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A straightforward method for measuring the range of apparent density of microplastics



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HIGHLIGHTS

- Bases of ethanol, ultrapure water and saturated Nal are optimal for the density gradient solutions.
- Density gradient solutions are easy to be prepared in a density range of 0.8–1.8 g/cm³.
- Density gradient solutions are feasible to measure the apparent density of microplastics.

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1. Introduction

Microplastic is an umbrella term that covers particles in varies physical and chemical properties, such as density, shape, size and polymer type (Lambert et al., 2017). Studies have demonstrated that certain properties, such as shape and polymer type, play important roles in the uptake of microplastics in aquatic organisms (Graham and Thompson, 2009; Gray and Weinstein, 2017). Therefore, physical and chemical properties of microplastics should be well characterized to

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GRAPHICAL ABSTRACT



ABSTRACT

Density of microplastics has been regarded as the primary property that affect the distribution and bioavailability of microplastics in the water column. For measuring the density of microplastis, we developed a simple and rapid method based on density gradient solutions. In this study, we tested four solvents to make the density gradient solutions, i.e., ethanol (0.8 g/cm³), ultrapure water (1.0 g/cm³), saturated NaI (1.8 g/cm³) and ZnCl₂ (1.8 g/cm³). Density of microplastics was measured via observing the float or sink status in the density gradient solutions. We found that density gradient solutions made from ZnCl₂ had a larger uncertainty in measuring density than that from NaI, most likely due to a higher surface tension of ZnCl₂ solution. Solutions made from ethanol, ultrapure water, and NaI showed consistent density results with listed densities of commercial products, indicating that these density gradient solutions were suitable for measuring microplastics with a density range of 0.8–1.8 g/cm³.

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explore the association between microplastic exposure and toxic effects in organisms (Andrady, 2017; Pottoff et al., 2017).

As an important physical parameter, density of microplastics invariably determines the distribution and bioavailability of microplastics in the water column (Wang et al., 2016). The positively buoyant plastics (with a density less than water), such as polyethylene (PE) and polypropylene (PP), share the upper water column and those plastics get available to zooplanktons, planktivores and filter feeders (Rochman et al., 2014; Li et al., 2016; Qu et al., 2018). Detritivores, benthic suspension and deposit feeders inhabiting benthos are likely to encounter negatively buoyant plastics, such as polyvinyl chloride (PVC) and nylon (Browne et al., 2013; Wright et al., 2013). Densities of plastics are not



Fig. 1. Microplastics (a_1-n_1) prepared from the original commercial plastic products were identified using μ -FT-IR (a_2-n_2) . Scale bar = 200 μ m.

always constant actually. Some additives and modification, as well as voids produced during the compounding and processing of plastic will result in significant changes in the density (Rani et al., 2017). For instance, density of PE increases from 0.92 to 1.28 g/cm³ by additives

introduction, which influence the ability to float or sink in water (Anon, 1988).

Although the densities of plastics can be measured based on the American Society for Testing and Materials (ASTM D792), the

Table 1

Formula of density gradient solutions (20 mL) in each density from 0.8 to 1.8 g/cm³, using the bases of ethanol (0.8 g/cm³), ultrapure water (1.0 g/cm³), NaI or ZnCl₂ (1.8 g/cm³). -', none.

Volume (mL/mL)	Density gradient solutions (g/cm ³)										
	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8
Ethanol/water	20/0	8/12	0/20	-	-	-	-	-	-	-	-
NaI/water	-	-	-	3/17	5/15	7/13	9/11	11/9	15/5	17/3	20/0
ZnCl ₂ /water	-	-	-	2/18	5/15	7/13	10/10	12/8	15/5	18/2	20/0



Fig. 2. Microplastics with known density were measured in the ethanol-water system. Scale bar = $200 \,\mu m$.

measurement of apparent density of microplastics is still a grand challenge due to the small size and light weight (Astm, 2008). Thereby, it is necessary and essential to develop a novel method to determine the densities of unknown microplastics encountered in different circumstances. The main purpose of the present study was to develop a straightforward method for measuring the apparent density of microplastics.

2. Materials and methods

2.1. Microplastics

Microplastics with known densities were purchased from Sigma-Aldrich, including PP (0.9 g/cm³) and polystyrene (PS, 1.06 g/cm³) pellets, PE (0.92 g/cm³) and nylon (1.14 g/cm³) fragments. The densities were provided by the vendor. Microplastics with unknown densities were prepared from original plastic products. Plastic products of 14 various polymer types were purchased from the market, including fragments of polyurethane (PU), polypropylene-polyethylene (PP-PE), PS, alkyd and polyethylene terephthalate (PET), fibers of PP, PE, nylon, acrylic, polyvinyl alcohol (PVA), polyester (PES), polyester-polyamide (PES-PA) and rayon, pellets of epoxy (Table S1). The PS, PET fragments and epoxy pellets were made into secondary microplastics in the laboratory. The hard PP-PE and alkyd products were scraped into fragments by razor blades. The soft PU and fiber products were cut with dissecting scissors into tiny pieces as small as possible (Kolandhasamy et al., 2018). Prepared microplastics were collected and passed through a 5 mm mesh size sieve for further density measurement.

Microplastics were observed with a Carl Zeiss Discovery V8 Stereo microscope (MicroImaging GmbH, Göttingen, Germany), and images were taken with an AxioCam digital camera (Yang et al., 2015). The polymers were identified using a micro-Fourier transform infrared spectroscopy microscope (LUMOS μ -FT-IR, Bruker) in attenuated total reflectance (ATR) mode (Dris et al., 2016). A total of 10 items of each polymer were identified to confirm their purities (Fig. 1).

2.2. Preparation of density gradient solutions

In this method, solutions with a gradient density of 0.8–1.8 g/cm³ (increment of 0.1 g/cm³) were established by mixing bases of ethanol (0.8 g/cm³), ultrapure water (Milli-Q, 1.0 g/cm³), sodium iodide (Nal, 1.8 g/cm³) and zinc chloride solutions (ZnCl₂, 1.8 g/cm³) (Schäfer and Synowietz, 1984; Horton et al., 2017). Densities of plastics mostly range from 0.89 g/cm³ (e.g. PP) to 1.58 g/cm³ (e.g. PVC) regardless of various additives (US EPA, 1992). These density gradient solutions seem to be suitable for measuring the apparent density of most microplastics because of the wide density range.

Stock solutions for both NaI and ZnCl₂ of 1.8 g/cm³ were prepared by dissolving NaI and ZnCl₂ powders in specific volume of ultrapure water. The density gradient solutions were prepared and stored at room



NaI-water system

ZnCl₂-water system

Fig. 3. Microplastics with known density were measured by the Nal-water and the ZnCl₂-water solutions, separately. Scale bar = 200 µm.

temperature (25 °C). Solution of each density from 0.8–1.8 g/cm³ was prepared 20 mL in a 30 mL vial for density measurement of microplastics. These density gradient solutions were prepared in the ethanol-water system (0.8–1.0 g/cm³), the Nal-water or ZnCl₂-water system (1.1–1.8 g/cm³) (Table 1). The densities of solutions were measured by comparing their mass to volume. Specifically, volume of 10 mL solutions was pipetted, and then the mass of the 10 mL subsample was weighed by electronic balance (BSA224S, Satorius). Measurements for each solution were performed at least 5 times, the relative standard deviations (RSD) were also calculated (Table S2).

Surface tensions of NaI and $ZnCl_2$ solutions in the density range of 1.1–1.8 g/cm³ were measured by pendant drop method using a contact angle instrument (JC2000D3, Powereach, China) at room temperature, and images were taken with a digital camera (EOS M10, Canon).

2.3. Measuring the density range of microplastics

The densities of 14 various polymer types of microplastics were measured. Fragments and pellets of 0.1 g or fibers of 0.01 g were dried under air at room temperature before adding to the density gradient solutions. Densities of microplastics were measured using a float or sink test in the density gradient solutions. The vials were shook for about 1 min to make microplastics re-disperse in solutions and kept in upright position. Fragments and pellets were hold for at least one day, fibers were hold for at least three days. Microplastics will sink or float in the density gradient solutions, owing to the higher or lower densities compared to the solutions. The largest density of solution where they sank and the smallest density of solution where they floated totally were considered to be the density range of microplastics. Floating or sinking statuses of the tested microplastics were observed, and images were taken by digital camera (DMC-LX100, Panasonic).

3. Results

3.1. Measuring the density range of microplastics with known density

The ethanol-water system with densities of 0.8, 0.9, and 1.0 g/cm³ were used to measure the densities of PP pellets and PE fragments. The largest density of solution where the PP pellets sunk down totally was 0.8 g/cm³, the smallest density of solution where the pellets floated totally was 0.9 g/cm³. Accordingly, the density of PP was measured as 0.8–0.9 g/cm³ (Fig. 2a, b). Similarly, based on the largest density of solution where they buoyed up totally, the density range of PE fragments was 0.9–1.0 g/cm³ (Fig. 2c, d). Densities of PP pellets and PE fragments measured by the ethanol-water system were in accordance with the given densities.

The Nal-water or ZnCl₂-water system with densities of 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, and 1.8 g/cm³ were used to measure the densities of PS pellets and nylon fragments. The density of PS pellets was measured as 1.0–1.1 g/cm³ in either Nal-water or ZnCl₂-water system (Fig. 3a–c). The density of nylon fragments was recorded as 1.1–1.2 g/cm³ using Nal-water system (Fig. 3d, e). However, the nylon was measured as 1.0–1.2 g/cm³ in the ZnCl₂-water system. In the ZnCl₂ solution of 1.1 g/cm³, part of fragments sank to the bottom and the other floated on the surface (Fig. 3f). Densities of PS pellets and nylon fragments measured by the Nal-water system were in accordance with the given densities. Densities measured by the ZnCl₂-water system showed a broader range than by the Nal-water system.

3.2. Comparing the NaI-water and the ZnCl₂-water system

The density of nylon fragments measured by the NaI-water system was different to the ZnCl₂-water system. Further comparison of the



Nal-water system

ZnCl₂-water system

Polyester (PES)

Fig. 4. Statuses of polyester microplastics were compared by the Nal-water and the ZnCl₂water system.

Nal-water and the ZnCl₂-water system was conducted by measuring the density of polyester microplastics. Polyester microplastics sank to the bottom in the Nal-water solutions of 1.1–1.3 g/cm³, and floated on the solution of 1.4 g/cm³ totally (Fig. 4a). In contrast, not all of the



Fig. 5. Surface tensions of NaI and ZnCl₂solutions in densities of 1.1–1.8 g/cm³.

microplastics sank down in the ZnCl₂-water solutions of 1.1-1.3 g/cm³ (Fig. 4b). Density measured by the ZnCl₂-water system showed a broader range than by the NaI-water system. NaI seemed to be a better base for the density gradient solutions than ZnCl₂.

In cases of the same density from $1.1-1.8 \text{ g/cm}^3$, surface tensions of the Nal solutions always lower than the ZnCl₂ solutions (Fig. 5). Microplastics showed different statuses in the Nal-water and the ZnCl₂-water systems with same density, which may be related to the different surface tensions.

3.3. Measuring the density range of microplastics with unknown density

Densities of microplastics with unknown density were measured using the density gradient solutions mixing by ethanol, water and NaI. Statuses of PP fibers and PP-PE fragments were presented in the ethanol-water system (Fig. 6a, b). Statuses of alkyd fragments and epoxy pellets were presented in the NaI-water system (Fig. 6c, d).

Densities of these 14 polymer types of microplastics were showed in the Table 2. PP, PE fibers, PU and PP-PE copolymer fragments showed densities $<1.0 \text{ g/cm}^3$. The other 10 types of microplastics showed densities $> 1.0 \text{ g/cm}^3$. Epoxy pellets showed the largest density $> 1.8 \text{ g/cm}^3$, which may be due to the inclusion of hardener.

4. Discussion

4.1. Bases selection for the density gradient solutions

The results of this work provide evidence that the density gradient solutions based on ethanol, water and Nal solutions have fair to excellent compatibility with microplastics. Serial dilutions of ethanol in ultrapure water have been used to measure the density of PE microplastics via a float or sink test (Jung et al., 2018). The ethanol-water system with a density range of 0.8–1.0 g/cm³ seems to be suitable for the density gradient solutions. Nal solutions with densities of 1.6–1.8 g/cm³ have been widely applied to separate microplastics from water, sediments and organisms (Dekiff et al., 2014; Ling et al., 2017). Meanwhile, other researchers used ZnCl₂ solutions with densities of 1.5–1.7 g/cm³ (Liebezeit and Dubaish, 2012; Imhof et al., 2012; Horton et al., 2017). These two high-density solutions have been used widely for separating microplastics from various environmental media.

Differences of NaI-water and ZnCl₂-water systems in measuring densities of microplastics were also compared in this study. In the present study, microplastics will float in ZnCl₂ solution while sink in NaI solution with the same density. This phenomenon might be related to the different surface tensions between NaI and ZnCl₂ solutions. The force exerted by the solution surface offset the gravitational pull causing microplastics to float. The larger the surface tension of ZnCl₂ solution is, the more microplastics float. Likewise, studies have proven that the surface tension of solution is an important factor influencing the flotation of solids as mineral (Kramer et al., 2012), sediment grain (Stolte et al., 2015) and plastic (Karvelas et al., 1996). Moreover, $ZnCl_4(H_2O)_2^2$ ions are proved to exist in stoichiometric zinc chloride solutions. The $ZnCl_4(H_2O)_2^{2-}$ ions are easy to hydrolysis and form white flocculent precipitates in the high-density ZnCl₂ solutions (Irish et al., 1963). The densities of both NaI and ZnCl₂ solutions were measured again one month later. Results showed that the ZnCl₂ solutions were unstable with increased densities after a long time storage. However, the NaI solutions were stable with unchanged densities. Thereby, NaI was considered as a better choice for the density gradient solutions.

4.2. Feasibility of the density gradient methodology

The density gradient solutions with a density range of $0.8-1.8 \text{ g/cm}^3$ are made up by three bases: ethanol (0.8 g/cm^3), ultrapure water (1.0 g/cm^3) and saturated NaI (1.8 g/cm^3). Due to the relatively small volume of solutions for density measurements, the cost of these three

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Fig. 6. Microplastics with unknown density were measured by the density gradient solutions based on the ethanol, ultrapure water and Nal.

bases is relatively low, which guarantees the economic feasibility of this method to determine the densities of polymers. Eleven densities of solutions which form the density gradient solutions are shown as follows: ethanol-water system (0.8, 0.9, 1.0 g/cm³), Nal-water system (1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8 g/cm³). The ethanol and saturated Nal should be stored in brown bottles in the shadows to avoid light. For better

Table 2

Density range of 14 polymer types of microplastics were measured using density gradient solutions. "1", sink; "1", float; "-", partly float or sink in solutions. Abbreviation: PP, polypropyrene; PU, polyurethane; PP-PE, polypropyrene-polyethylene; PE, polyethylene; PS, polystyrene; PVA, polyvinyl acetate; PET, polyethylene terephthalate; PES, polyester; PES-PA, polyester-polyamide.

Microplastic	Density gradient solutions (g/cm ³)										Measured		
	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	density (g/cm ³)	
PP	\downarrow	Ť										0.8-0.9	
PU	\downarrow	↑										0.8-0.9	
PP-PE	\downarrow	↑										0.8-0.9	
PE		\downarrow	1									0.9-1.0	
PS			\downarrow	1								1.0-1.1	
Nylon			\downarrow	1								1.0-1.1	
Acrylic			\downarrow	1								1.0-1.1	
Alkyd			\downarrow	-	î							1.0-1.2	
PVA						\downarrow	1					1.3-1.4	
PET						\downarrow	1					1.3-1.4	
PES						\downarrow	1					1.3-1.4	
PES-PA							\downarrow	1				1.4-1.5	
Rayon										\downarrow	1	1.7-1.8	
Ероху											\downarrow	>1.8	

observing, long glass containers with small diameter are recommended for density gradient solutions. For further laboratory ecotoxicity testing, if ethanol is employed as the solvent to test the sample density, the microplastics cannot be reused in ecotoxicity testing, since ethanol may modify the characteristics of the microplastics and desorb additives or other pollutants from the surface.

Densities of microplastics are variable with the introduction of additives, polymer modifications, and production of voids. In this study, with the wide measurement range, the density gradient solutions are feasible to measure most microplastics that were prepared in the laboratory. In terms of microplastics sampled from the field, bacterial, chemical contaminants, and absorbed organic matter also add to complexity of microplastics and may affect the accuracy of density measurement using this approach (Galloway et al., 2017). In that case, we recommend adding some diluted oxidants such as hydrogen peroxide to remove/reduce particle-associated biofilm.

5. Conclusions

Ethanol (0.8 g/cm³), water (1.0 g/cm³) and saturated NaI (1.8 g/cm³) are considered as the best choices for the density gradients solutions. These density gradient solutions with density range of 0.8–1.8 g/cm³ are suitable for measuring the density of most of the microplastics. Measurement of density is considered as an essential prerequisite for the laboratory test of microplastics. The developed method in this study provides a useful tool for characterizing microplastics in the laboratory experiments and has the potential to be applied for field samples.

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Appendix A. Supplementary data

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