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Method Article

Improving cost-efficiency for MPs density separation by zinc chloride reuse

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A B S T R A C T

The methodology used to extract and quantify microplastics (MPs) in aquatic systems are still not standardized. Salt saturated solutions, such as sodium chloride (NaCl), zinc chloride ($ZnCl_2$) and/or sodium iodide (NaI), are normally added to separate dense plastics from aquatic samples. However, the most effective reagents are also the most expensive (e.g. $ZnCl_2$ and NaI). To decrease this cost, a reuse process of the salt solutions should be applied. The reuse process has been widely investigated for the NaI solution neglecting the $ZnCl_2$. Hence, the aim of this study was to present a simple methodology to reuse the $ZnCl_2$ solution ensuring the efficiency of the product. Results of the present study showed that $ZnCl_2$ solution could be reused at least five times maintaining an efficiency above 95 %.

- The $ZnCl_2$ reuse decreases the cost of the methodology.
- The efficiency of $ZnCl_2$ solution after five filtrations remains above 95 % (all polymers are detected and recovered).
- The use of this salt solution is the most cost-effective methodology to isolate MPs from aquatic samples.

A R T I C L E I N F O
Method name: Microplastics isolation using reused zinc chloride solution
Keywords: Microplastics, Separation methodology, Zinc chloride, Aquatic systems, Reuse process
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Specification Table

Subject Area:	Environmental Science
More specific subject area:	Aquatic science
Method name:	*Microplastics isolation using reused zinc chloride solution*
Name and reference of original method:	*Density separation:* J. Masura, J. Baker, G. Foster, C. Arthur, Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments., 2015. M. Zobkov, E. Esiukova, Microplastics in Baltic bottom sediments: Quantification procedures and first results, Mar. Pollut. Bull. 114 (2017) 724–732. doi:10.1016/j.marpolbul.2016.10.060. M.O. Rodrigues, A.M.M. Gonçalves, F.J.M. Gonçalves, H. Nogueira, J.C. Marques, N. Abrantes, Effectiveness of a methodology of microplastics isolation for environmental monitoring in freshwater systems, Ecol. Indic. 89 (2018) 488–495. doi:10.1016/j.ecolind.2018.02.038. M. Zobkov, E. Esiukova, Microplastics in Baltic bottom sediments: Quantification procedures and first results, Mar. Pollut. Bull. 114 (2017) 724–732. doi:10.1016/j.marpolbul.2016.10.060. *Vacuum filtration:* V. Hidalgo-ruz, L. Gutow, R.C. Thompson, M. Thiel, Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification, Environ. Sci. Technol. 46 (2012) 3060–3075. doi:dx.doi.org/10.1021/es2031505. S. Mintenig, Planktonic Microplastic in the North Sea - A new extraction method for the detection by Fourier Transform Infrared Spectroscopy (FTIR), Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany, 2014. W. Urgert, Microplastics in the rivers Meuse and Rhine. Master thesis., Open University of the Netherlands, Heerlen, Netherlands, 2015. M.O. Rodrigues, A.M.M. Gonçalves, F.J.M. Gonçalves, H. Nogueira, J.C. Marques, N. Abrantes, Effectiveness of a methodology of microplastics isolation for environmental monitoring in freshwater systems, Ecol. Indic. 89 (2018) 488–495. doi:10.1016/j.ecolind.2018.02.038.
Resource availability	Material: • Lab coat; • Gloves; • Volumetric balloon; • Metal spatula; • Density separator; • Magnetic stirrer; • Stir bars; • Aluminium foil; • Forceps; • Glass Pasteur pipette; • Glass bottles/beakers/Erlenmeyer flasks; • Squirt bottle filled with distilled water; • Vacuum system apparatus: Kitasato flask, vacuum pump, sand funnel/glass funnel, rubber bung, rubber tubing, filtering cup, clamp; • 0.45 μm clean membrane filter; • Fridge (4 °C); • Petri dishes; • Oven; • Stereomicroscope; • Analytical laboratory balance; • Fourier-transform infrared spectroscopy (FTIR). Reagent: • Zinc chloride ($ZnCl_2$).

Method details

Background

Microplastics (particles with a diameter of 1–5000 μm [1]; MPs) are emerging aquatic contaminants since they are persistent, can reach high densities and interact with abiotic and biotic environments. However, there is still not a standardized method for isolating MPs in aquatic systems which could result in inconsistent data not allowing spatial and temporal comparison between studies [2,3]. The choice for a simple, low-cost and accurate methodology is one of the main challenges currently. One of the most adopted methodologies is the density separation with a high concentrated or saturated salt solution (e.g. sodium chloride, zinc chloride and/or sodium iodide) [3–10]. These salts are normally mixed with the samples and shaken [3], notwithstanding they are not equally effective. Zinc chloride ($ZnCl_2$) is the most effective (density of 1.6–1.8 g/cm^3) and least expensive method (when compared with sodium iodide; NaI) [5], however the substance is very hazardous, corrosive [9] and requires large amounts of product [7]. Hence, careful handling, disposal and reuse is necessary to guarantee the most efficient and safe use of the product. The reuse process has been widely investigated for the NaI solution [11], while for $ZnCl_2$ there is lack of information. Hence, the aim of this study was to present a methodology for MPs isolation using reused $ZnCl_2$ solution. The methodology proposed focused on the density separation methodology, vacuum filtration and visual inspection, based on [7].

Sample processing

This methodology could be used for MPs isolation (particles with size between 1 and 5000 μm).

Density separation

1 Assembly of the density separator.
2 Add 50 mL of $ZnCl_2$ solution (concentration = 700 g/L) to the dried sample. Note: Stir the ZnCl2 solution during at least 24 h before adding to the sample.
3 Place the sample in the density separator.
4 Rinse the glass bottle with $ZnCl_2$ solution to transfer all remaining solids to the density separator.
5 Cover loosely with aluminium foil.
6 Allow solids to settle 1 h at room temperature.
7 Collect floating MPs into a flask with forceps and a glass Pasteur pipette.
8 Remove the Mohr's pinch clamp. Drain settled solids (if existing) and $ZnCl_2$ solution into a flask.
9 Rinse the density separator several times with a squirt bottle filled with distilled water to transfer all solids to the flask containing recovered MPs.
10 Stir the flask with MPs and the flask with $ZnCl_2$ for 10/15 min.

Vacuum filtration

11 Filter the $ZnCl_2$ solution and settled solids (if existing) through a 0.45 μm clean membrane filter using a sand funnel connected to a vacuum system (*see* Fig. 1). Note: Do not rinse with distilled water, it can cause precipitation of $ZnCl_2$.
12 Store the reused solution in a glass bottle at 4 °C.
13 Filter the plastic samples through a 0.45 μm clean membrane filter using the same filtration process (*see* Fig. 1).
14 Rinse the filtration setup with a squirt bottle filled with distilled water several times to ensure that no MPs were lost.
15 Once filtration was complete, carefully remove the membranes and put them in Petri dishes.
16 Place the Petri dishes in 40 °C drying oven for 3–5 days.

Visual inspection

17 Stereomicroscope *Optika* using 1.5× magnification: select the MPs.
18 Count and/or weigh the MPs (using an analytical laboratory balance).

Identification

19 Fourier-transform infrared spectroscopy (FTIR).

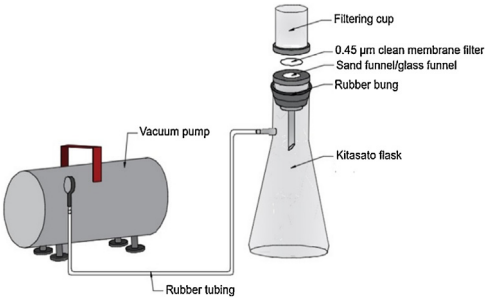

Fig. 1. Vacuum system (figure copyright: Mariana O. Rodrigues).

Contamination mitigation

To prevent MPs contamination during isolation procedure:

- Wear lab coats (preferably 100 % cotton).
- Change gloves between steps.
- Use glass vials during all steps of sample treatment. However, if plastic ware was used instead, cover it with aluminium foil.
- Clean every material and cover on top immediately (with cap or aluminium foil) after washing and after each step allowing the amount of time that a sample is exposed to air is very limited.
- Clean the workplace before and during each procedure.
- Since all polymers were characterized in the beginning of the experiment, any others, especially fibers, are discarded and not accounted for the final number of recovered MPs.

To reuse the $ZnCl_2$ solution, repeat the methodology described above (density separation, vacuum filtration and visual inspection) using the reused solution (solution obtained in step 12) in the second step of the density separation methodology. It is not necessary to stir again the solution.

Methods validation

MPs and sample preparation

Three different plastic products (i.e. water bottles, pipe and fabric) widely used in everyday life were cut and fragmented using a coffee grinder. These particles were sieved in a 5000 µm mesh and material sized >5000 µm was discarded (also named MPs). Based on previous FTIR analysis, each plastic product was characterized by its polymer type [7]. Table 1 summarizes both chemical and morphological characterization of plastic products (*see* Table 1).

These three plastic products included some of the most common and dense types of polymers [12] such as polyvinyl chloride (PVC) and polyethylene terephthalate (PET). Five particles of each type of plastic were added per sample. For each sample three replicates were prepared.

Table 1
Morphological and chemical characterization of the 3 types of plastic products used in this experiment.

Source	Polymer type	Size (µm)	Shape	Colour	Density (g/cm^3)
Water bottles	Polyethylene terephthalate (PET)		Fragment	Blue	1.37–1.45
Fabric		<5000	Fiber	Blue	
Pipe	Polyvinyl chloride (PVC)		Fragment	Grey	1.16–1.58

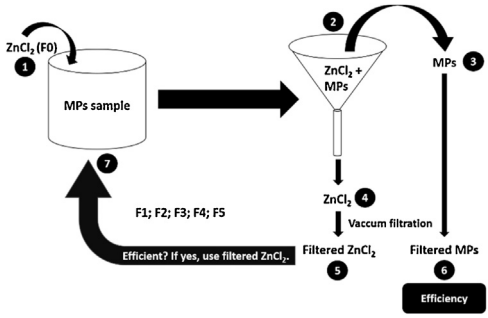

Fig. 2. Experimental setup for the $ZnCl_2$ reuse, based on a density separation methodology: Step 1 – Addition of the $ZnCl_2$ solution to the sample; Step 2 – Density separation; Step 3 – Recovering of MPs; Step 4 – Recovering of $ZnCl_2$ solution; Step 5 – Filtered $ZnCl_2$ (after vacuum filtration); Step 6 – MPs recovered (after vacuum filtration and stereomicroscope inspection); Step 7 – Reuse of the filtered solution in a new sample (after calculation of the solution efficiency). Solutions were reused to a maximum of five times (F1 to F5): F0 = Initial $ZnCl_2$ solution (concentration = 700 g/L); F1 = $ZnCl_2$ solution after being reused once; F2 = $ZnCl_2$ solution after being reused twice; F3 = $ZnCl_2$ solution after being reused three times; F4 = $ZnCl_2$ solution after being reused four times; F5 = $ZnCl_2$ solution after being reused five times (figure copyright: Mariana O. Rodrigues).

Experimental design

Following the methodology described above, samples were subjected to a density separation methodology with $ZnCl_2$, vacuum filtration and stereomicroscope inspection (*see* Fig. 2).

After vacuum filtration (step 5), $ZnCl_2$ was available to be reused. The reuse process was repeated five times, membranes (steps 5 and 6) were recovered and inspected for MPs presence. The MPs recovered were counted for further calculation of the efficiency of initial and filtered/reused solutions (%).

The efficiency was calculated following the equation : $$\text{Efficiency } (\%) = \left(\frac{number\ of\ MPs\ recovered\ on\ top\ of\ density\ separator}{number\ of\ MPs\ added\ in\ sample\ preparation}\right) \times 100$$

.

Since normality test failed, One-way ANOVA on Ranks was used to assess the significant diff ;erences between the efficiency of solutions. The significance level of all statistical analyses was set at 0.05.

Results

- The efficiency of the density separation was not affected by the reuse process (H= 1.289; DF = 5; p *value* = 0.936; *see* Fig. 3).
- All types of polymers and shapes (PVC and PET – fragments and fibers) were recovered from the top of the funnel.
- Fibers (from fabric – PET) tend to deagglomerate making difficult their recovery.
- Pipe (PVC) and water bottle (PET) particles were fully recovered in all treatments.

Hence, results pointed-out that $ZnCl_2$ solution could be reused at least five times with an efficiency above 95 %. Moreover, it is important to note that although this work was done with pristine MPs, there is no scientific evidence that aging or complexation processes change their density and therefore this process can be used for all MPs particles as long as they have a density lower than 1.6–1.8 g/cm^3.

Disadvantage of the reuse process:

- During recovering of MPs, the volume of $ZnCl_2$ solution decreased in approximately 10 %.

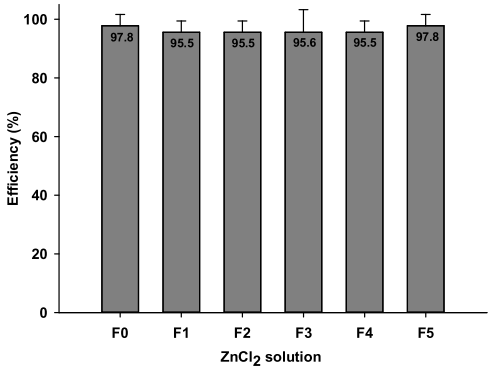

Fig. 3. Density separation efficiency (based on the number of particles recovered) of the initial and reused $ZnCl_2$ solutions (F0 = Initial $ZnCl_2$ solution (concentration = 700 g/L); F1 = $ZnCl_2$ solution after being reused once; F2 = $ZnCl_2$ solution after being reused twice; F3 = $ZnCl_2$ solution after being reused three times; F4 = $ZnCl_2$ solution after being reused four times; F5 = $ZnCl_2$ solution after being reused five times). Each bar has an indication, at the top, of the efficiency (%) of the initial and reused $ZnCl_2$ solutions.

CRediT authorship contribution statement

M.O. Rodrigues: Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **A.M.M. Gonçalves:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **F.J.M. Gonçalves:** Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **N. Abrantes:** Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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.

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