Proposal for a measurement method for use in investigations of microplastics in tap water

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1   Background

In the period October 2017-January 2018, the DCE worked for the Danish Environmental Protection Agency on developing a reliable method to analyse microplastics in drinking water. The point of departure was that, as part of the method, sampling equipment was to be developed that could be fitted directly onto the taps from which the samples were taken in order to eliminate as far as possible the risk of contamination of the samples while they were being taken.

Focus was initially on analysing microplastic particles as fragments and filaments/fibres with sizes/lengths of more than 100 μm. This lower limit for particle sizes is the same limit as in the Danish and American studies of drinking water employed previously (Strand 2017a,b).

2 Description of method

2.1 Sampling

The sampling equipment developed is based on the following components

- A horizontal conical filtration chamber made of stainless steel, which retains both high-density and low-density particles.

- A filter consisting of a stainless-steel mesh with mesh size 20 μm to collect the particles.

- A flowmeter fixed to the water outflow after filtration to measure the volume of water that has passed through the filtration chamber during sampling.

With this system water samples are filtered directly at the outflow from taps. The volume of samples has so far been 50 litres. The samples have been taken at a water flow rate of ~2.5 litres per minute, i.e. over a period of approx. 20 minutes. Larger sample volumes could also be taken if this is considered relevant.

After the end of sampling, the sampling system is reversed, so the system can be flushed clean from the rear (reverse direction of sample collection). First 20 ml of filtered concentrated detergent solution (sulfo) is added from behind so that the plastic particles that have settled can run off the surfaces more easily. The particles collected on the steel filter are then collected on a white MCE filter (mixed cellulose ester, which consists of nitrocellulose, ADVANTEC) with a pore size of 5 μm by flushing the system again in the reverse direction with 5-10 litres of filtered water.

Both the flush water and detergent solution are filtered with 5 μm filters before use.

2.2 Preparation of the sampling and analysis equipment

The sampling chamber and 20 μm stainless steel filters are cleaned before use. First by washing with detergent solution, then by rinsing in filtered water and finally by wrapping them in aluminium foil and heating them to 550°C for 2 hours in a muffle furnace to remove any organic material and thereby avoid internal contamination with microplastics. All the glassware (petri dishes, flasks etc.) used is cleaned in the same way. Black rubber gaskets, which are used to seal the sampling system, are rinsed clean before use, first in a filtered sulfo solution with ultrasonication and then in filtered water. During storage, sensitive surfaces and openings in the sampling equipment are wrapped in aluminium foil until the equipment is taken into use. The MCE filters are cleaned before use with a micro vacuum cleaner under a stereo microscope to remove any interfering particles.

2.3 Analysis method

The analysis method is based on two steps.

The first step consists of visual characterisation and quantification of potential microplastic particles on the MCE filters by using a stereo-microscope at 10x - 100x magnification. The potential microplastic particles are characterised and quantified in accordance with recommendations for analyses of microplastics in aquatic environment samples from JRC (2013), i.e. according to their type (e.g. filaments, fragments, film etc.), colour and size fraction. In general, it has been assessed that only particles with sizes of >100 µm can be visually characterised in this way with reasonable certainty.

The second step is validation of the visual characterisation of the potential microplastic particles using µFT-IR microscopy. In this way, it can be verified whether the particles found consist of plastic polymers or primarily of other types of material (e.g. cellulose, protein, metals, etc.).

The DCE uses Agilent Cary 6210/670 with FPA (Focal Planar Array) for these analyses, and this device covers the wavelength range 875 - 3800 cm^{-1}. With µFT-IR, the analyses can be carried out with the device set at either transmission or reflectance mode, or they can be analysed with µATR. The particles are transferred to the relevant discs (e.g. ZnSe or MirrIR), depending on whether they are to be analysed in transmission or reflectance. The preferred setup is for the analyses to be carried out in transmission using ZnSe discs.

Using spectroscopic mapping, particles within a "field of view" with an area of 704 µm x 704 µm (pixel resolution of 5.5 x 5.5 µm) can either be analysed one by one, or several particles can be analysed simultaneously, for example by making a mosaic covering a larger area composed of several "fields of view."

It is recommended that any larger particles (> 500 µm) are analysed with normal FT-IR with an ATR detector, as this provides better quality spectra, covers a larger wavelength range of 400 - 4000 cm^{-1} and is a faster method of analysis.

Identification of the particle composition is carried out either by experience-based visual recognition of the spectra recorded, or, in more uncertain cases, by comparing the FT-IR spectra recorded with spectra of known materials collected in a library database. This library consists of the laboratory's own database of reference spectra and a commercial database (Know it all) with spectra of polymers and other materials. However, final determination of the constituents of the particles should still include a final expert assessment of the quality of the library-search match.

The particles are identified as microplastics if their primary constituents are made up of synthetic polymeric materials such as PE, PP, PET, PVC, PTFE, PA, PS, PU, PB, EVA PMMA, acrylate, etc., whereas particles consisting of natural or semi-synthetic polymers such as cellulose (e.g., cotton) or viscous (rayon), are not characterised as microplastics.

It has been assessed that the current procedure to identify potential microplastic particles using stereo-microscopy (step 1) takes approx. half a working day per sample. While µFT-IR analyses of 10 - 20 potential microplastic particles (step 2) take approximately one working day. Therefore, for reasons of cost efficiency, for each sample, it is recommended to FT-IR analyse up to 10 - 20 representative particles that have been characterised as potential microplastic particles in the visual identification.

3 Validation of the measurement method

Validation of the measurement method (sampling and analysis) has included the following four steps:

1. Determination of the recovery rates and reproducibility of the sampling system developed by adding different types and sizes of reference particles, which are quantified using stereo-microscopy.
2. Investigations of blank samples to assess any internal contamination, for example from airborne particles or particles from the flush water.
3. Comparison of sampling with the sampling equipment directly attached to taps with situations where the sampling is based on random samples of the water samples collected with annealed 5 l glass flasks (red cap).
4. Testing the sampling procedure with the initial investigations of microplastics in drinking water from three selected taps.

Re 1. To start, a series of tests were performed with a relatively simply designed sampling system using 20 µm mesh filters with a diameter of approximately 20 mm inserted into a ½-inch plumbing fitting between 2 rubber membranes. Using this model, it turned out to be hard to achieve acceptable recovery of reference particles. This was due to the fact that a significant part of the PE spheres (microspheres from Cospheric) used as reference particles were broken, probably due to a high flow rate and turbulence inside the filter system. Therefore, sampling equipment was then built with 20 µm filters, but with a larger diameter of 90 mm in which the flow rate could also be better adjusted downwards.

The investigations using reference particles showed that the new sampling equipment had more efficient retention for microplastic particles. The recovery rates were more than 90% for four of the five reference particles used. The lowest recovery rate of 75.0 ± 14.1 % was found for thin filaments (table 1). The recovery rate of thin filaments could perhaps be improved if filters with a mesh size of 10 µm were used instead of 20 µm.

Table 1. Recovery rates for reference particles added to the sampling equipment..

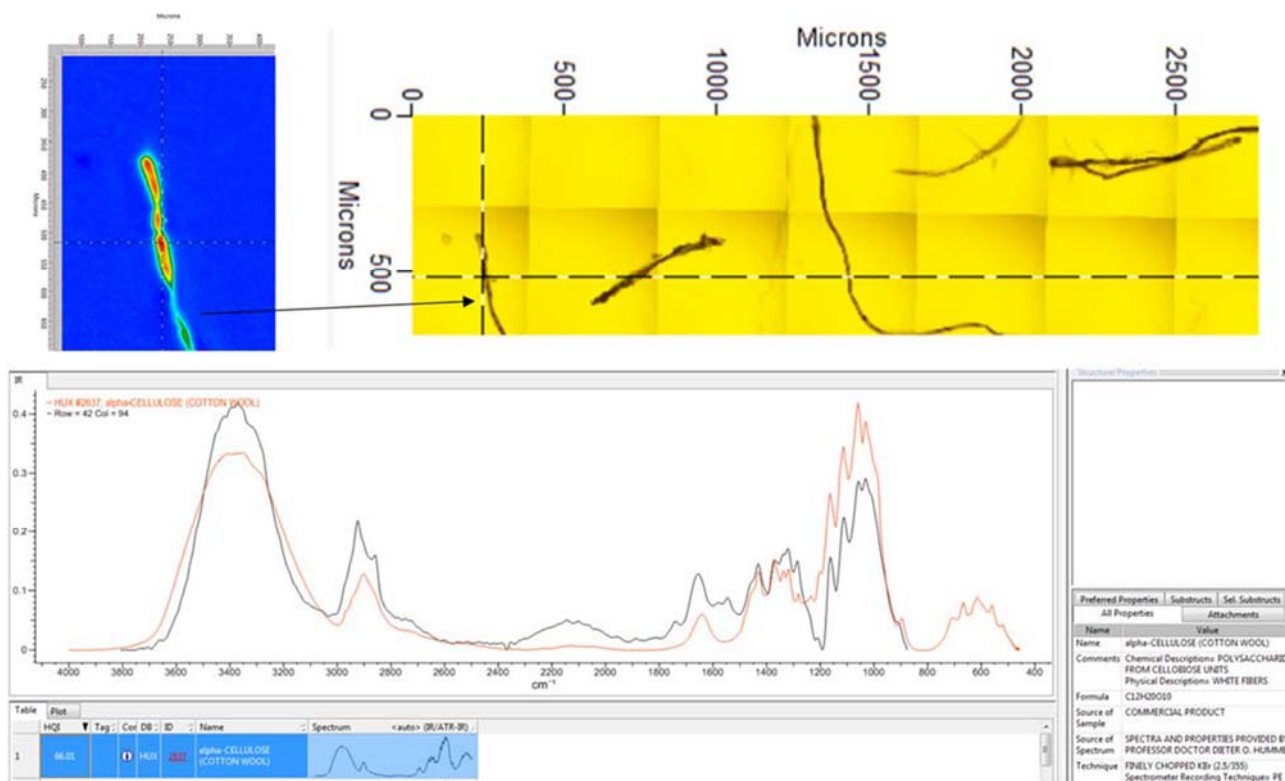| Particle type	Size	Polymeric material	Recovery (%)
Spheres	60-70 µm	PE	98.2 ± 2.0
Spheres	210-220 µm	PE	95.6 ± 3.8
Spheres	500-600 µm	PE	97.5 ± 4.3
Fragments	~80 x ~500 µm	PP	86.9 ± 11.4
Filaments	~10 x ~1000 µm	PET	75.0 ± 14.1

Figure 1. μFT-IR identification of filaments from blank samples, here determined as consisting of cellulose and confirmed with library searches on 1st derivatives of their spectra.

Re 2. Investigations of blank samples for the presence of potential micro-plastic particles, such as filaments and fragments showed that some filaments may occur at the MCE filters, even if the sampling equipment is thoroughly cleaned and the MCE filters are handled with great care.

On average, so far 3.9 (range 0 - 8) microplastic-like filaments have been found per blank sample, with colours transparent, grey, blue or black. Of these, 19 filaments have been analysed with μFT-IR, and they were all identified to consist of cellulose-like materials and thus cannot be characterised as microplastics. It is currently difficult to determine in which parts of the procedure contamination with these filaments was introduced.

In addition to filaments, relatively high amounts of small inorganic fragments occurred in the blank samples, including metallic fragments. These have been assessed to come from the sampling equipment, the aluminium foil or the pipe system from which the filtered flush-water was taken.

It has been assessed that the data for the blank samples is of sufficient quality, especially if μFT-IR analyses are subsequently used to validate the particles found visually as microplastic, which is particularly relevant for filaments.

Re 3. For comparison, recovery rates has been investigated during sampling using random samples of water collected in annealed 5 l glass flasks (red cap). This showed a recovery rate of >90% for 3 of the 4 types of reference particles used, which was also good quality. The lowest recovery rate was for the thin filaments and for the sampling equipment developed to attach to taps. Data from blank samples was also generally good, with only 0 - 3 microplastic-like filaments per blank sample, all of which could be identified with μFT-IR as consisting of cellulose as the primary constituent. Examinations of tap water

(drinking water) collected with the same 5-litre glass flasks at three households initially showed that, in addition to individual cellulose filaments, the sample could also contain other types of potential microplastic particles. These were identified with µFT-IR as being white PTFE filaments (teflon) and red thermoplastic PET fragments. Following comparative FT-IR analyses, similar particles were assessed to have come from plastic materials in the cap of the glass flasks (red cap), which thereby indicates that there is a risk of internal contamination in the sampling process.

It has been assessed that taking samples by filling cleaned glass flasks is an alternative option to the sampling equipment developed to attach to taps. However, it is important to be aware of the risk of internal contamination from plastic caps as well as the higher detection limits due to the lower sampling volume usually used.

Re 4. Finally, a preliminary investigation of the occurrence and analysis of microplastic particles with sizes of >100 µm in three selected samples from taps was performed to test the sampling procedure. Taps were selected on the basis of internal logistics and based on criteria that they represented different types of water supply. The taps were located at kitchen sinks in three locations on Zealand in Denmark at Roskilde (Risø campus), Ølstykke and Amagerbro in Copenhagen. A total of 50 litres of water was filtered at each location. The examinations showed that 4 - 7 microplastic-like filaments and no fragments occurred per 50-litre sample in all three samples (table 2). These levels were comparable with the occurrence of filaments in the blank samples. µFT-IR identification also showed that all the filaments primarily consisted of cellulose, except for a single microplastic filament that consisted of PET. This was found in the analysis of the water sample from Amagerbro.

Table 2. Microplastic (MP)-like particles with sizes of >100 µm and their primary constituent identified with µFT-IR collected after filtration of 50 litres of tap water from three locations on Zealand, Denmark.

Location	Number of MP-like filaments	Number of MP-like filaments	µFT-IR identification of primary constituen
Risø campus	4	0	Cellulose (4)
Ølstykke	7	0	Cellulose (7)
Amagerbro	5	0	Cellulose (4), PET (1)

The levels of microplastic-like particles described in the previously mentioned Danish and American studies of microplastics in drinking water in among others Strand (2017a,b) could therefore not immediately be confirmed with these three analyses. However, it is important to mention that the three samples cannot be considered representative of all types of water supply in Denmark, where variations over time are also likely. Furthermore, it cannot be ruled out that microplastics sized <100 µm can occur in the samples, as these have not been included in the analysis.

3.1 Re. analysis of microplastic particles sized <100 µm

The method of measurement described here has been developed and validated for particles >100 µm. If future analyses of tap water are also to include analyses of particles with sizes <100 µm (e.g. in the range of 20 - 100 µm), it is not recommended that these are based on an initial step comprising visual

identification of potential microplastic particles using stereo-microscopy. Instead, a spectroscopic mapping will be necessary of all the particles collected with µFT-IR. It would also be an advantage to use filters made of stainless steel with smaller mesh size, e.g. 10 µm.

Spectroscopic mapping with µFT-IR of all the particles collected in the filters used in the sampling equipment means that they must first be transferred to either a disc or a filter that is compatible with analysis with µFT-IR. Such sampling may include filtration of the whole sample with a special Anodisc membrane filter, for example, with 5 µm pore size, Whatman or by settling of particles in a subsample of 50 µl in a particle suspension in ethanol on a ZnSe disc. Both ZnSe discs and Anodisc filters are compatible with µFT-IR measurements carried out in transmission.

However, there are some limitations in these methods. Use of Anodisc limits the spectroscopic wavelength range that can be recorded with FT-IR to 1300 - 3800 cm^{-1}. This means that information in the fingerprint range of <1300 cm^{-1}, which can be important because sufficiently accurate identification of several types of polymeric materials, e.g. PET, PVC, acrylate, PTFE, etc., is not included.

The disadvantage of using settling of particles in a sub-sample of, e.g., 50 µl of a particle suspension in ethanol on a ZnSe disc is that there may be a relatively high degree of uncertainty in the quantitative determinations. This is because it may be difficult to extract a representative sub-sample from a particle suspension consisting of a complex mixture of different types of particles with different sizes and densities.

In the long term, development of large-area µATR will also be an option to analyse complex mixtures of small microplastic particles. The DCE expects to commence testing of this new detection method in the coming year.

Furthermore, note that µFT-IR mapping is a time-demanding method to identify which of the small particles are microplastics and their polymeric constituents in a complex sample. More automated methods should be developed to manage the large volumes of data generated that then have to be interpreted as part of the analysis. Therefore, depending on the composition and the number of particles, it can take several days to go through a single sample.

4   Conclusion

On the basis of the validation, it has been assessed that the measurement method developed by the DCE (sampling and analysis) has sufficiently good quality to be used in analyses of microplastics in tap water. A method like this can therefore be taken into use, if an actual investigation into the occurrence and composition of microplastic in tap water in Denmark is to be initiated. The measurement method is based on sampling by filtering the water through a closed sampling chamber mounted directly on taps. This is assessed to be a better alternative to initially taking samples by filling cleaned sampling glass flasks.

For particles with sizes of >100 µm, an initial characterisation and quantification of potential microplastic particles can be performed visually by using stereo-microscopy. Although there is a need for subsequent validation using µFT-IR spectroscopy to verify that the constituents of the relevant particles consist of plastic polymers. This is important in order to rule out particles consisting of natural and semi-synthetic polymers or other materials. For example, filaments consisting of cellulose can occur in samples due to slight background contamination.

Investigation of the occurrence of the smaller microplastic particles with sizes of <100 µm can also be initiated. However, it is necessary to be aware that there are still some methodological challenges here, among other things due to a relatively large degree of uncertainty regarding the quantitative determinations. Furthermore, analysing for microplastic particles sized <100 µm is significantly more resource-demanding, and this will affect the length of time an investigation will take.

5 References

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