approx. 50% and for the largest analyzed PS particles (6 µm), only approx. 25% (Fig. 1a + d). Standard deviations of mean NPOC concentrations are very high for particle sizes between 1 and 6 µm (Fig. 1a). This indicates strongly varying and thus unreliable measurement results for these particles. Similar results were observed previously. Aiken et al. reported low recoveries for TOC measurements of different sources of POC. For PS beads the recoveries ranged from 1.4 to 115.8%, for dried bacteria from 9.4 to 132.5%, and for leaf material from 53.6 to 99.8% [14].

Based on the low recovery, we assume that the PS particles are not completely oxidized to CO_2 during the high-temperature catalytic oxidation in the instrument. Interestingly, with increasing concentration the ratio between expected and measured C concentration for one particle size does not increase. This implies that the concentration of PS (at least in the tested range) is not a limiting factor for a complete oxidation of PS particles to CO_2 in the TOC analyzer. Instead, we assume that the particle size is the limiting factor as the recovery decreases with increasing particle size (Fig. 1d). This means that only a certain fraction of each PS particle is converted to CO_2 during the oxidation process of the NPOC measurement and subsequently quantified with the NDIR detector. The remaining, not fully oxidized part of the particle (which increases with particle size) is thus responsible for the low recoveries of NPOC measurements. With increasing particle size, the surface-to-volume ratio of PS particles is decreasing (Fig. 2). The degradation of small plastic particles proceeds faster compared to larger ones due to a higher surface-to-volume ratio [21, 22]. Therefore, we assume that this parameter is also responsible for the more effective oxidation of smaller PS particles because a larger surface area is available for chemical attacks (Fig. 2).

Addition of metal hydroxides to PS samples improves the recovery of the measurement substantially. After addition of 10 mg/L Fe or Al hydroxide to the samples, the recovery of 0.5-2 µm PS particles only slightly deviates from the expected C concentration (Fig. 1b-c). The total recovery increased for all particles sizes and was

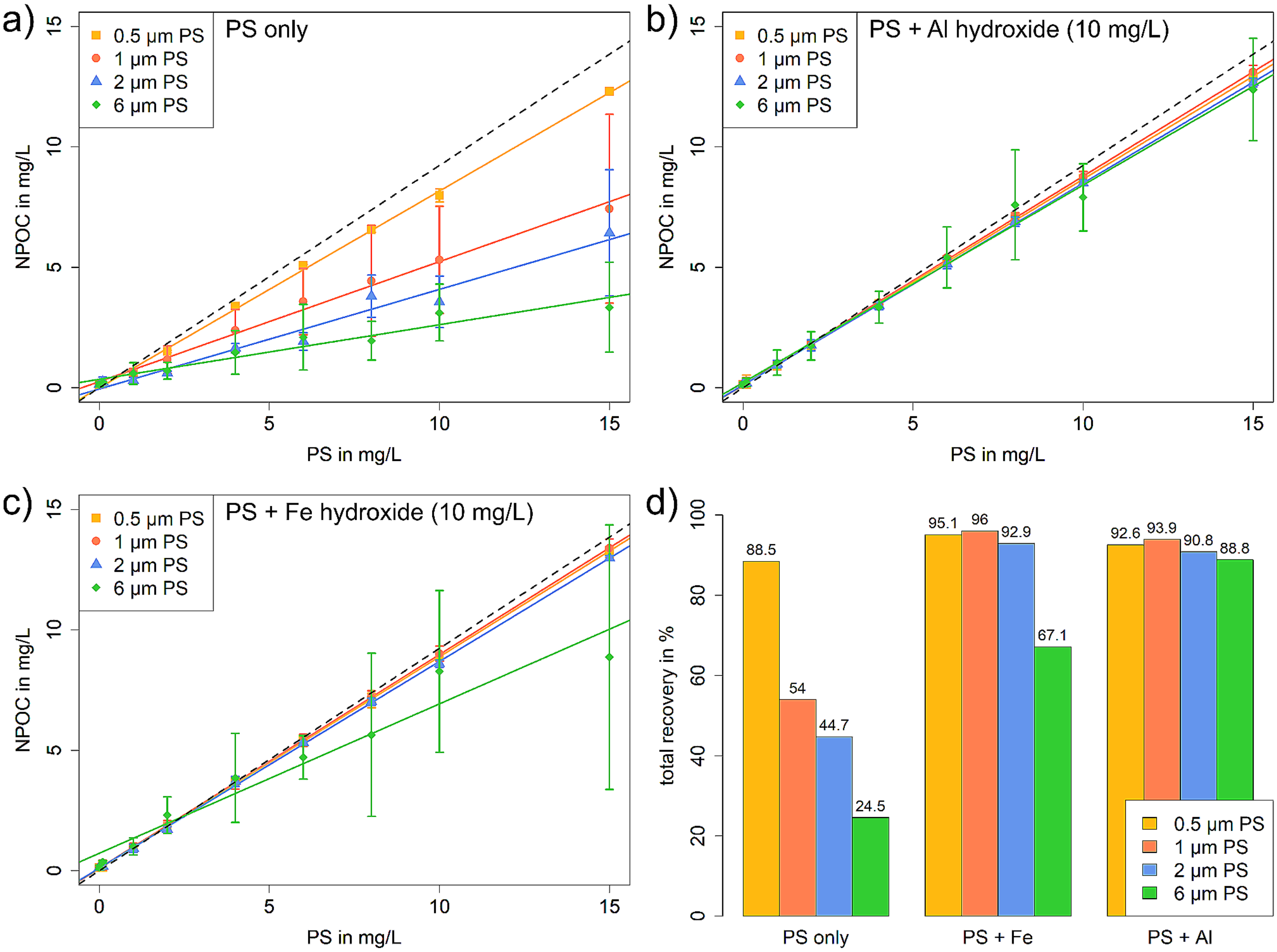

Fig. 1 Mean measured NPOC concentrations of PS samples plotted against the PS concentration: **(a)** in absence of metal hydroxides, **(b)** in presence of 10 mg/L Al hydroxide, and **(c)** in presence of 10 mg/L Fe hydroxide. The error bars represent the standard deviation of the mean. The dashed black line represents the reference NPOC concentration considering the mass percent of C in PS (92.3%). **(d)** Total recovery in percent for each particle size in presence and absence of Fe or Al hydroxide to the PS samples

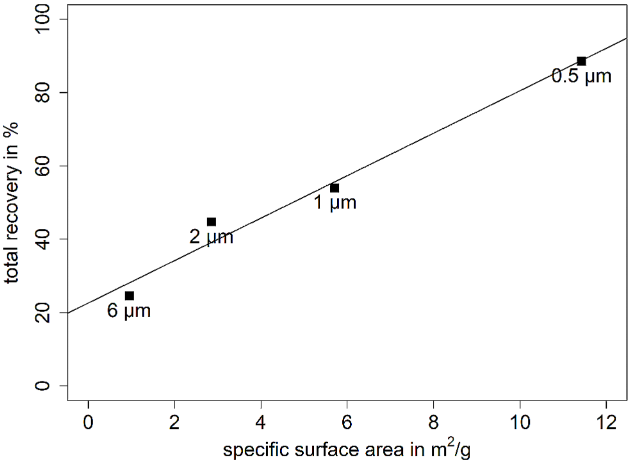

Fig. 2 Recovery of PS particles in absence of metal hydroxides in dependency of the calculated specific surface area (SSA) of PS particles

above 90% for 0.5-2 µm PS particles (Fig. 1d). For the largest particles (6 µm), the total recovery increased to 89% in the presence of Al hydroxide and to 67% in the presence of Fe hydroxide. High standard deviations of mean NPOC concentrations were observed for this particles size, especially for the set of particles that were amended with Fe hydroxide. In contrast, the high standard deviations observed in the samples with 1 and 2 µm PS particles without addition of metal hydroxides, were clearly reduced after addition (Fig. 1a-c). For 1 µm PS, the coefficient of variation was 0.42 without addition of metal hydroxides and was reduced to 0.23 and 0.19 after Fe and Al hydroxide addition, respectively. For 2 µm PS, the coefficient of variation was 0.36. After addition of Fe and Al hydroxides, it had been decreased to 0.18 and 0.21, respectively. These findings indicate that not only the recovery of NPOC measurements increased with addition of metal hydroxides, but also the precision of the measurements. It appears that the presence of metal hydroxides in the samples catalytically accelerates the combustion to CO_2 and that the catalyst in the TOC device is not sufficient for the conversion of MP particles and other organic particles [14] to CO_2.

In the TOC analyzer used in this study, Pt is used as catalyst, a common noble metal catalyst for the oxidation of organic compounds. However, studies on the thermocatalytic degradation of organic compounds like VOCs found that not only precious or noble metals can act as a catalyst for the oxidation of VOCs but also metal oxide-based catalysts (e.g. Mn, Fe, Al) [23]. The catalytic activity of Pt/Al_2O_3 to oxidize CO at room temperature was enhanced by deposition of Fe-oxide on Pt/Al_2O_3 at room temperature [24]. We therefore conclude that the Fe and Al hydroxides used in this study increase the catalytic activity and allow for fully oxidation of particles and their subsequent TOC measurement. However, an upper limit in particles size seems to exist as indicated by the relatively low recoveries and high standard deviations found for 6 µm particles despite the addition of metal hydroxides.

Conclusions

Without the addition of Fe or Al hydroxides, PS particles were only incompletely oxidized to CO_2 and consequently incompletely quantified using TOC analysis. The presence of metal hydroxides strongly improves the recovery of the determination of PS concentration with TOC analysis. We therefore propose that TOC measurements are a fast, simple, and cheap alternative to commonly used methods for MP quantification in experiments studying the environmental behavior of MP in the sub-micrometer and nanometer range. We recommend the application of this method for samples in which no other organic substances are present, except for MP. It remains to be tested whether in cases where NOM is present, removal of NOM by Fenton reaction may be a useful technique to expand the proposed TOC method also to more complex systems including other organic substances. In this study, we showed the applicability of TOC analysis for PS particles, as they are commercially available in the applied size range. The availability of polymers other than PS in the lower micrometer range is currently very limited. Therefore, it remains to be tested in future studies whether the presented method is also suitable for other types of polymers.

Abbreviations

MP	Microplastic
FTIR	Fourier-transform infrared spectroscopy
GC-MS	Gas chromatography - mass spectroscopy
TOC	Total organic carbon
POC	Particulate organic carbon
DOC	Dissolved organic carbon
NOM	Natural organic matter
PS	Polystyrene
NPOC	Non-purgable organic carbon
NDIR	Non-dispersive infrared
SSA	Specific surface area
VOC	Volatile organic compound

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43591-024-00080-y.

Supplementary Material 1

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Author contributions

JS performed the experiments, analyzed and interpreted the data and was major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets generated and analysed during the current study are available in the zenodo repository: https://doi.org/10.5281/zenodo.10350414.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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