Method Article

Hydrophobicity–water/air–based enrichment cell for microplastics analysis within environmental samples: A proof of concept

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A B S T R A C T

The analysis of microplastics in sediments, soils or beach samples is commonly paired with a separation step to enrich microplastics or to remove non-plastics, respectively. Those steps are often very time consuming and are performed in presence of high concentrated solvents. The latter are also suspected to corrode or decompose the analyte particles, which hamper further identification processes. This paper describes a new fast and effective microplastics separation apparatus for analytical issues that was based on hydrophobic adhesion of microplastics and fine air bubbles. The presented prototype could successfully enrich over 90 $\%_{wt}$ of 30ppm$_w$ microplastics in 200 g sand in 20 min. Additionally, it could be demonstrated that the new separation technique was very suitable for further microplastics identification by FTIR microscopy. In this context, a sample with different polymers and matrix components was analyzed and the results were presented within this article.

- *Microplastics were enriched selectively by hydrophobic adhesion.*
- *No additional chemicals except water and air were used.*
- *Separation took only 20 min and 90 $\%_{wt}$ of microplastics were recovered.*

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Specification Table

Subject Area:	*Chemistry*
More specific subject area:	*Environmental Microplastics Analysis*
Method name:	*Hydrophobicity–Water/Air–Based Enrichment Cell For Microplastics (μSEP)*
Name and reference of original method:	*Separation of microplastic particles for analytical issues*
Resource availability:	*Cura:* https://ultimaker.com/software/ultimaker-cura

Introduction

The analysis of microplastics within environmental samples is often complicated by highly variable matrices. On the one hand, non–plastics can accumulate on the microplastics surfaces, which leads to interferences and misinterpretations of the measurement signals [1–3]. On the other hand, non–plastics particles significantly extend analysis times due to an increased measuring and evaluation effort [4,5]. The latter is commonly countered with a microplastics selective density–based separation/enrichment step [4,6]. However, this procedure could be time intensive (more than 10 h per sample) and is commonly applied in presence of highly concentrated density modifiers, e.g., NaCl, $ZnCl_2$, Na_2WO_4 or EtOH [4,6–8]. In this context, a change in the microplastics species and/or additional disturbance effects due to the chemicals used cannot be ruled out. Furthermore, there is no clear distinction between the densities of microplastics and the highly variable matrix components. In the worst case, low dense non–plastics are unintentionally enriched, while high dense microplastics are held back.

This paper describes a hydrophobicity–based microplastics enrichment approach, which is deduced and modified from similar techniques to purify minerals [9,10]. The non–polar microplastics should adhere to fine air bubbles and be transported from the separation system onto a fine filter for further analytical investigations [11]. In this context, a promising microplastics–separator design approach (μSEP), which functions without any additional chemicals except water and air, will be presented and discussed.

Method details

Materials for the μSEP prototype

The developed microplastics separator had to meet special geometric requirements to maximize the recovery and to avoid dead volumes. In this context, the prototype nature required many alterations and customizations. To fit with this challenge, most construction parts were highly individualized and created using a 3D–printer (Ultimaker 3, Ultimaker, Utrecht, NL) fed with an acrylonitrile butadiene styrene (ABS) filament from ICE Filaments, Ham, BE. For further details, all specifications and print parameters can be obtained from Table S1. The printed ABS modules were connected with four poly(methyl methacrylate) (PMMA) tubes sealed with hemp tow and the recirculation of the water overflow was realized via polytetrafluoroethylene (PTFE) hoses. Of course, most of these materials are not suitable for microplastics analysis but fitted the purpose for method/design development.

Microplastics reference sample

The microplastics separator was developed using an artificial microplastics / sand mixture to simulate the microplastics analysis within a real beach sample. For method development 20 mg micro granules of polyoxymethylene (POM), 200 g aquarium sand and a mixture of both were investigated. Additionally, 5 mg micro granules of polyamide (PA), polyvinyl chloride (PVC), polyoxymethylene (POM) and milled shrimp shells (chitin) were mixed with 200 g aquarium sand. Further details can be obtained from Table S2. All sample mixtures were homogenized in a crystallizing dish by stirring with a metal spatula.

Determination of the recoveries

The particles were collected on fine stainless-steel filters with a mesh width of 20 μm. These were weighed after drying at 80 °C for 2 h against the respecting tared mass of the empty filters. In a second step, the estimated net masses were compared with the input sample masses.

Basic concept

If fine air bubbles stream vertically through an aqueous micro particle suspension, it can be observed that hydrophobic particles adhere to the air bubbles. This concept is commonly used during mineral processing but seems to be suitable for microplastics enrichment from soils, sediments or beach samples, because most microplastics consist of hydrophobic polymers. In the simplest case, the sample is mixed with water in a column, and at the bottom of the apparatus there are air stones which release fine air bubbles. The rising bubbles transport the microplastics to the water surface, where they can be extracted by overfilling the vessel [4].

However, the hydrophobicity–based microplastics separation takes place at the boundary phase of air bubbles and microplastics [12,13]. In common approaches the sample was introduced to the separation column as a loose bulk, where microplastics were surrounded by sand particles, which suppressed a free flow of air bubbles and hampered the microplastics moving. In consequence, the recoveries of microplastics decreases significantly, which could be demonstrated by Imhof et al. [4]. Therefore, the sample was not fed all at once, but finely trickled against the air bubble flow, which can be seen in Fig. 1.

To increase the separation yield, the sample was introduced into the air bubble flow not only once, but multiple times in a cyclic process. This was realized using a bypass to transport the already sedimented particles upwards again. This approach substituted continuous circulation of the suspension by a stirrer, which would place high mechanical stress on the sample. To ensure that particles were not damaged during transport, a secondary air stream consisting of large bubbles was introduced in the bypass. The air bubbles filled the diameter of the bypass and pushed the suspension upwards in a frictionless way. Henceforth, the terms separation bubbles and transport bubbles are used for a clearer distinction of the two different kinds of air bubbles.

During the separation process, the microplastic particles are attached to separation bubbles of the primary air stream and are transported upwards to the overflow. Passing this part, all particles are collected within a filter cascade for further analysis, e.g., by infrared microscopy. Sand particles are not attached to the air bubbles and remained in the cyclic transport process. The water passing through the filter cascade is returned to the bypass to keep the water level constant. In summary, the whole microplastics separator consists of two connected loops. In the sample loop, the particles sedimented
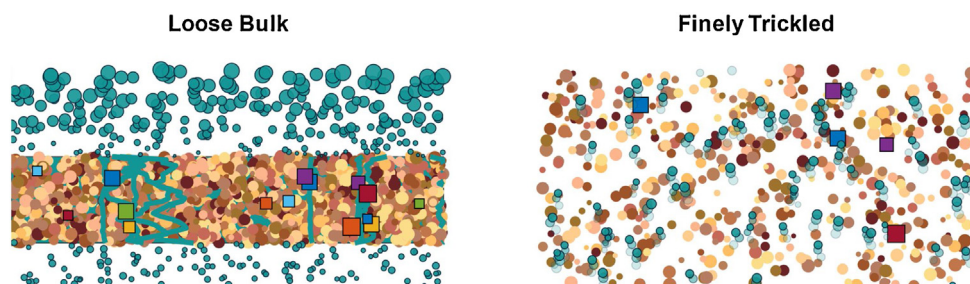
Loose Bulk   **Finely Trickled**

Fig. 1. Schematic comparison of sample introduction as loose bulk or finely trickled. As the air bubbles (cyan) pass the loose bulk, they combine, which decreases the total effective contact surfaces significantly. Moreover, microplastics (colorful) are completely enclosed in the sand bulk (brown), which hampers the air bubble assisted particle transport. All these disadvantages cannot be observed introducing the sample by fine trickling, as there is much more space between the individual particles.

and are transported upwards again. The separation bubble stream conveyed the microplastics into the enrichment loop, where particles are collected on a filter, while the overflowing water is returned to the sample loop again. The described approach is also illustrated in Figs. 2 and S1.

Design of the component geometry

Mainly, the presented microplastics separator consists of commercially available PMMA tubes and four highly customized ABS fitting elements, which can be obtained from Fig. 2.

All individual components as well as the assembled separator showed no inner edges and consisted of a single closed surface. This minimized dead spaces that could otherwise serve as particle sinks. The Y–connector at the bottom part was most challenging and will be presented in detail, while the other components are described in the supporting information, as they are mostly self–explanatory by an engineering drawing.

The Y–connector coupled the separation column with the bypass and had a gas inlet at its lowest point to introduce the transport bubbles. To prevent the air stream from entering the separation column instead of the bypass, there was a serrate backflow preventer implemented. As a result, the particles could only move in one direction and blocked the way back. This increased the back pressure and re-directed the transport bubbles into the bypass. Moreover, proper mixing of transported particles with water in the bypass had to be ensured to avoid agglomeration, which could lead to bypass blocking. Therefore, the overflowing water was returned to the bypass after passing the filter cascade at the upper end of the separation column, which also ensured a constant water level of the whole system. In addition, an interface for a fine bubble diffuser was implemented at the Y–branch that was connected to the separation column. An illustration of the Y–connector is shown in Fig. 3.
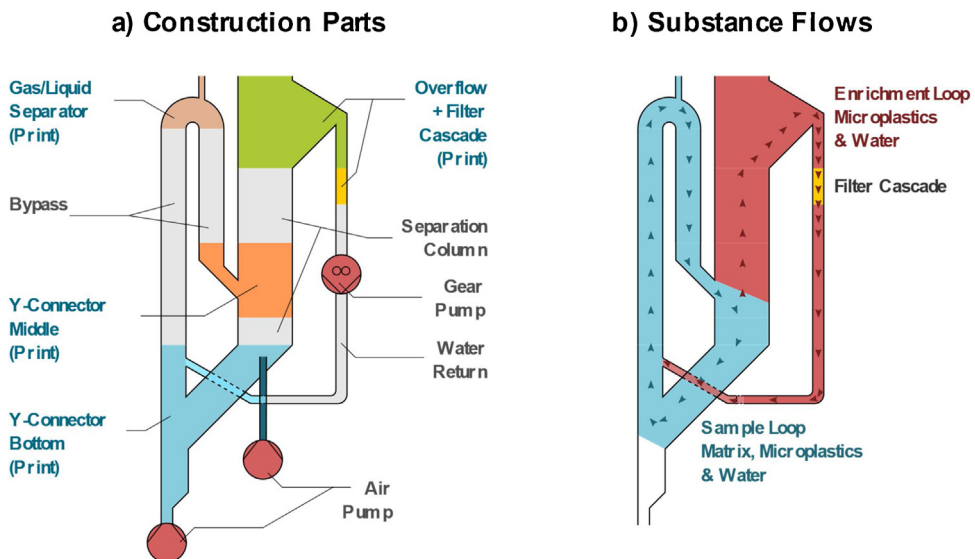

a) Construction Parts   b) Substance Flows

Fig. 2. Scheme of the microplastics separation apparatus. a) The separator consists of four 3D printed parts, which are connected by PMMA tubes (bypass & separation column). Additionally, the outlet of the filter cascade is returned to the bottom part of the separator by a PTFE hose. b) The separator consists of two coupled loops. In the sample loop (blue) the sediment / microplastics / water suspension is circulated using a stream of large air bubbles, which is introduced at the bottom part of the apparatus. In the enrichment loop (red) microplastics were transported upwards by small air bubbles within the separation column. Sand particles will reside in the sample loop, as they do not adhere to the small air bubbles. All particles in the enrichment loop are flushed onto a fine filter for further analysis.
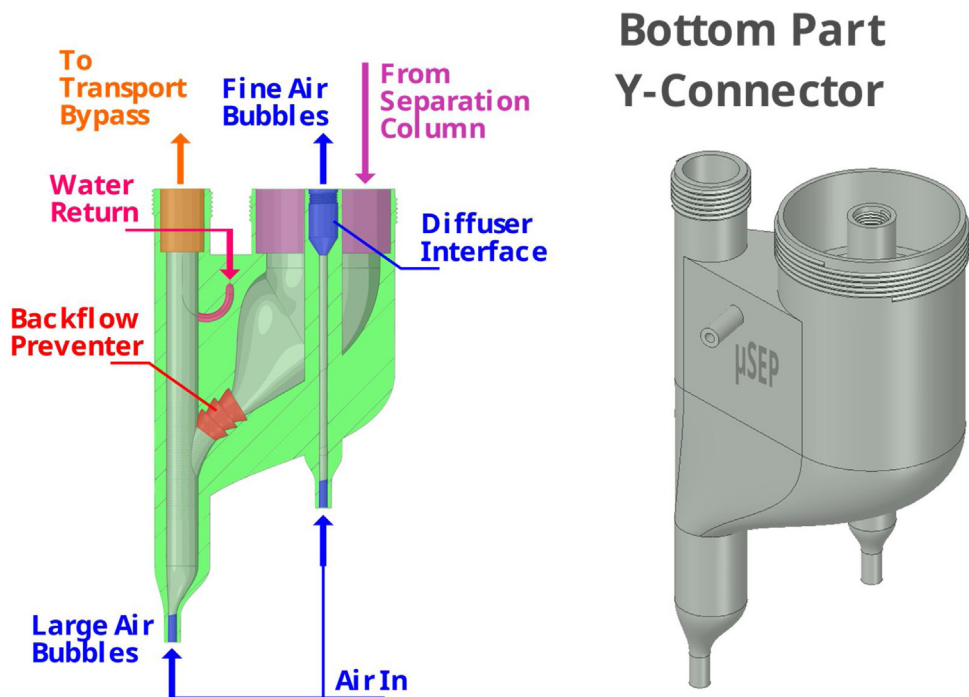
Fig. 3. Scheme of the bottom part of the microplastics separator. This part connected separation column and transport bypass. Additionally, air inlets for separation und transport air bubbles as well as the water return were implemented.

Optimizing the air flow

The presented microplastics separator used two different kinds of air bubbles: for transport and for separation. On the one hand, a very dense air bubble stream can transport particles upwards based on the buoyancy force, which increases with the diameter of the bubbles [14]. This process was used within the bypass of the presented system to convey the whole sample in a loop. However, this phenomenon should not take place within the separation column, as this would not separate microplastics from sand particles. On the other hand, a fine and lower dense air bubble stream can transport particles upwards based on hydrophobic interactions [15,16]. This is the required transport mechanism within the separation column. To that end, the size and density of the separation bubble stream should be optimized for maximum yield of microplastics.

Based on pure sand and POM samples, air bubble volume flows were varied, and the dry masses of the trapped particles were determined after 30 min, which can be seen in Fig. 4a.

The recovery of particles showed a sigmoid dependency to the air flow of the separation bubble stream. However, microplastics were significantly enriched, as their recoveries were ≈50′000 times higher than the recoveries of sand and ranged up to ≈100 %. From the experimental data, an air flow of 24–30 mL/min seemed to be suitable. However, above 26 mL/min a low but significant amount of sand particles was trapped, and therefore, an air flow of 24–26 mL/min could be recommended.

Time resolved separation

The presented microplastics enrichment method is a continuous process. Every microplastic particle in the separation system circulated n times before it was trapped on the filter, which happened with a certain probability. As a result, the amount of collected particles increased over time. However, the presented microplastics separator should be a practical, and therefore, a fast sample
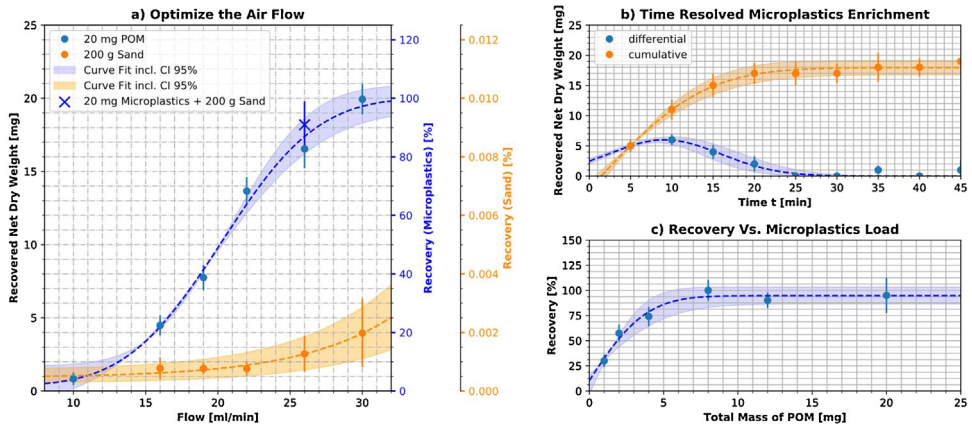
Fig. 4. a) Dependency of air flow and particle recovery on the filter. The asymptotic upper and lower limits can be deduced to the control range of the valve. The separation process took 30 min each. b) Dependency of separation time and particle recovery on the filter. After a separation time of 20 min, the total dry mass did not change significantly. The airflow was set to 26 mL/min. c) Dependency of particle load in the sample and particle recovery on the filter. Above 6 mg of POM, a constant recovery level could be observed. However, below this limit only qualitative analyses were possible. a–c) The recovery curves could be described numerically by a Gaussian sigmoid and its differential function. Error bars based on the standard errors with n = 2.

preparation tool, as a common disadvantage of current microplastics analysis are time intensive processes. To that end, a sample of 200 g sand and 20 mg POM was investigated, and the filter was changed and weighed every 5 min to obtain a time resolved overview of the enrichment process, which can be seen in Fig. 4b.

According to the data obtained, a separation time of at least 20 min is already sufficient for recovery of POM. This experimental result underlines the practicability of the presented microplastics separator.

Recovery

To determine the quantitative micro particle composition, sufficient recoveries on a constant level ranging between 80–120 % should be ensured. Therefore, the recoveries were investigated while the mass load of microplastics (POM) was varied from 1 to 20 mg while the amount of sand remained constant at 200 g, which can be obtained from Fig. 4c.

A statistically sufficiently reliable quantitative analysis was assured for a mass load >5 mg. Below this limit of quantification recoveries decreased significantly which only allowed qualitative statements. One reason for the decrease of the recoveries in case of lower particle loads could be associated with the prototype nature of the separator. In concrete terms, it was observed that particles occasionally adhered to the wall of the apparatus. The amount POM particles adhered did not seem to correlate with the total amount of POM in the sample, and therefore, wall adhesion became more significant for lower concentrations.

Examination of a sand–microplastics mixture

The microplastics separator was used to analyze an artificial sample, which consisted of 200 g sand and 20 mg of a micro particle mixture (PVC, POM, PA and chitin), which can be obtained in detail from Table S2. Considering the performed parameter optimizations, the air flow was set to 26 mL/min and the separation process was stopped after 30 min. The weight of particles collected were 18 mg dry mass (recovery of 90 %). However, beyond total mass the chemical composition of particles had to be checked to ensure that no sand particles and sufficient amounts of PVC, POM and PA were enriched. Therefore, the micro particles were placed as homogenously as possible on a reflecting sample carrier

for FTIR microscopy analysis. This step was necessary to isolate all particles, as they covered each other on the filter, which would hamper the identification of the individual microplastics. A representative partial area (10 × 10 mm) of the sample carrier was investigated by FTIR microscopy, and the recorded FTIR spectra were automatically evaluated using the μIDENT$_{mod}$ algorithm [3]. In this context, significant vibrational bands of every recored FTIR spectrum were automatically integrated by multi curve fitting. The obtained peak areas were transformed into highly characteristic patterns based on peak positions and peak area ratios. For microplastics identification, these patterns were compared with an in-house reference database. Finally, the identification results were transferred to the optical image of the FTIR microscope for false coloring. The results of this mapping experiment are shown in Fig. 5.

From the results of the microplastics mapping it can be inferred that all three types of microplastics were enriched on the filter, while sand particles were not observed. This underlines the microplastics separator's suitability for different polymer types. Additionally, ABS or PMMA from the separator itself was detected. Chitin as a representative non–plastic matrix components was also collected on the filter, which was not astonishing due to its very low density. These kinds of materials do not sediment in water but float on the water surface.

Looking at the chemical compositions of the particles based on the calculated weights, PA was underestimated. However, 59 % of the 595 particles detected were identified as PA. On the one hand, a reason for this phenomenon could be the critical process of transferring the collected particles from the filter onto the FTIR sample carrier. Particle size discriminating effects could not be excluded. However, this task was not in the focus of this paper and has to be investigated in further research projects. On the other hand, the calculated micro particle weights were associated with large statistical uncertainties. These were based on the equivalent spherical volumes, which were estimated from the two dimensional image projections of the particles. Therefore, a precise extent of particle size discriminating effects could not be estimated.

To ensure that no significant amounts of microplastics resided within the sand sample, the separation process was continued for 6 h. The second filter (net dry mass: 0,47 mg) was inspected using an optical microscope, but there were no significant numbers of particles observable.
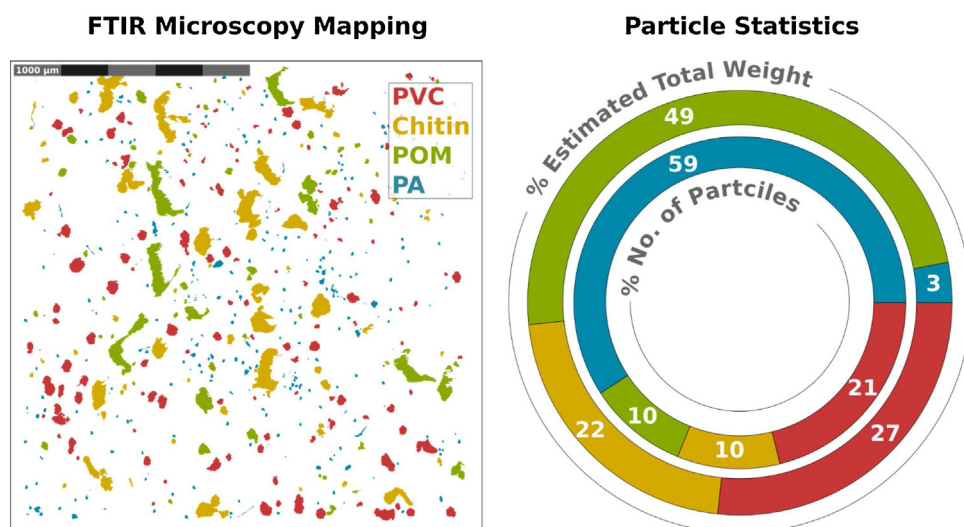

Fig. 5. FTIR microplastics mapping of a 10 × 10 mm area. The effective spatial resolution was set to 50 × 50 μm, and the FTIR spectra were measured with a spectral resolution of 16 cm^{-1}. In total, n = 595 *particles* were detected. The chemical composition was evaluated under the consideration of the no. of particles and the estimated weight. The latter was calculated using the summed equivalent spherical volumes of the individual particle areas and the respecting material densities.

Conclusion & outlook

The concept of a fast and efficient hydrophobicity–based microplastics separator (μSEP) was successfully demonstrated. Due to the continuous particle circulation and fine trickling against a stream of upwards moving small air bubbles, the separation process took only 20–30 min, and recoveries of ≈90 % were observed. The presented prototype was suitable for microplastics concentrations down to 30 mg/kg and could handle sample volumes up to 200 g. As no additional chemicals except air and water were used for the separation process, further microplastics analysis, e.g., FTIR microscopy could be performed without any limitations. With all these features, the hydrophobicity–based microplastics separator should be a good alternative to already established sedimentation technique.

However, the current prototype is only conditionally suitable for separating real environmental samples, as the materials of the individual components are made of ABS and PMMA. Although it could be demonstrated that the apparatus was inert in this respect. In this context, the next generation of μSEP is already planned, which will be made of glass. Moreover, the air bubble size or generation process, respectively, within the separation column was not varied yet. For this purpose, (sub)micro air bubble generators (similar to nebulizers) or water decomposition by electrolysis are promising approaches and will be studied in further research projects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mex.2019.11.006.

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