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Fang, Zheng, Sallach, Brett and Hodson, Mark Edward (2023) Ethanol, not Water, Should Be Used as the Dispersant When Measuring Microplastic Particle Size Distribution by Laser Diffraction. Science of the Total Environment. 166129. ISSN: 1879-1026

https://doi.org/10.1016/j.scitotenv.2023.166129

Science of the Total Environment 902 (2023) 166129

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Ethanol, not water, should be used as the dispersant when measuring microplastic particle size distribution by laser diffraction

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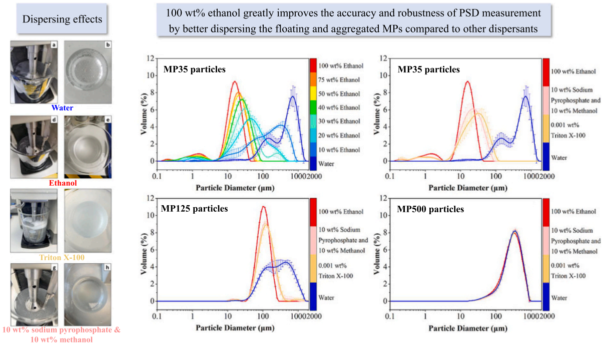
HIGHLIGHTS

- Microplastics aggregate and float in water.
- Poor dispersion causes inaccuracies in size measurement by wet laser diffraction.
- A range of chemicals have better dispersion properties than water.
- 100 wt% ethanol as a dispersant gives the most accurate measurements.
- Effects are most significant for smaller particle sizes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yi Yang

Keywords:
MPs
Aggregation
Floating
Laser granulometer
PSD
Polyethylene

ABSTRACT

Size distribution is a crucial characteristic of microplastics (MPs). A typical method for measuring this property is wet laser diffraction. However, when measuring size distributions of MPs, despite it being a poor dispersant for many MPs, water is commonly selected, potentially limiting the reliability of reported measurements. To evaluate dispersant suitability, different aqueous concentrations of ethanol (0, 10, 20, 30, 40, 50, 75, 100 wt%) and aqueous solutions of 0.001 wt% Triton X-100 and a mixture comprising 10 wt% sodium pyrophosphate and 10 wt% methanol were used as dispersants in a laser granulometer (Mastersizer 2000) to determine particle size distributions (PSDs) of granular polyethylene MP35, MP125 and MP500 particles (nominally <35, <125 and, < 500 μm in size). The reliability of the PSDs depended on the dispersant used and size of primary MPs. With increasing ethanol concentrations, PSD curves of MP35 particles shifted from multi-modal to mono-modal distributions. The measured size distribution reduced from 1588.7 to 4.5 μm in water to 39.9 to 0.1 μm in 100 wt% ethanol. Generally, as ethanol concentration increased, uncertainty associated with the PSD parameters decreased. Although Triton X-100 and the mixed solution also showed better dispersion than water, measured particle sizes and coefficient of variation (COV, %) were notably larger than those for 100 wt% ethanol. Similar trends were observed for larger-sized MP125 and MP500 particles, but differences in PSD curves, PSD parameters, and COV (%) among dispersants were less pronounced. In all dispersants, the volume weighted mean diameters (VWMD) in 100 wt% ethanol (MP35: 14.1 μm, MP125: 102.5 μm, MP500: 300.0 μm) were smallest and close to diameters determined from microscope observations (MP35: 14.6 μm, MP125: 109.0 μm, MP500:

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https://doi.org/10.1016/j.scitotenv.2023.166129
Received 31 March 2023; Received in revised form 21 July 2023; Accepted 6 August 2023

310.6 μm). Therefore, for accurate determinations of the PSDs of MP by wet laser diffraction, ethanol rather than water should be used as the dispersant.

1. Introduction

Microplastics (MPs) are usually defined as plastic debris having at least one dimension that is <5 mm in size (Frias and Nash, 2019). The size of MPs plays a crucial role in shaping their ecological and human health impacts by affecting their mobility (Waldschlager and Schuttrumpf, 2020), their ability to cross biological membranes (Yee et al., 2021) and their hydrophobic surface area (Pathak and Navneet, 2017). What is more, because surface area per unit volume increases with decreasing size, MP size also impacts on the adsorption of pollutants to (Fu et al., 2021), and the leaching of additives from (Hale et al., 2020), MPs. Therefore, the accurate determination of the particle size distribution (PSD) of MP particles is necessary for understanding the fate and behaviour of MPs in the environment.

Although wet laser diffraction is recognized as one of the most efficient methods to quantify the PSD of samples (Blott et al., 2004; Eshel et al., 2004; Rawle, 2015), researchers wanting to determine the PSD of MPs usually encounter two main obstacles when using this method. First, the particle densities of some common plastics (e.g., polyethyene: 0.915–0.970 g/cm^3, polypropylene: 0.900–0.910 g/cm^3) are less dense than water at typical earth surface temperatures and pressures (c. 0.998 g/cm^3) which is the most frequently used dispersant (Malvern Instruments, 2013; Stelray Plastic Products. Inc, 2023). As a result, MPs float on the surface of the water and cannot be analysed (Li et al., 2019). Second, given the hydrophobic nature of non-polar MP surfaces, the formation of persistent aggregates from primary MP particles is driven by hydrophobic attraction (Pathak and Navneet, 2017; van Oss, 2003). These aggregates change their sizes via disintegration and reaggregation and can be recognized as large size fractions during measurement. Thus, PSD measurements made using water as the dispersant can be unreliable and fail to meet the requirements of the ISO 13320 (2009) standard, e.g. a COV (%) of <3 % for D50, and <5 % for D10 and D90 (ISO, 2009) where DX is the percentage by volume of particles which have a lower diameter than the value stated.

To prevent these issues in PSD analyses, compatible dispersants are strongly recommended by manufacturers (Malvern Instruments, 2007b; Malvern Instruments, 2013). Despite this, many studies continue to use water as the dispersant (Bell et al., 2021; Curlin et al., 2021; Gonzalez-Doncel et al., 2022; Li et al., 2019; Lopez et al., 2022) or do not mention the dispersant used in their methods (Gardon et al., 2022; Li et al., 2022). In some studies surfactants are added to the dispersant to reduce MP aggregation in water (Al-Azzawi et al., 2020; Seghers et al., 2022), but these surfactants have varying dispersing effects and may generate bubbles that can be mistaken for MPs (Malvern Instruments, 2013). Furthermore, the use of surfactants does not stop the density-driven issue of floating MPs. The only MP study of which we are aware that reports using an alternative dispersant to determine PSDs is Renner et al. (2022) in which a mixture of 10 % sodium pyrophosphate solution and 10 % methanol is used to disperse polyethylene MPs recovered from personal care products. However, the mean particle sizes determined by laser diffraction are still 1.9–4.9 times that of the sizes determined by image analysis (Renner et al., 2022). It can be seen that a general lack of consideration for a compatible dispersant has limited the reliability of reported measurements.

Ethanol has been applied in pycnometer studies to determine the particle density of MPs (e.g. see below in this study and Porizka et al., 2023), and also used as a dispersant for measuring the PSD of other cohesive particles (e.g., lactose powders; Adi et al., 2007). We therefore hypothesised that the use of ethanol as a dispersant for PSD measurement using wet laser diffraction would reduce floatation and aggregation leading to a reduction in the measured particle size and increased similarity between particle size measured by wet laser diffraction and direct measurement using microscope observations. The experiments reported here aim to (i) examine the effectiveness of ethanol in the PSD measurement of granular polyethylene MPs using the laser diffraction method and (ii) investigate whether ethanol has a better dispersing effect than other dispersants which have been used in previous MP studies. The reduced health impacts, and easier accessibility of ethanol, relative to other reagents which are also less polar and less dense than water (e.g., methanol and cyclohexane), represent additional considerations for selecting ethanol (Beg et al., 1993; Carr and Riddick, 1951; Malvern Instruments, 2013).

2. Material and methods

2.1. Microplastics

We selected polyethylene as our test material because it is one of the most highly used plastics (Hale et al., 2020) and studies report that it dominates the MP load of sewage sludge (El Hayany et al., 2022). Pristine PE particles nominally described as <35, <125, <500 μm in size were bought from Goonvean Fibres Ltd. (Devon, UK, EX15 1UY) and are referred to in this study as MP35, MP125 and MP500. The particles were confirmed as polyethylene by FTIR (Alpha, Bruker Optics, Karlsruhe, Germany) (Fig. S1). Particles, as observed by optical microscope (SC100, Olympus Corporation, Tokyo, Japan) were equidimensional but irregular in shape (Fig. S1). The particle densities of the MP particles were measured using 1.0 g of material, 5 mL pycnometers (Pomex Glassware, Beijing, China) and absolute ethanol (VWR Chemicals BDH, Leicestershire, United Kingdom) at 20 °C.

2.2. Dispersants

Mixtures of deionized water and absolute ethanol (VWR Chemicals BDH, Leicestershire, UK, LE17 4XN) resulting in ethanol concentrations of 0, 10, 20, 30, 40, 50, 75, 100 wt% were used as dispersants. The dispersing effects of dispersants used in previous MP studies, including an aqueous solution of Triton X-100 surfactant (0.1 wt%) (Seghers et al., 2022) and a mixed (mass ratio of 3:5) 10 wt% sodium pyrophosphate and 10 wt% methanol aqueous solution (Renner et al., 2022), were also investigated. However, due to bubble formation during the use of the 0.1 wt% Triton X-100 solution (see below), the majority of experiments using the Triton X-100 used a 0.001 wt% solution. The densities of the dispersants were determined using 5 mL pycnometers (Pomex Glassware, Beijing, China) at 20 °C (aqueous solution of Triton X-100 and mixed solution) or were taken from published data (aqueous solutions of ethanol).

2.3. Laser granulometer analyses

The PSD (2000–0.02 μm) of MPs was determined by a Mastersizer 2000 (Malvern Panalytical, Malvern, UK, WR14 1XZ) laser granulometer with manual entry of operating conditions and material properties. Mie theory rather than the Fraunhofer method was chosen to calculate the PSD because Mie theory can be used for various sizes of particles and is the only advised method for particles <50 μm (Malvern Instruments, 2007a). The following values were used for refractive indices of the materials: polyethylene MPs (1.520) (Malvern Instruments, 2007b), water (1.332), aqueous solutions of ethanol (10 wt%: 1.340; 20 wt%: 1.346; 30 wt%: 1.352; 40 wt%: 1.356; 50 wt%: 1.359; 75 wt%: 1.363; 100 wt%: 1.359) (Lapeira et al., 2016), aqueous solution of Triton X-100 (1.332), and the mixed solution of 10 wt% sodium

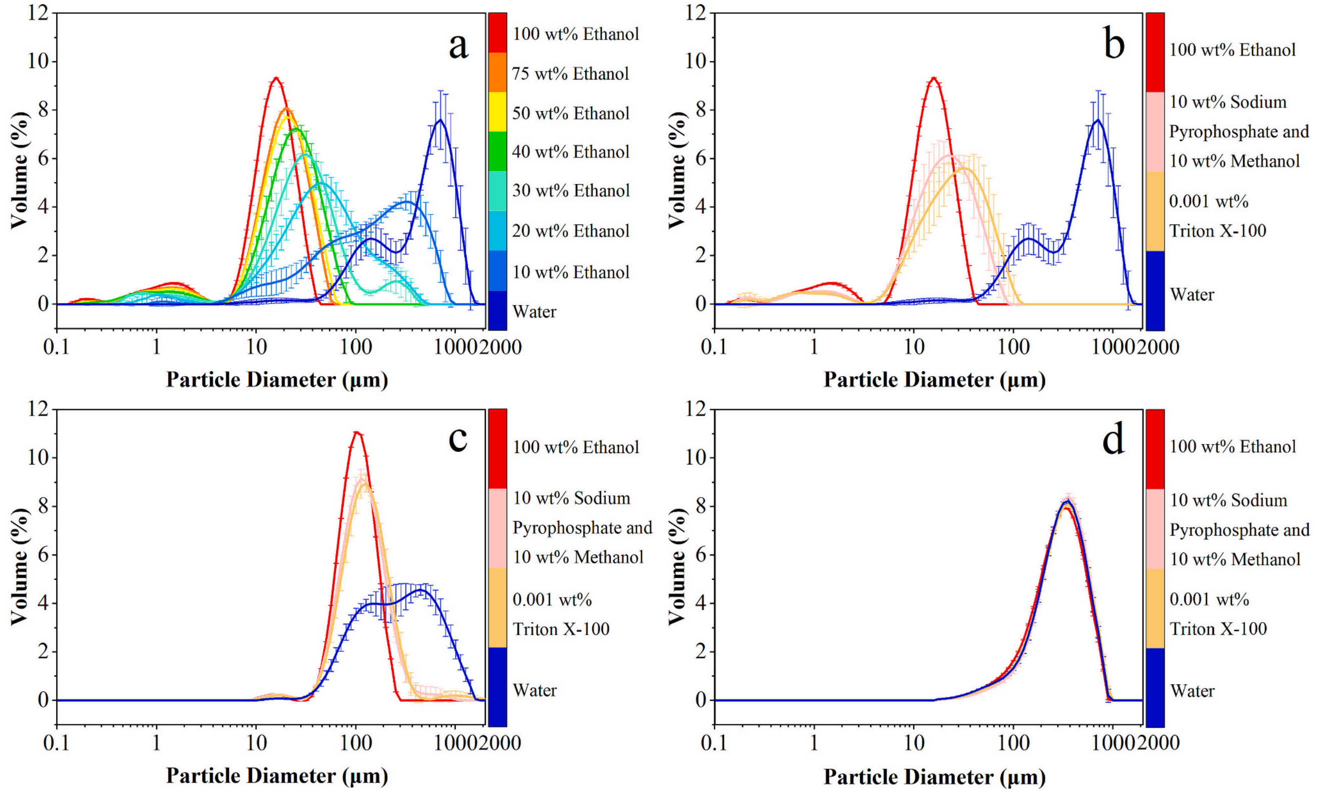

Fig. 1. PSD (log10 scale) curves (mean ± SD, $n = 3$) of (a) MP35 particles measured in increasingly strong concentrations of aqueous solutions of ethanol, (b) MP35 particles measured in different dispersants, (c) MP125 particles measured in different dispersants, (d) MP500 particles measured in different dispersants.

pyrophosphate and 10 wt% methanol (1.332). As the polyethylene MPs were irregularly shaped, transparent particles (Fig. S1), and light could be absorbed by the irregularities on their surfaces, the imaginary absorption index of MPs was set as 0.1 (University of Warwick, 2000). We used our measured values for the densities of MPs (MP35: 0.934 g/cm^3, MP125: 0.952 g/cm^3, MP500: 0.936 g/cm^3). The measured densities of aqueous solutions of Triton X-100 and the mixture of 10 wt% sodium pyrophosphate and 10 wt% methanol aqueous solution were 0.999 and 1.009 g/cm^3, respectively. The density of water and the ethanol solutions were set as 0.998 (water), 0.983 (10 wt%), 0.960 (20 wt%), 0.948 (30 wt%), 0.929 (40 wt%), 0.910 (50 wt%), 0.852 (75 wt%) and, 0.796 g/cm^3 (100 wt%), respectively (Lapeira et al., 2016).

MPs can easily adhere to the sides of the tubing used in laser granulometers. Therefore, in this study before starting a new measurement, the laser granulometer was rinsed seven times with deionized water placed in the sample introduction beaker and a pump speed set to 4000 rpm. Two drops of washing-up liquid were added to the first and third run. Each run lasted 30s and the inbuilt ultrasonic probe (power: 20 W) was used throughout. After cleaning, the instrument was rinsed with 700 mL of the dispersant to be used for the next measurement.

When performing the PSD measurements, 700 mL of dispersant was placed in the sample introduction beaker, the pump speed was set to 3000 rpm and the ultrasonic probe (power: 20 W) was turned on for 30s to fully homogenize the dispersants. The pump speed was then reduced to 1500 rpm and the ultrasonic probe turned off for 20 s to eliminate bubbles in the instrument before making a background measurement. After the background measurement, the pump speed was increased to 3000 rpm, and the ultrasonic probe switched back on (power: 20 W). MPs were slowly added to the sample introduction beaker. When obscuration reached 10 % and stabilized, no more MPs were added, pump speed was reduced to 1500 rpm, the ultrasonic probe switched off and the instrument was left for 20 s prior to measuring the sample. The pump speed was set as 1500 rpm during measurement to avoid generating bubbles which are recognized as particles by the instrument. The obscuration rate was maintained at about 10 %, because excessive particle concentrations can cause multiple scattering effects that lowers the accuracy of PSD measurements (Malvern Instruments, 2013).

In the PSD measurements of MP35 particles, each dispersant mentioned in Section 2.2 was tested. However, the presence of bubbles caused by the Triton X-100 surfactant at concentrations of 0.1 wt% and also at a lower concentration of 0.01 wt% led to laser intensity values below the minimum threshold required for background measurements. A much lower concentration of Triton X-100 surfactant (0.001 wt%) was therefore used in the experiments (Fig. S2). In the PSD measurements of MP125 and MP500 particles, we only tested the dispersing effects of water, aqueous solution of Triton X-100 surfactant (0.001 wt%), the mixture of 10 wt% sodium pyrophosphate and 10 wt% methanol, and 100 wt% ethanol. There were 3 consecutive tests in each treatment, giving 54 measurements in total.

For the MP35 particles D10, D50 and D90 values, defined as the diameter that 10 %, 50 % and 90 % of particles, by volume, are less than, were measured. Average particle size as the volume weighted mean diameter (VWMD) (Eq. 1) was also measured (Adi et al., 2007; Malvern Instruments, 2007a).

$$VWMD = \sum_{i=1}^{n} V_i d_i \tag{1}$$

where d_i is the diameter of each size fraction (μm), and V_i is the volume proportion of the sample corresponding to that size fraction.

2.4. Validation

The major axis (maximum Feret diameter) and the minor axis (minimum Feret diameter) of the MP particles ($n = 50$) was measured manually under an optical microscope (SC100, Olympus Optical, Japan), as an independent method, to compare with the results of the

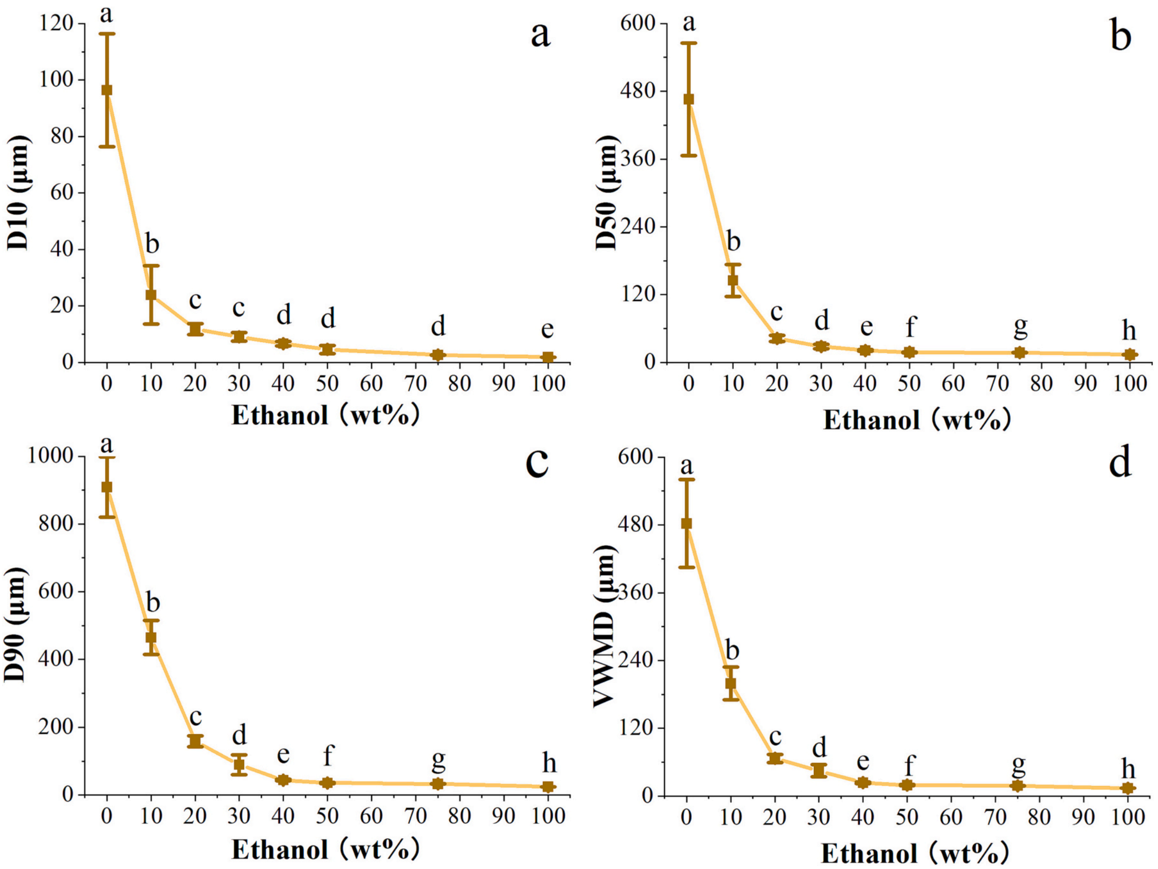

Fig. 2. The particle size distribution parameters (a) D10 (10 % by volume of particles have lower diameters than this value), (b) D50 (50 % by volume of particles have lower diameters than this value), (c) D90 (90 % by volume of particles have lower diameters than this value), and (d) VWMD (volume weighted mean diameter) of MP35 particles measured with water or aqueous solutions of ethanol as the dispersant. Data are means (± SD, n = 3). The non-parametric Kruskal-Wallis H tests reveal significant difference between treatments ($p = 0.002$). Superscript letters indicate significant differences ($p < 0.05$) between the treatments.

laser granulometer measurements (Bowen, 2002) (See SI for details). The equivalent spherical diameter of each particle was calculated as the square root of the product of its major and minor axes. These diameters were used to calculate the VWMD of the MPs using Eq. (1).

2.5. Statistical analyses

Statistical analysis was carried out using IBM SPSS Statistics 24. The normality and homogeneity of variance of the data were assessed using Shapiro-Wilk's test and Levene's test, respectively. The differences in size distribution parameters were analysed by one-way ANOVA (dispersant as the fixed factor) followed by Tukey post-hoc tests. For data sets which failed to meet the assumptions of normality and equal variance between treatments, a transformed data approach was employed prior to ANOVA analyses. If the assumptions were still not met, data were analysed using one-way non-parametric ANOVA (Kruskal-Wallis H test) followed by Dunn's post-hoc test. A p value of ≤0.05 was considered to indicate significant differences.

3. Results

During measurements, MPs of each size fraction in water floated on the surface and formed persistent aggregates (Fig. S3 a-c). As the concentration of the ethanol in the dispersant increased, the amount of floating particles and aggregation decreased, with no visible floating and aggregated MPs in 100 wt% ethanol (Fig. S3 d-f). In comparison, the mixture of 10 wt% sodium pyrophosphate and 10 wt% methanol only partially reduced particle floating and aggregation (Fig. S3 g-i). Despite having greatly reduced the concentration of Triton X-100 surfactant to 0.001 wt%, there was still significant bubble formation with MPs mixed in with the foam (Fig. S2).

When measuring small-sized MP35 particles, values for measured PSDs and particle size descriptors decreased significantly when measured in 10 % ethanol compared to the values obtained in water, which were all notably greater than the nominal size of MPs (< 35 μm) ($p < 0.01$) (Figs. 1a and 2, Table S1). Values continued to reduce significantly as ethanol concentration increased, but the degree of reduction was not so marked ($p < 0.01$) (Fig. 2, Table S1). These decreases were accompanied by a shift in the PSD curves from multi-modal to mono-modal distributions (though a minor peak slightly grew at 3–0.3 μm), from broad distributions to narrow distributions, and from larger to smaller sizes (Fig. 1a). Generally, with increasing ethanol concentration, the coefficient of variation (% COV) of these PSD parameters also decreased (Fig. 3), while the fit of the Mie model to the measured PSD increased (Table S1). Triton X-100 and the mixed solution achieved better dispersing effects than water, but the measured values obtained in these dispersants remained markedly larger than those obtained in 100 wt% ethanol (Figs. 1b, 4 and 5, Table S1). The VWMD of MP35 particles in 100 wt% ethanol (14.1 ± 0.3 μm) was smallest and closest to the value of 14.6 μm (21.6–0.5 μm, $n = 50$) obtained from microscopy (Figs. 2d and 4, Tables S1 and S2).

Larger-sized MP125 particles also showed significant reductions in measured PSDs and particle size descriptors when replacing water with other dispersants ($p < 0.05$) (Figs. 1c and 4, Table S3). However, the differences among dispersants were less pronounced compared to those observed for the MP35 particles (Figs. 1b, c and 4, Tables S1 and S3). Among different dispersants, the measured PSD parameters of MP125 particles and their COV (%) were the smallest in 100 wt% ethanol

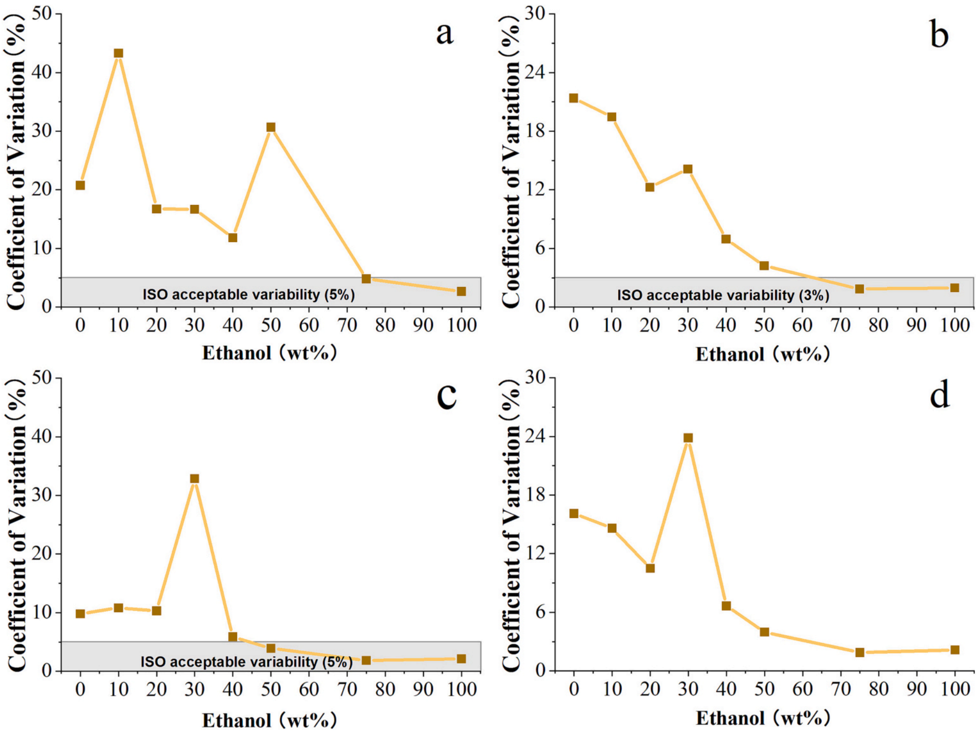

Fig. 3. The coefficient of variation (%) of the particle size distribution parameters (a) D10 (10 % by volume of particles have lower diameters than this value), (b) D50 (50 % by volume of particles have lower diameters than this value), (c) D90 (90 % by volume of particles have lower diameters than this value), and (d) VWMD (volume weighted mean diameter) of MP35 tested with water and aqueous solutions of ethanol.

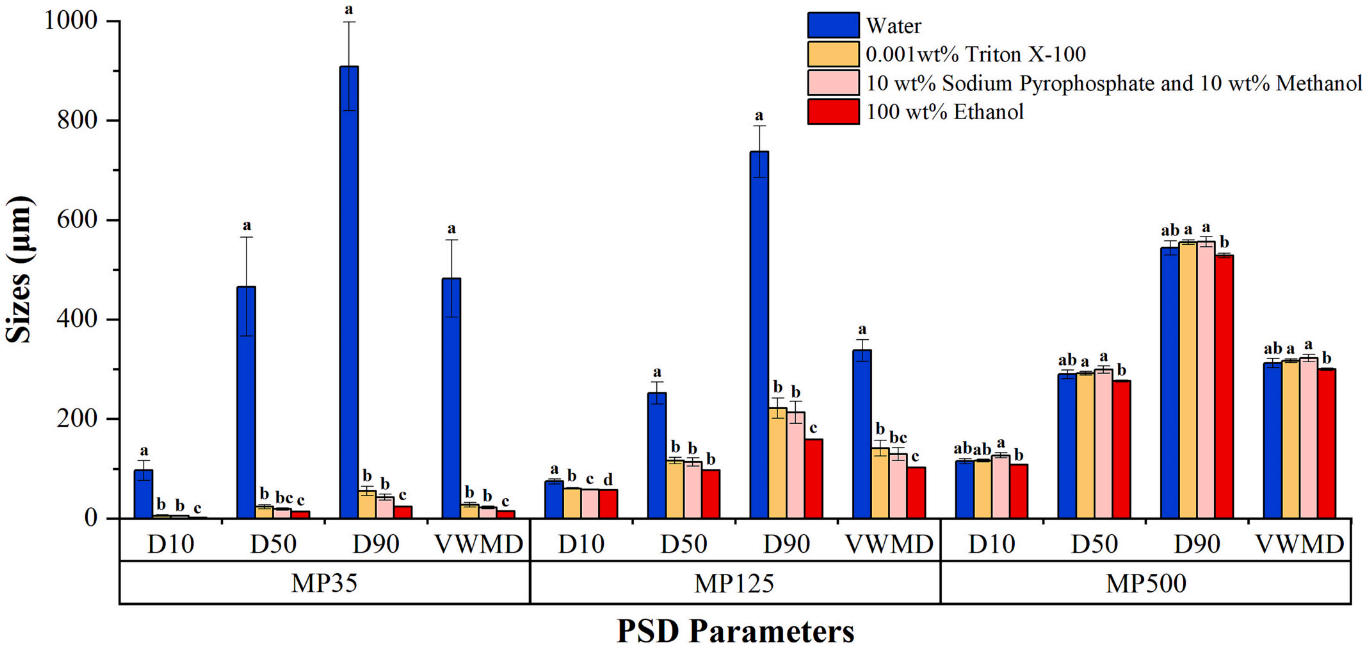

Fig. 4. The particle size distribution parameters of different size fractions of MP particles measured with different dispersants. Data are means (± SD, n = 3). The one-way ANOVA tests reveal significant difference between treatments ($p < 0.03$). Superscript letters indicate significant differences ($p < 0.05$) between the treatments.

(Figs. 4 and 5), and the VWMD of MP125 particles obtained in 100 wt% ethanol (102.5 ± 0.1 μm) was closest to the value of 109.0 μm (145.3–15.1 μm, n = 50) obtained from microscopy (Tables S3 and S4). As the size of MP particles further increased to MP500 particles, the differences in PSD curves, PSD parameters, COV (%) among different dispersants continued to be smaller (Figs. 1c, d, 4 and 5, Tables S3 and S5). Nevertheless, those measured values obtained in 100 wt% ethanol remained the smallest (Figs. 1d, and 4–5, Table S5). The VWMDs of MP500 particles in different dispersants ranged from 322.5 ± 7.4–300.0 ± 2.4 μm, and were all close to the value of 310.7 μm (423.2–42.2 μm, n = 50) obtained from microscopy (Tables S5 and S6).

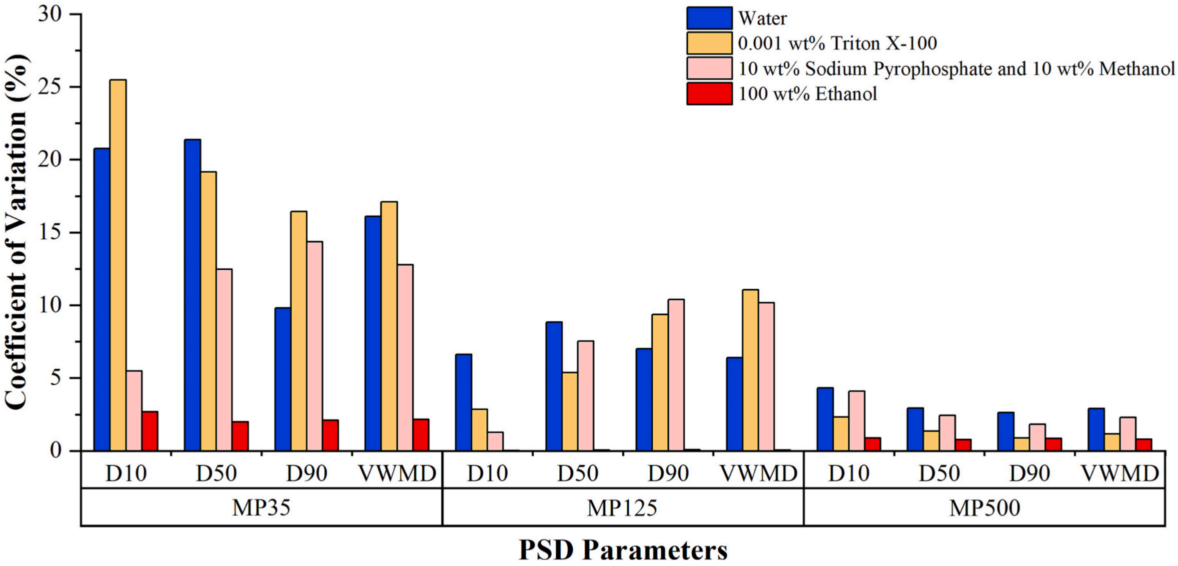

Fig. 5. The coefficient of variations (%) of the particle size distribution parameters including D10 (10 % by volume of particles have lower diameters than this value), D50 (50 % by volume of particles have lower diameters than this value), D90 (90 % by volume of particles have lower diameters than this value), and VWMD (volume weighted mean diameter) of different size fraction of MPs tested with different dispersants.

4. Discussion

For PSD determination of fine particles, the accuracy and robustness of wet laser diffraction methods primarily depends on the degree of sample dispersion (Malvern Instruments, 2013). The poor dispersal of MPs by water limits its application in MP studies. The hydrophobic attraction between particles immersed in water is caused by the AB free energy of cohesion among the polar water molecules which surround particles in water (van Oss, 2003). As the non-polar hydrophobic surface of pristine polyethylene MPs tends to reduce the total area of MP surface in contact with water, the induced attractive force results in the aggregation of MP particles (Fig. S3 a-c). In this study, the degree of MP aggregation increased notably as the size of primary MP particles decreased from MP500 to MP35 particles, as demonstrated by the increased differences between the VWMD analysed by laser diffraction (water as a dispersant) and by microscopy (Fig. 4, Tables S1 - S6). Similar results were also found in the study by Renner et al. (2022). This is explained by the fact that smaller particles have more surface area per unit volume, thus enhancing the hydrophobic attraction between MP particles. In addition electrostatic charges on highly insulating materials, such as the tested MPs, can be produced during processing and transportation due to triboelectrification (Mort, 2003); induced electrostatic attraction due to these charges can strengthen the aggregation of MPs in water (Visser, 1989). Additionally, given that polyethylene MPs (0.934 g/cm^3) are less dense than water (0.998 g/cm^3), buoyancy can offset the gravitational pull and the hydrodynamic forces of the circulating water (Rawlins et al., 2013). The resulting floating MPs ‘escape’ from being analysed (Fig. S3 a-c).

Ethanol is less polar than water due to its methyl functional group. Its presence in the dispersant reduced the hydrophobic attraction of MPs, thus effectively reducing their aggregation (Fig. S3 d-f). In addition, the presence of ethanol decreased the overall density of the dispersant, thus mitigating, and at higher concentrations, eliminating, the floating problem of polyethylene MPs (Fig. S3 d-f). In comparison, Triton X-100 and the mixed solution only partly reduced the MP aggregation and failed to resolve the density-driven issue of floating MPs (Figs. S2, S3 g-i). Furthermore, induced foam formation due to the addition of Triton X-100 surfactant interfered with the measurements, as these bubbles can be mistaken for MP particles, adsorb added MPs and retain these MPs on the dispersant surface (Fig. S2). These issues limit the application of these dispersants in MP studies.

The reduction in aggregation and floating caused the PSD parameters of MP35 particles to decrease with increasing ethanol concentration (Fig. 2). The increased precision of these parameters was indicated by the trend of decreasing COV (%) with increased ethanol concentration (Fig. 3). Although Triton X-100 and the mixed solution also significantly reduced the measured PSD parameters compared to water ($p < 0.05$) (Fig. 4), their measured COV (%) were notably higher than those of 100 wt% ethanol in the PSD measurement of MP35 particles (Fig. 5). In all of the dispersants, only the results of 75 wt% ethanol and 100 wt% ethanol met the requirements of the ISO 13320 (2009) for COV (%), indicating their suitability for determining the PSD of polyethylene MPs (Fig. 3). Of the two concentrations, the significant ($p < 0.05$) decreases in the PSD parameters from the 75 wt% to 100 wt% ethanol concentration and the 100 wt% ethanol result being closer to the microscopy result suggests that 100 % ethanol is the most suitable dispersant for MP35 particles.

With increasing primary MP particle sizes, the differences in PSD curves, PSD parameters, and COV (%) among different dispersants became less pronounced (Figs. 1b-d, 4 and 5, Tables S1, S3 and S5), mainly caused by the generally decreasing degree of MP aggregation in dispersants. For polyethylene MP125 particles, significant differences in the accuracy and precision of laser diffraction resulted from different dispersants (Figs. 1c, 4 and 5, Table S3). 100 wt% ethanol exhibited the smallest PSD parameters (Fig. 4), was the only dispersant satisfying the ISO 13320 (2009) requirements for COV (%) (Fig. 5), and was more consistent with microscopy result (Tables S3 and S4). The influences of dispersant on the results of laser diffraction measurements notably decreased for polyethylene MP500 particles (Figs. 1d, 4, and 5, Table S5). However, the PSD parameters and COV (%) values remained smallest in 100 wt% ethanol. Therefore, despite observing similar measured results in different dispersants, it is still recommended to use 100 wt% ethanol as the dispersant due to its better dispersion property and robustness, especially when characterising a heterogenous sample of different particle size MPs.

5. Conclusions

This study measured the particle size distribution of polyethylene MPs using ethanol instead of water and other dispersants used in previous MP studies. Our findings are widely applicable to other MP types as they result from fundamental physical and chemical properties – density differences between the MP and the dispersant, and aggregation due to the hydrophobic nature of the material. Whilst microfibres may behave differently in terms of clumping, PSD of microfibres should not be measured using laser diffraction methods anyway given their geometry and the assumptions behind laser diffraction methods. Our results suggest that the choice of dispersant can significantly affect the accuracy and precision of PSD measurements by laser diffraction. Particularly for finer primary MP particles, the selection of a compatible dispersant becomes critical. 100 wt% ethanol significantly increases the accuracy and precision of the laser diffraction results when used to measure the PSD of MPs by preventing the floatation and aggregation of MPs.

CRediT authorship contribution statement

Zheng Fang, Brett Sallach and Mark Hodson designed the study. Zheng Fang carried out the practical work and data analysis and wrote the first draft of the paper. Brett Sallach and Mark Hodson provided edits and worked on later drafts.

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.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the University of York – China Scholarships Council Joint Research Scholarship (CSC NO: 202208890030).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166129.

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