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Method Article

Think positive: Proposal of a simple method to create reference materials in the frame of microplastics research

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ABSTRACT

In the context of the harmonization of methodologies employed to isolate and count microplastics in samples or to organize ring trials tests, the use of reference materials, i.e. samples with controlled amount of particles is required. The method proposed here uses transparent, sealed capsules containing in-house generated microplastics as a convenient way to generate microplastic reference materials. This method is a simple approach for adding particles to samples without risk of loss during particle extraction or transporting/handling.

- Low-cost and easy-to-use preparation of heterogeneous mix of microplastic reference particles
- Possibility to control microplastic size, shape, and polymeric composition
- Applicable to many protocols and wide range of applications on water, sediments and biota.

Specifications table

Subject area:	Environmental Science
More specific subject area:	QC for microplastics research
Name of your method:	Production of reference material in the frame of microplastics research
Name and reference of original method:	De Frond, H., L. Thornton Hampton, S. Kotar, K. Gesulga, C. Matuch, W. Lao, S. B. Weisberg, C. S. Wong, and C. M. Rochman. 2022. "Monitoring microplastics in drinking water: An interlaboratory study to inform effective methods for quantifying and characterizing microplastics." *Chemosphere* **298**: 134,282. doi: https://doi.org/10.1016/j.chemosphere.2022.134282
Resource availability:	Transparent, gelatin or HPMC capsules sold by retailers worldwide. Plastic wastes or items present in laboratories. Synthetic sewing thread spool sold by retailers worldwide

Background

Research of microplastics (MPs) has been growing at an exponential rate for the last decade [29]. MPs are now studied in every environmental compartment, including seawater [22,24], marine sediments [11,22], freshwater [17,20], freshwater sediments [17,27], terrestrial soils [10,28], air [23,26], biota [2,20] and food [5]. Despite the increasing number of publications, there is still a lack of harmonization and method comparison. Harmonization should be better considered in order to help risk assessors and policy makers to access reliable data [1,9,12].

The difficulties in harmonizing methodologies is partly the result of the absence of reference material, as recently mentioned by Seghers et al. [21] in the frame of the organization of a ring trial. Reference materials can be defined in the terms of ISO Guide 30:2015

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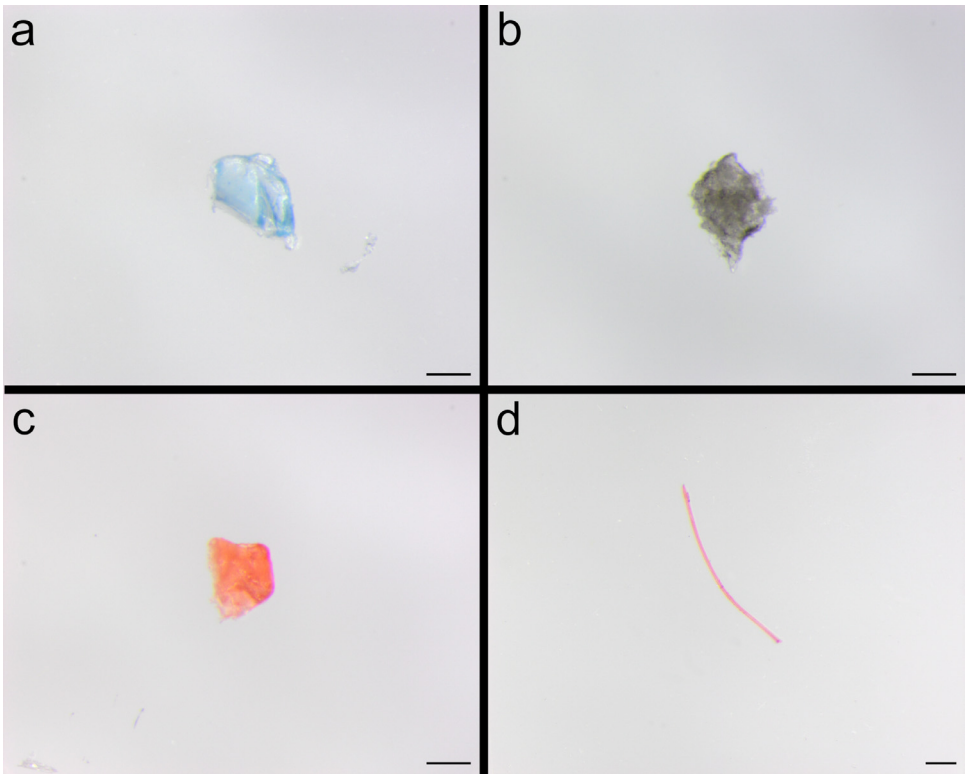

Fig. 1. Pictures of microplastics fragments and fiber used to fill the capsules including: (a) blue high-density polyethylene (HDPE), (b) gray polypropylene (PP), (c) red polystyrene (PS) fragments and (d) red polyester (PES) fiber. Black bars at the right bottom of each picture represents a length of 100 μm.

with notions of homogeneity and stability with respect to desired quantity properties and prepared to be suitable for its intended use [14]. So far, the few studies concerning interlaboratory tests require complex procedures for the preparations of microplastic reference materials, including sending bottles, filtered solutions, stabilizers or even detergents [15,19,21]. Recently, in the frame of the development of standardized methods for analysis of microplastics in drinking water and the organization of a ring trial, De Frond et al. [7] proposed the use of gelatin capsules in order to add MPs in water and create spiked samples to test. During the development of spiked samples, authors mentioned difficulties of dissolution necessitating heating at 48 °C and addition of malic acid and sodium bicarbonate to accurately dissolve capsules.

Beyond interlaboratory studies, another application that would need to use a reference material is the addition of positive control (PC) concomitantly with the analysis of sample(s) in order to contribute to the evaluation of the quality of the analysis [3,4,13,16]. This kind of controls is extremely important because it allows the investigator to check different parameters, like the impact of the isolation process on the integrity of polymers, or evaluate if all spiked MPs are recovered at the end of the extraction. So far, the protocols are not very clear on how to spike the samples and this is probably done by hand, which can be time consuming.

For these reasons, that the use of capsules containing different compositions fo MPs is proposed here as an inexpensive and simple method to create in-house reference materials. These materials can either be used directly in the lab or send by mail within the frame of ring trial tests.

Preparation of in-house microplastics fragments

Three types of fragments and a single type of fiber composed of different polymers were employed in this study (Fig. 1). High-density polyethylene (PE), polypropylene (PP) and polystyrene (PS) originated respectively from a blue milk bottle cap, a gray fragment of a waste harvested on a beach, and a red document file box. All plastic items were cut using grinding repetitions of a few seconds with a household Moulinex DPA141 La Moulinette (Ecully, France) blender and sieved in order to get particles of ca. 100, 200 and 500 μm. Fibers, from ca. 500 to 700 μm long and 11 μm thick, were produced using a Prima polyester (PES) spool of thread (Villepinte, France). Thread sections were hand-sliced with a scalpel and a fiber Yuchengtech slice Y172 (Beijing, China) as employed by Ma et al. [18]. The polymeric composition of each items was ascertained using a FT-IR (Fig. S1). Briefly, an item of each polymer particle/fiber was placed under the crystal of the μ-ATR tool from a Perkin Elmer Spotlight^{TM} 400 Fourier-transform device (Villebon-sur-Yvette, France), equipped with a MCT detector. Each spectrum was acquired from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}, 25 acquisitions being performed for each item. Particles were identified by comparing spectra and Flopp/Flopp-e databases [6].

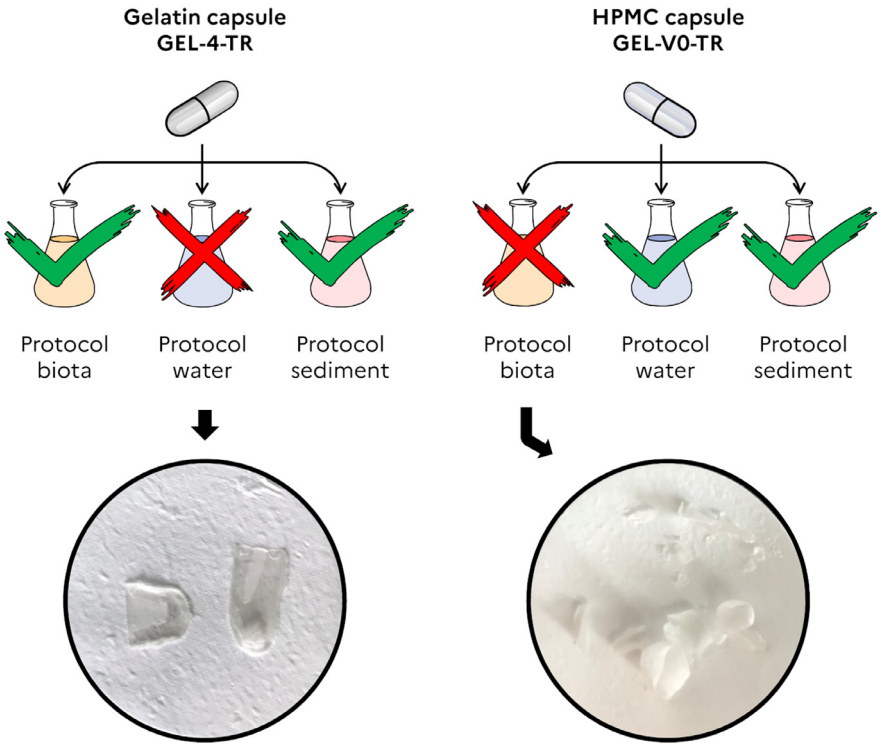

Fig. 2. Main results obtained after filtration of solutions containing capsules from the three tested protocols. GEL-4-TR capsules were not efficiently dissolved with water and GET-V0-TR capsules were not efficiently dissolved with 10% (w/w) KOH.

Selection of capsules

Two types of capsules acquired from ID Labo (Pierres, France) were tested. The first was transparent capsules composed of hydroxypropyl methylcellulose (HPMC) (GEL-V0-TR) and the second was a transparent gelatin capsule (GEL-4-TR). The respective volumes of GEL-V0-TR and GEL-4-TR capsules were of 0.68 mL and 0.21 mL.

Each types of capsule was first tested using a protocol of digestion used for MPs in biota matrix, described by Dehaut et al. [8] and optimized by Treilles et al. [25]. Samples were incubated into a Binder 240 drying oven (Tüttlingen, Germany) at 40 ± 1 °C for 24H within 100 mL of 10% (w/w) potassium hydroxide (KOH) solution (Chimie plus, Saint-Paul-de-Varax, France) without agitation. Additional tests were performed with the two capsule categories at room temperature (18.7 ± 0.2 °C) for 24H, within 100 mL of 30% (w/w) hydrogen peroxide (H_2O_2) solution (Carlo Erba, Val-de-rueil, France), as used for sediment analysis, and at room temperature (19.7 ± 0.2 °C) for 24H within 1000 mL of water for analysis conform to EN-ISO 3696 grade 3 (Carlo Erba, Val-de-rueil, France) without agitation.

All experiments were carried out in triplicate in clean Erlenmeyer flasks or glass bottles. At the end of the incubation, contents were filtered using a glass Büchner device composed of a glass funnel and a fritted filter holder. A 47 mm diameter GF/A glass fiber filter with a porosity of 1.6 µm (Whatman, Maidstone, United Kingdom) was placed between funnel and holder. Duration of filtration was consigned and filters were photographed and stored at room temperature in glass Petri dishes. The steps of preparation and filtration for all experiments were carried out under a Thermofisher Scientific Herosafe 2030i laminar flow cabinet (Saint-Herblain, France).

From this experiment and as presented in Fig. 2, the gelatin capsules can only be used with the protocols using either 10% KOH or 30% H_2O_2 solutions, while HPMC capsules only dissolve in water and 30% H_2O_2 solutions.

Application of the method with mussels

As the aim of this article is to provide a methodological development and not a monitoring study, no strict procedure of contamination management was applied. Nonetheless, the preparation of particles and capsules was carried out in a first room. Then digestions and filtrations were carried out in another room into a Thermofisher Scientific Herosafe 2030i laminar flow cabinet.

In order to assess the feasibility of the process with biota samples, an experiment was conducted with ten mussels (*Mytilus edulis*) bought in a local supermarket and conserved in a freezer at −20 °C. After being held at 1 °C for 24H, mussels were shelled and put into Erlenmeyer flasks before being weighted. The average tissue weight was 3.3 ± 0.6 g. Then, GEL-4-TR capsules containing a mixture of in-house microplastics fragments were added in Erlenmeyer flasks containing mussel. Ten capsules were manually filled with five

microplastics particles of each for polymers (PE, PP, PS) on average < 300 μm and five microfibers of PES from ca. 500 to 700 μm, leading to the presence of $n = 20$ MPs for each capsule. Then, 100 mL of 10% (w/w) KOH were poured into the ten Erlenmeyer flasks containing each a mussel, as well as a capsule containing MPs. Erlenmeyer flasks were placed into an Binder BD 240 drying oven for 24H at 40 ± 1 °C under an agitation of 200 rpm onto 2mag MIXDrive 6 HT (Munich, Germany). Afterwards, the digestates were filtered under vacuum with a Büchner through 90 mm diameter 1.6 μm pore size glass fiber filters GF/A. A specific rinsing protocol was employed in order to recover a maximum number of particles. It was composed of three rinsing steps as follow: first Erlenmeyer flask, then the funnel of Büchner, and finally the edge of funnel just above the sintered glass support. Each rinsing step corresponded to three successive sub-steps using a succession of rinses with filtered water, with a filtered 70% ethanol solution, again with filtered water. The obtained filters were placed in Petri dish and dried at room temperature for 24H before counting using an Olympus SZX16 stereomicroscope equipped with a SDFPLAPO PF 1x/0.15 objective and a UC90 camera.

In the cases, GEL-4-TR capsules were successfully dissolved and particles of each type were recovered onto filters. Overall, the average recovery rate of particles for the ten capsules was 84.0 ± 11.5%. Depending on polymers, the average number of particles ranged from 76 ± 24.6% for PE fragments to 96 ± 8.4% for PES fibers. PES fibers and PP fragments were the particles recovered most easily during the test.

The method presented in this paper it is an easy approach to create reference materials containing controlled amounts of different microplastics, which are necessary in order to implement positive controls or ring trials. Depending on the protocol to be followed, two types of capsules are put forward: gelatin-based for 10% KOH and 30% H_2O_2 or HPMC-based for 30% H_2O_2 and water. New found that chemical additive within capsule was required in order to facilitate their dissolutions.

Ethics statements

No ethic statements to be declared.

Declaration of Competing Interests

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.

CRediT authorship contribution statement

Alexandre Dehaut: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Funding acquisition. **Charlotte Himber:** Investigation, Writing – review & editing. **Mathilde Colin:** Investigation. **Guillaume Duflos:** Funding acquisition, Resources, Supervision, Writing – review & editing.

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2023.102030.

References

[1] W. Cowger, A.M. Booth, B.M. Hamilton, C. Thaysen, S. Primpke, K. Munno, et al., Reporting guidelines to increase the reproducibility and comparability of research on microplastics, Appl. Spectrosc. 74 (2020) 1066–1077, doi:10.1177/0003702820930292.
[2] E. Danopoulos, L.C. Jenner, M. Twiddy, J.M. Rotchell, Microplastic contamination of seafood intended for human consumption: a systematic review and meta-analysis, Environ. Health Perspect. 128 (2020) 126002, doi:10.1289/EHP7171.
[3] K. Davidson, S.E. Dudas, Microplastic Ingestion by Wild and Cultured Manila Clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia, Arch. Environ. Contam. Toxicol. 71 (2016) 147–156, doi:10.1007/s00244-016-0286-4.
[4] P. Davison, R.G. Asch, Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre, Mar. Ecol. Prog. Ser. 432 (2011) 173–180, doi:10.3354/meps09142.
[5] G.E. De-la-Torre, Microplastics: an emerging threat to food security and human health, J. Food Sci. Technol. 57 (2020) 1601–1608, doi:10.1007/s13197-019-04138-1.
[6] H. De Frond, R. Rubinovitz, C.M. Rochman, μATR-FTIR Spectral Libraries of Plastic Particles (FLOPP and FLOPP-e) for the Analysis of Microplastics, Anal. Chem. 93 (2021) 15878–15885, doi:10.1021/acs.analchem.1c02549.
[7] H. De Frond, L. Thornton Hampton, S. Kotar, K. Gesulga, C. Matuch, W. Lao, et al., Monitoring microplastics in drinking water: an interlaboratory study to inform effective methods for quantifying and characterizing microplastics, Chemosphere 298 (2022) 134282, doi:10.1016/j.chemosphere.2022.134282.

[8] A. Dehaut, A.L. Cassone, L. Frere, L. Hermabessiere, C. Himber, E. Rinnert, et al., Microplastics in seafood: benchmark protocol for their extraction and characterization, Environ. Pollut. 215 (2016) 223–233, doi:10.1016/j.envpol.2016.05.018.
[9] A. Dehaut, L. Hermabessiere, G. Duflos, Current frontiers and recommendations for the study of microplastics in seafood, TrAC Trends in Anal. Chem. 116 (2019) 346–359, doi:10.1016/j.trac.2018.11.011.
[10] J.J. Guo, X.P. Huang, L. Xiang, Y.Z. Wang, Y.W. Li, H. Li, et al., Source, migration and toxicology of microplastics in soil, Environ. Int. 137 (2020) 105263, doi:10.1016/j.envint.2019.105263.
[11] P.T. Harris, The fate of microplastic in marine sedimentary environments: a review and synthesis, Mar. Pollut. Bull. 158 (2020) 111398, doi:10.1016/j.marpolbul.2020.111398.
[12] E. Hermsen, S.M. Mintenig, E. Besseling, A.A. Koelmans, Quality Criteria for the Analysis of Microplastic in Biota Samples: a Critical Review, Environ. Sci. Technol. 52 (2018) 10230–10240, doi:10.1021/acs.est.8b01611.
[13] E. Hermsen, R. Pompe, E. Besseling, A.A. Koelmans, Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria, Mar. Pollut. Bull. 122 (2017) 253–258, doi:10.1016/j.marpolbul.2017.06.051.
[14] ISO. ISO GUIDE 30:2015(E) Reference materials — Selected terms and definitions. 2015. URL: https://www.iso.org/standard/46209.html
[15] A. Isobe, N.T. Buenaventura, S. Chastain, S. Chavanich, A. Cozar, M. DeLorenzo, et al., An interlaboratory comparison exercise for the determination of microplastics in standard sample bottles, Mar. Pollut. Bull. 146 (2019) 831–837, doi:10.1016/j.marpolbul.2019.07.033.
[16] J. Li, X. Qu, L. Su, W. Zhang, D. Yang, P. Kolandhasamy, et al., Microplastics in mussels along the coastal waters of China, Environ. Pollut. 214 (2016) 177–184, doi:10.1016/j.envpol.2016.04.012.
[17] H.-.C. Lu, S. Ziajahromi, P.A. Neale, F.D.L. Leusch, A systematic review of freshwater microplastics in water and sediments: recommendations for harmonisation to enhance future study comparisons, Sci. Total Environ. (2021) 781, doi:10.1016/j.scitotenv.2021.146693.
[18] C. Ma, L. Li, Q. Chen, J.S. Lee, J. Gong, H. Shi, Application of internal persistent fluorescent fibers in tracking microplastics in vivo processes in aquatic organisms, J. Hazard. Mater. 401 (2021) 123336, doi:10.1016/j.jhazmat.2020.123336.
[19] Y.K. Muller, T. Wernicke, M. Pittroff, C.S. Witzig, F.R. Storck, J. Klinger, et al., Microplastic analysis-are we measuring the same? Results on the first global comparative study for microplastic analysis in a water sample, Anal. Bioanal. Chem. 412 (2020) 555–560, doi:10.1007/s00216-019-02311-1.
[20] N.N. Phuong, T.T. Duong, T.P.Q. Le, T.K. Hoang, H.M. Ngo, N.A. Phuong, et al., Microplastics in Asian freshwater ecosystems: current knowledge and perspectives, Sci. Total Environ. 808 (2022) 151989, doi:10.1016/j.scitotenv.2021.151989.
[21] J. Seghers, E.A. Stefaniak, R. La Spina, C. Cella, D. Mehn, D. Gilliland, et al., Preparation of a reference material for microplastics in water-evaluation of homogeneity, Anal. Bioanal. Chem. 414 (2022) 385–397, doi:10.1007/s00216-021-03198-7.
[22] L. Simon-Sanchez, M. Grelaud, M. Franci, P. Ziveri, Are research methods shaping our understanding of microplastic pollution? A literature review on the seawater and sediment bodies of the Mediterranean Sea, Environ. Pollut. 292 (2022) 118275, doi:10.1016/j.envpol.2021.118275.
[23] S. Sridharan, M. Kumar, L. Singh, N.S. Bolan, M. Saha, Microplastics as an emerging source of particulate air pollution: a critical review, J. Hazard. Mater. 418 (2021) 126245, doi:10.1016/j.jhazmat.2021.126245.
[24] Thompson R.C., Napper I.E. Microplastics in the Environment. In: Harrison RM, Hester RE, editors. Plastics and the Environment 2019, pp. 60–81.
[25] R. Treilles, A. Cayla, J. Gasperi, B. Strich, P. Ausset, B. Tassin, Impacts of organic matter digestion protocols on synthetic, artificial and natural raw fibers, Sci. Total Environ. 748 (2020) 141230, doi:10.1016/j.scitotenv.2020.141230.
[26] A. Xu, M. Shi, X. Xing, Y. Su, X. Li, W. Liu, et al., Status and prospects of atmospheric microplastics: a review of methods, occurrence, composition, source and health risks, Environ. Pollut. 303 (2022) 119173, doi:10.1016/j.envpol.2022.119173.
[27] L. Yang, Y. Zhang, S. Kang, Z. Wang, C. Wu, Microplastics in freshwater sediment: a review on methods, occurrence, and sources, Sci. Total Environ. 754 (2021) 141948, doi:10.1016/j.scitotenv.2020.141948.
[28] X. You, S. Wang, G. Li, L. Du, X. Dong, Microplastics in the soil: a review of distribution, anthropogenic impact, and interaction with soil microorganisms based on meta-analysis, Sci. Total Environ. 832 (2022) 154975, doi:10.1016/j.scitotenv.2022.154975.
[29] M. Zhou, R. Wang, S. Cheng, Y. Xu, S. Luo, Y. Zhang, et al., Bibliometrics and visualization analysis regarding research on the development of microplastics, Environ. Sci. Pollut. Res. Int. 28 (2021) 8953–8967, doi:10.1007/s11356-021-12366-2.