

Diverse Solutions for Improving the Marine Environment

# Analytical and Measuring Instruments for Microplastics



# Diverse Solutions for Improving the Marine Environment

Tiny plastic fragments on the order of several  $\mu\text{m}$  to 5 mm are referred to as microplastics. In recent years, there have been growing concerns that microplastics are having a negative impact on marine environments and ecosystems. Not only the microplastic materials themselves but also the additives they contain, as well as harmful substances absorbed in the environment, may have a latent impact on humans via the food chain.

Shimadzu provides analytical and measuring instruments for the study of a variety of plastic materials: for R&D, characteristic evaluation of raw materials, quality control for plastic products, and deterioration analysis. Utilizing these diverse techniques, Shimadzu provides optimal solutions for challenges in microplastic research, addressing improvements to environmental issues.



## Microplastics and Their Effects in the Marine Environment

There are two types of microplastics. Primary microplastics consist of the plastic beads used in industrial polishing powders, scrubbing agents, and so on. Secondary microplastics consist of small fragments created by physical wear and UV degradation after plastics are released into the environment.

Their impact on the marine environment is becoming a serious problem. When plastics disposed of as rubbish are swept out into the sea, they degrade into microplastics through physical wear and ultraviolet light. These microplastic pieces are then inadvertently consumed by marine animals. In addition to the risk of physical obstruction or damage to the digestive organs and digestive tracts in marine animals, the additives (such as flame retardants, plasticizers, and antioxidants) contained in the microplastics themselves, or harmful substances (such as PCBs and DDT) which adhere to them within the environment, are accumulated in the organs, and gradually become biomagnified.



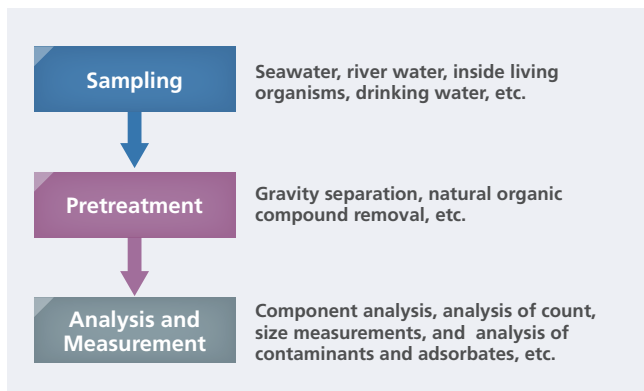
## Risks to Health

There is a concern that microplastics taken in by marine animals will also have an impact on human health as they pass through the food chain. While the main focus is on pollution in oceans and other environmental water, there are also reports that microplastics have been detected in the air. At the current time, however, the impact of microplastics on human health is not sufficiently understood, and further investigation is required.



## Experimental Workflow for Microplastics

Investigations of microplastics are underway around the world. Assessment of the current situation regarding microplastics discharged into the environment or taken in by living organisms (including their quantity, distribution density, size, composition, and adsorbates) is progressing. At present, microplastics approximately 300 µm or larger are targeted for investigation in environmental monitoring. On the other hand, in discussions of the impact on the ecosystem, an important focus is the investigation of microscopic plastics smaller than 300 µm, which are inadvertently consumed even by organisms at the bottom of the food chain.

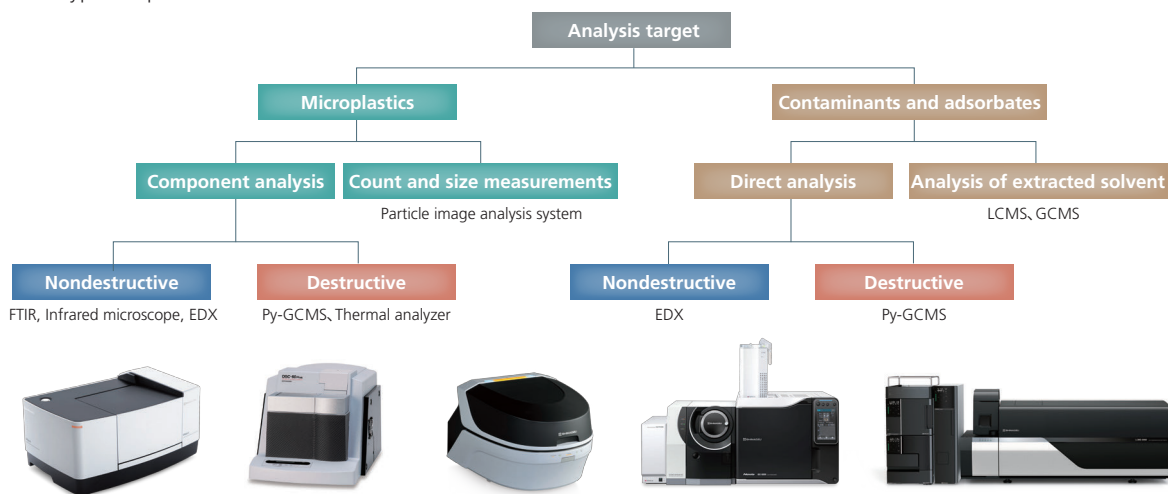


When sampling from the environment, a variety of substances are intermixed. After sampling, pretreatment including the removal of contaminants, oxidative decomposition, gravity separation, and extraction is performed in order to collect adsorbates or the microplastics targeted for analysis and measurement.

Standard analysis methods for microplastics are still a matter of discussion. There are advantages and disadvantages to the respective methods, and it is necessary to select the optimal analysis and measurement method to suit the investigation and research objective.

## Workflow for Selecting an Analysis and Measurement Method

The choice of microplastic analysis and measurement methods differs depending on what is being measured. A flowchart for selecting the optimal analysis and measurement method is shown below. Py-GCMS and other thermal analyses, which analyze the components of microplastics as well as contaminants and adsorbates directly, destroy the measurement sample when the measurement is performed. As a result, the measurement sample cannot be used for other applications. On the other hand, an advantage of these techniques is that they can analyze the substances contained inside the material, and enable simultaneous analysis even when multiple different types of plastic are mixed.



### Applications for Microplastics

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# Qualitative and Mapping Analysis of Microplastic

A Fourier transform infrared spectrophotometer (FTIR) is suited to the analysis of microplastics, as FTIRs are optimal for the qualitative analysis of organic compounds. Using the AIM-9000 infrared microscope, highly-sensitive analysis of samples under 100  $\mu\text{m}$  is made possible. Results can be referenced immediately against the comprehensive library provided as standard.

## Qualitative Analysis of a Primary Microplastic

Primary microplastic contained in a scrub was prepared as a sample and measured.

Microplastic was extracted from the scrub by dissolving the scrub in water and filtering it several times. Fig. 1 shows an optical microscopic image of the microplastic on the filter.

One piece of microplastic was taken from the filter and then compressed in a diamond cell for measurement by infrared transmission microspectroscopy. Fig. 3 shows the measurement result which indicates that the microplastic is polystyrene (PS).

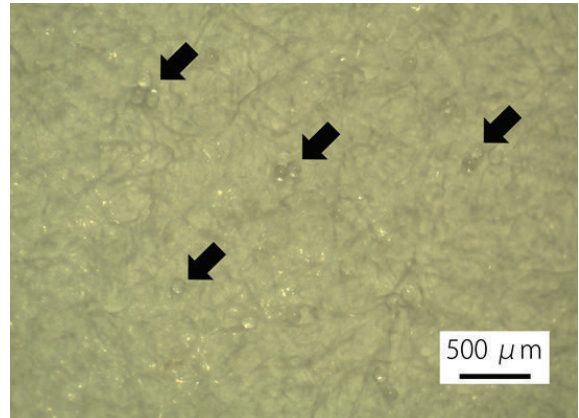


Fig. 1 Microplastic on Filter Paper (Indicated by black arrows)

### Measurement Conditions

Instrument	: IRTracer-100, AIM-9000
Resolution	: 8 $\text{cm}^{-1}$
Accumulation time	: 40
Apodization function	: Sqr-Triangle
Aperture size	: 50 $\mu\text{m}$ $\times$ 50 $\mu\text{m}$
Detector	: MCT

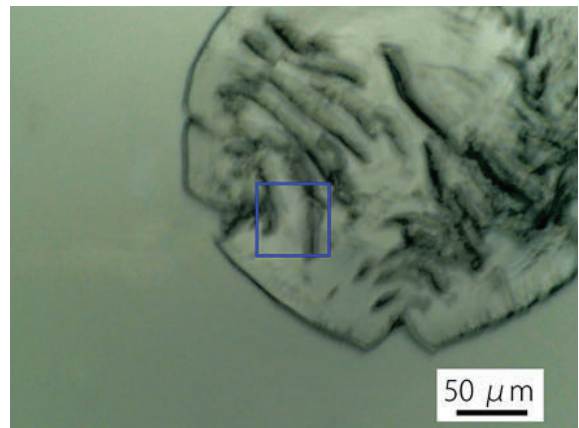


Fig. 2 Measurement Sample

## AIM-9000 Infrared Microscope

An infrared microscope system is capable of acquiring information about minute areas with high sensitivity by adjusting the infrared beam diameter using an aperture.



IRTracer-100 Fourier Transform Infrared Spectrophotometer (Left)  
AIM-9000 Infrared Microscope (Right)

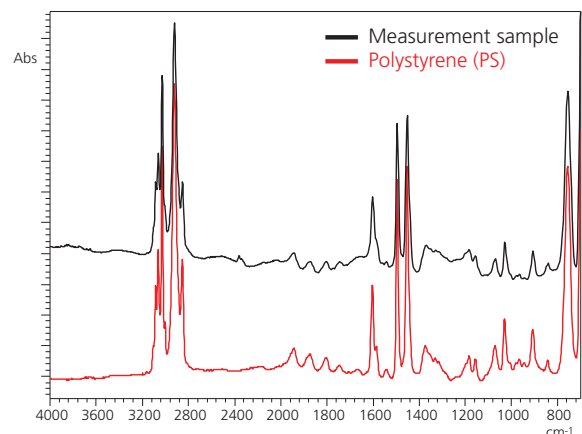


Fig. 3 Measurement Results

## Mapping Analysis of Secondary Microplastics

Secondary microplastics similar to those found in oceans and rivers were prepared as a sample and measured.

Microplastics dispersed in water were filtered and collected using a polytetrafluoroethylene (PTFE) filter. Since PTFE only has an infrared absorption band near  $1200\text{ cm}^{-1}$ , it is convenient for measuring transmission spectra with the sample left on the filter.

In order to identify the many microplastics collected on the filter, mapping analysis was performed using infrared transmission microspectroscopy. Fig. 4 shows an optical microscopic image of the microplastics on the filter. Figs. 5-(a) to (c) show the measurement results. The area of the PTFE filter where no sample was adhered was set as the background.

The measurements revealed that the microplastics in the sample were polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET). Figs. 5-(a) to (c) are colored based on corrected peak height values (the peak height from the baseline) of the characteristic peak of each plastic (PE:  $718\text{ cm}^{-1}$  caused by  $\text{CH}_2$  rocking vibrations, PP:  $2839\text{ cm}^{-1}$  caused by  $\text{CH}_2$  stretching vibrations, PET:  $1724\text{ cm}^{-1}$  caused by  $\text{C}=\text{O}$  stretching vibrations). Red areas indicate points where the plastic component is present in high amounts and blue areas indicate points with low amounts. Fig. 6 shows typical infrared spectra from the areas in Figs. 5-(a) to (c).

### Measurement Conditions

Instrument	: IRTracer-100, AIM-9000
Resolution	: $8\text{ cm}^{-1}$
Accumulation time	: 1
Apodization function	: Sqr-Triangle
Aperture size	: $50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m}$
Measurement interval	: $50\text{ }\mu\text{m}$
Mapping area	: $1800\text{ }\mu\text{m} \times 2600\text{ }\mu\text{m}$
Detector	: MCT

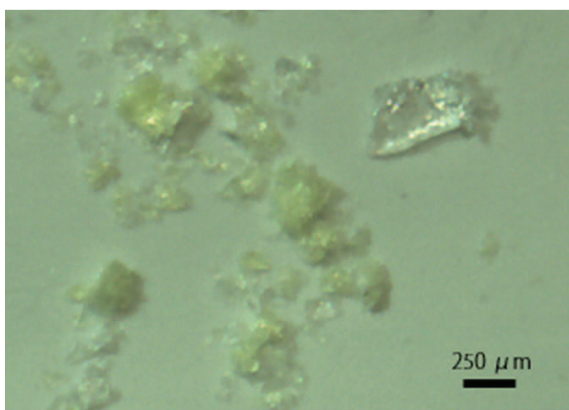


Fig. 4 Microplastics on Filter Paper

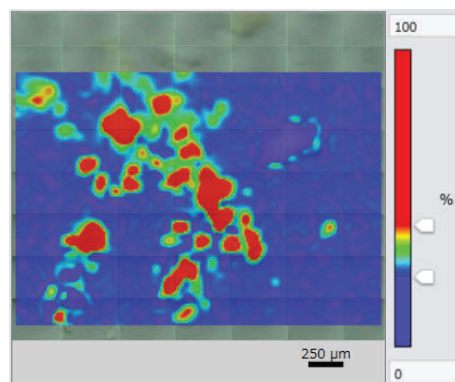


Fig. 5-(a) PE Distribution  
(Based on the corrected peak height value of  $718\text{ cm}^{-1}$ )

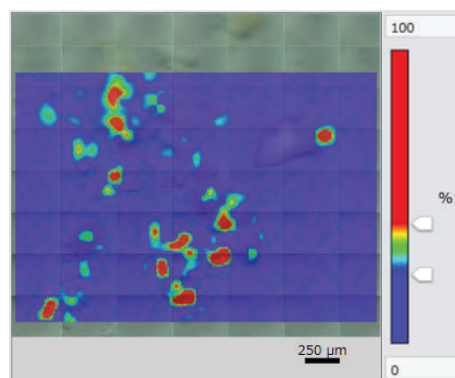


Fig. 5-(b) PP Distribution  
(Based on the corrected peak height value of  $2839\text{ cm}^{-1}$ )

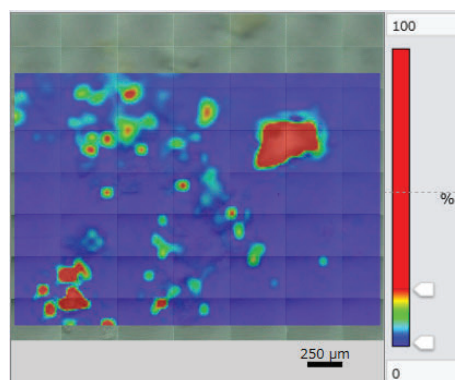


Fig. 5-(c) PET Distribution  
(Based on the corrected peak height value of  $1724\text{ cm}^{-1}$ )

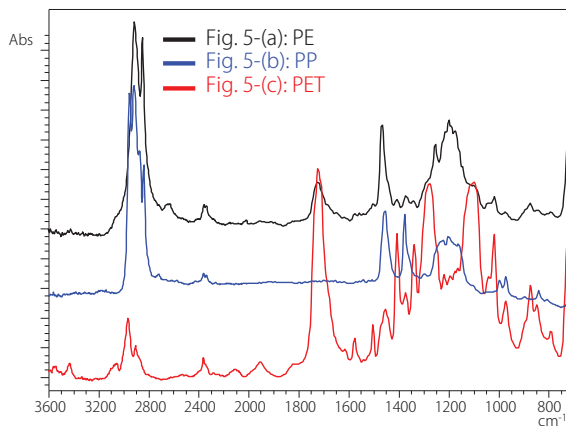


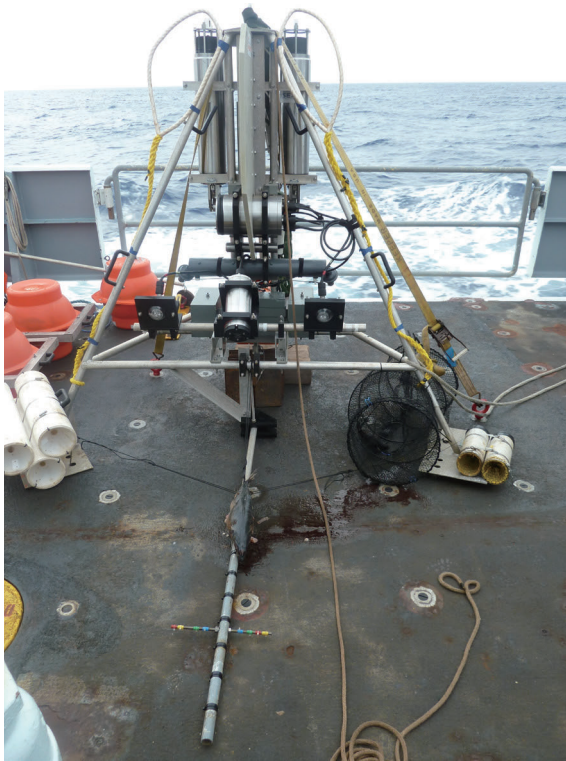
Fig. 6 Typical Infrared Spectra from Areas in Figs. 5-(a) to (c)

# Analysis of Microplastics Collected from Marine Species

Pollution by marine debris including microplastics has become a serious environmental problem, and scientists all over the world are exploring the situation by carrying out surveys on microplastics accumulated in marine species. The effects of marine debris have spread through the food chain to marine species such as polar cod living in the Arctic Ocean and deepwater shrimp (order Amphipoda), which should have been hard for the impact of pollution to reach. Microplastics have even been found in the polar ice.

A group of scientists from Newcastle University in the UK and Wageningen Marine Research in the Netherlands collected the stomach contents of various marine creatures and separated microplastics of approximately 100  $\mu\text{m}$  in size as part of a survey on the impact of marine debris <sup>(1)</sup>.

This article introduces an example of analysis using an infrared microscope on microplastics collected from polar cod and deepwater shrimp.



Marine Survey Equipment

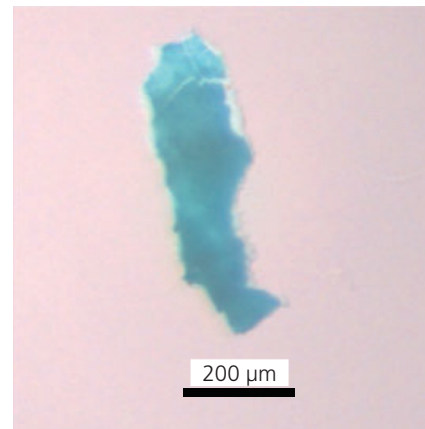


Fig. 1 Microplastic Collected from Polar Cod

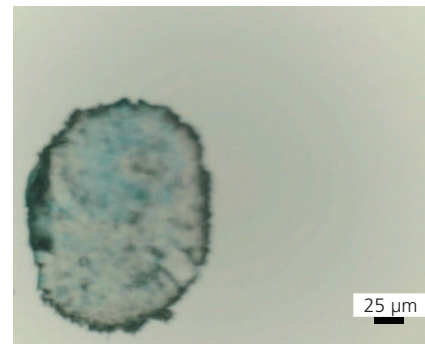


Fig. 2 Microplastic Collected from Deepwater Shrimp

## Sample Pretreatment and Precautions

Microplastics analysis requires scrupulous attention to prevent contamination of samples. Direct touching could transfer sebum or dust and contaminate samples. Microfibers from clothing and minute particles floating in the air could also contaminate samples.

If samples are contaminated, proteins and other residues need to be removed using an organic solvent or water. However, there is a possibility that the properties intrinsic to the samples may be lost by using an organic solvent.

In this experiment, samples were washed with potassium hydroxide solution which removes organic contaminants without affecting the samples.



Deepwater Shrimp

## Measurement Results

The blue microplastic piece collected from polar cod shown in Fig. 1 was measured with the microscopic ATR method (Fig. 3), and the microplastic piece collected from deepwater shrimp shown in Fig. 2 was compressed in a diamond cell and measured with the microscopic transmission method (Fig. 4).

From Fig. 3, we can see that the main component of the microplastic piece collected from polar cod was PMMA (polymethylmethacrylate) and that kaolin (aluminum silicate) was included as an additive. PMMA is a tough, lightweight resin with excellent resistance to weather, water and impacts, and is therefore used for everyday items and miscellaneous goods.

From Fig. 4, it can be seen that the main component of the microplastic piece collected from deepwater shrimp was a mixture of PE (polyethylene), CaCO<sub>3</sub> (calcium carbonate) and kaolin (aluminum silicate). PE is a common general-purpose resin used for packing materials and containers. It is often detected in microplastics.

### Measurement Conditions

Instrument	: IRTracer-100, AIM-9000
Resolution	: 8 cm <sup>-1</sup>
Accumulation time	: 100 times (Fig. 1), 50 times (Fig. 2)
Apodization function	: Happ-Genzel (Fig. 1) Sqr-Triangle (Fig. 2)
Aperture size	: 25 μm × 25 μm (Fig. 1) 15 μm × 15 μm (Fig. 2)
Detector	: MCT

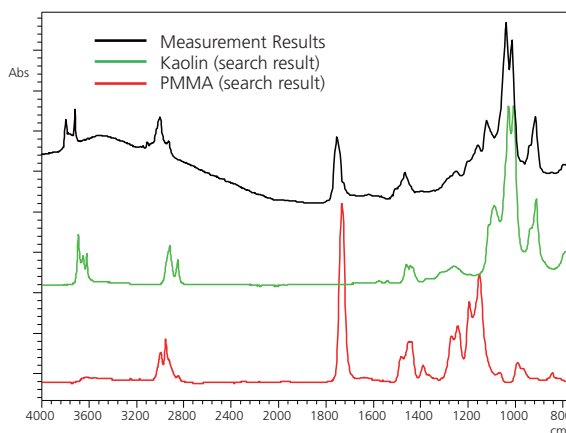


Fig. 3 Measurement Results for Microplastic Collected from Polar Cod

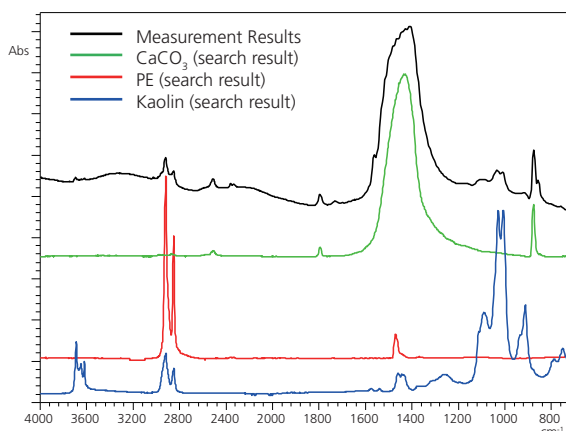


Fig. 4 Measurement Results for Microplastic Collected from Deepwater Shrimp

### AIM-9000 Infrared Microscope

For analysis of microplastic particles of sizes from several tens to hundreds of μm, the resin components and additive components can be quickly determined by using an infrared microscope that enables qualitative observations of organic matter and some inorganic matter.



IRTracer-100 Fourier Transform Infrared Spectrophotometer (Left)  
AIM-9000 Infrared Microscope (Right)

### References

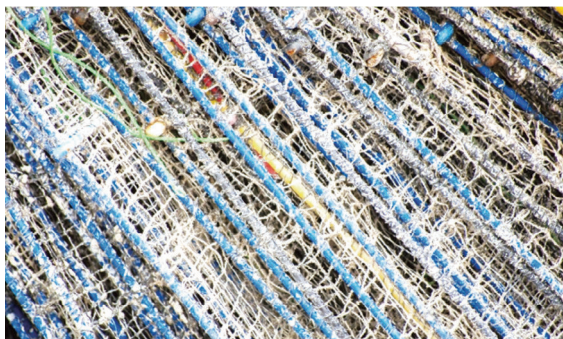
- (1) In every ocean, at every depth - microfibrers and microplastics, Micro FTIR analysis of smallest particles from deep sea to polar ice; Susanne Kühn, Wageningen Marine Research, The Netherlands Alan Jamieson, Newcastle University, Great Britain Robert Keighley, SUK, Great Britain Marion Egelkraut-Holtus, Shimadzu Europa GmbH, Germany, SHIMADZU NEWS, 2. 2018

## Qualitative and Elemental Analysis of Marine Debris

Fishing nets, trawling nets, fishing lines, etc., used to be made from natural materials, but nowadays they are usually made of synthetic resin, which provides greater functionality. However, when such materials are not properly managed or disposed of, they become marine debris and are a factor in environmental destruction. Therefore, it is hoped that this kind of marine debris will be collected and reused as a raw material for new fishing equipment.

### Effects of Copper on Fish and Challenges in Recycling

The causes of deterioration and breakup of fishing nets are contact with fish, algae, and stones in the sea and on beaches, as well as ultraviolet radiation from the sun. An example of a way to prevent this kind of naturally occurring damage is to give the surface of fishing nets used for aquaculture a protective coating of copper (1). In the past, paints containing toxic tributyltin (antifouling paints for ships) were used as a protective coating on fishing nets, but copper came to be used instead in an effort to protect the environment. The heavy element copper (Cu) has an antibacterial effect and not only protects against bacteria and viruses but also has the function of preventing fouling, so it plays an important role in the manufacture of fishing nets. However, in recent years adverse effects of copper on fish have also been reported, and Marina Nikolaou et al. report in their literature that these effects are manifested at 58 mg of copper sulfate per liter in the case of tilapia (freshwater fish from the family Cichlidae in the order Perciformes), and 70 mg per liter in the case of catfish. It is suggested that exposure of fish to high concentrations of copper sulfate over a long period of time may lead to damage to the gills, liver, kidneys and nervous system (2).



Fishing Net Used for Aquaculture

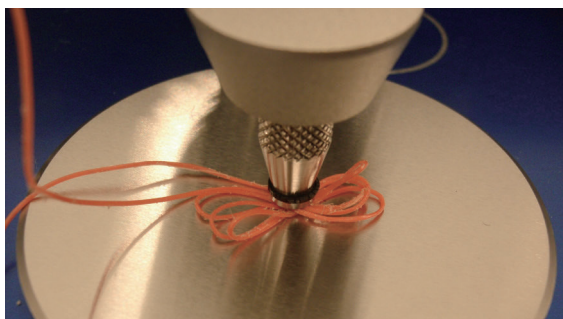


Fig. 1 ATR Measurement in Progress

### Measurement Samples and Conditions

The measurement samples were fishing nets collected on the beach of Majorca island in Spain and others collected at a recycling plant. These samples were analyzed without undergoing any processing or special pretreatment.

The ATR method of FTIR stands for Attenuated Total Reflection. By measuring all of the light reflected by the sample surface, the absorption spectrum of the sample surface can be obtained. The penetration depth of the light is several  $\mu\text{m}$ .

Fluorescent X-ray spectroscopy is a technique for analyzing the composition of a sample by irradiating it with X-rays and measuring the X-ray fluorescence generated from the elements contained in it. A 3 mm $\varnothing$  collimator (irradiation diameter) was selected in accordance with the size of the sample.

#### FTIR Measurement Conditions

Instrument	: IRTracer-100, Quest (Diamond prism)
Resolution	: 4 $\text{cm}^{-1}$
Accumulation time	: 45
Apodization function	: Happ-Genzel
Detector	: DLATGS

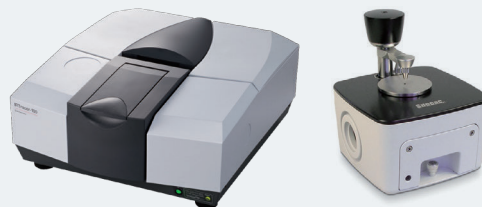
#### EDX Measurement Conditions

Instrument	: EDX-8000
X-Ray tube target	: Rh
Voltage / current	: 50 kV (Al-U) / Auto, 15 kV (C-Sc) / Auto
Atmosphere	: Vacuum
Analysis diameter	: 3 mm $\varnothing$
Filter	: None
Integration time	: 50 seconds

#### IRTracer-100 Fourier Transform Infrared Spectrophotometer

#### EDX-8000 X-ray Fluorescence Spectrometer

FTIR can determine the materials of the filaments used in fishing nets and fishing lines, while EDX facilitates the elemental analysis of copper, etc., used in protective coatings, so both these instruments can be utilized in the management of recycled materials.



IRTracer-100 (Left), Quest (Right)



EDX-8000



## Measurement Results

The results of measurement using FTIR showed that, for the samples in Figs. 2 (a) and (b), polyethylene was the main component in many cases, and the other components were polypropylene and, as additives, calcium carbonate and silicate. From the results of qualitative quantitative analysis by EDX, it was found that the Cu content of the samples was less than 0.03 wt%, and that they had no Cu protective coating.

On the other hand, it was found that various types of polymer including polyethylene, polypropylene and polyamides were used in the samples in Figs. 2 (c) and (d). In addition, the Cu content was estimated to be 15 wt% in the sample in Fig. 2 (c), and 8 wt% for that in Fig. 2 (d), which is more than others, so it could be inferred that it was part of a fishing net with a Cu protective coating.

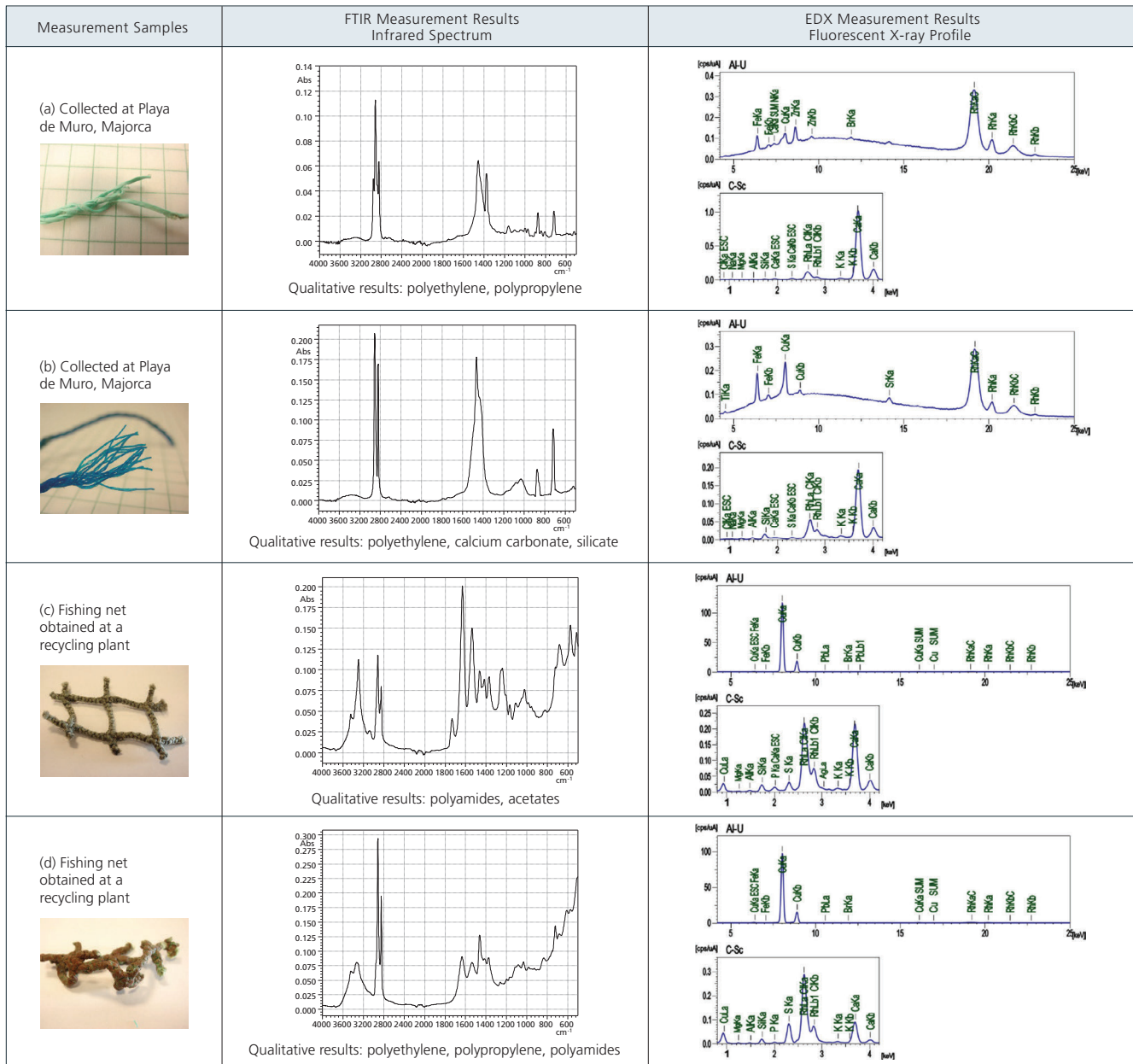


Fig. 2 Measurement Samples and Measurement Results

### References

- (1) "Biofouling in the Marine Aquaculture Industry, with Particular Reference to Finfish – Current Status and Future Challenges", Mark G. J. Hartl, Douglas Watson and John Davenport, Nov. 2006, Marine Estate Research Report, (AQU/06/03), The Crown Estate
- (2) "Fish farming and anti-fouling paints: a potential source of Cu and Zn in farmed fish", Marina Nikolaou, Nikos Neofitou, Konstantinos Skordas, Ioanna Castritsi-Catharios, Lamprini Tziantziou, June 2014, Vol.5: 163-171, Aquaculture environment interactions

### Acknowledgments

We would like to thank Albert van Oyen (Carat GmbH, Bocholt, Germany), who is a collaborative researcher.

## Qualitative and Elemental Analysis of Plastic Pellets

Large quantities of water are consumed for both domestic and industrial use during the course of daily life. Although the Earth has abundant water resources, the amount of safe, drinkable water within these resources is extremely limited. Securing drinking water through the process of recycling wastewater is therefore a crucial issue.

The water treatment processes employed at wastewater treatment plants involve primary treatment for physically separating and removing solid material (physical treatment) and secondary treatment for removing organic matter using microorganisms (biological treatment). During biological treatment, cultivated microorganisms feed on the organic matter dissolved and suspended in wastewater which results in the oxidative decomposition of the organic matter. Plastic pellets (carriers for water treatment) serve the function of holding the microorganisms on the surface to improve purification of the waste water. Plastic pellets play a role in purifying water used in a wide range of applications. However, there is concern that these pellets may turn into marine pollution (microplastics etc.) by flowing into the sea and river systems when heavy rain or other circumstances cause wastewater to overflow.

This article introduces an example of using FTIR and EDX to analyze plastic pellets before and after use in water treatment.

### Measurement Samples and Conditions

Plastic pellets are approximately 5 mm in diameter, as shown in Fig. 1 (left). A large number of pores are visible upon inspection of the cross section as in Fig. 1 (right).

Fig. 2 shows the measurement samples of unused and used plastic pellets. The used pellets have lost their original shape and exhibit significant unevenness on their surface.

Analysis was performed using a system comprised of an IRTracer-100 Fourier transform infrared spectrophotometer connected to a Quest single-reflection ATR accessory, and an EDX-8000 energy dispersive X-ray fluorescence spectrometer. There was no processing or special pretreatment of samples before analysis.



Fig. 1 (Left) Approx. 5-mm Diameter Plastic Pellet  
(Right) Cross Section



Fig. 2 (Left) Unused (Right) Used

#### FTIR Measurement Conditions

Instrument	: IRTracer-100, Quest (Diamond prism)
Resolution	: 4cm <sup>-1</sup>
Accumulation time	: 100
Apodization function	: Happ-Genzel
Detector	: DLATGS

#### EDX Measurement Conditions

Instrument	: EDX-8000
X-Ray tube target	: Rh
Voltage / current	: 50 kV (Al-U) / Auto, 15 kV (C-Sc) / Auto
Atmosphere	: Vacuum
Analysis diameter	: 10 mmø
Filter	: None
Integration time	: 100 seconds

### IRTracer-100 Fourier Transform Infrared Spectrophotometer EDX-8000 X-ray Fluorescence Spectrometer

Since FTIR is capable of performing qualitative analysis of organic matter and some inorganic matter, this method was able to quickly identify the main components of plastic pellets. Moreover, the element information obtained using EDX was able to reveal the existence of contaminants and wear due to additives as well as minute material differences.



IRTracer-100 (Left), Quest (Right)

EDX-8000

## Measurement Results

Fig. 3 shows the results of measurement with FTIR and EDX. Measurement with FTIR was performed on both the sample surface and a cross section.

From the results of FTIR measurement, we found that the surface is a mixture of polyethylene and cellulose and the cross section is polyethylene for both the unused and used plastic pellets.

The results of qualitative and quantitative analysis using EDX show that <sup>15</sup>P (red frame in Fig. 3) was detected in the unused plastic pellets but not in the used pellets. However, since there were no significant differences in composition between the two samples, we consider that contaminants that adhered to the surface or components that rubbed against the samples during use may be present in trace amounts.

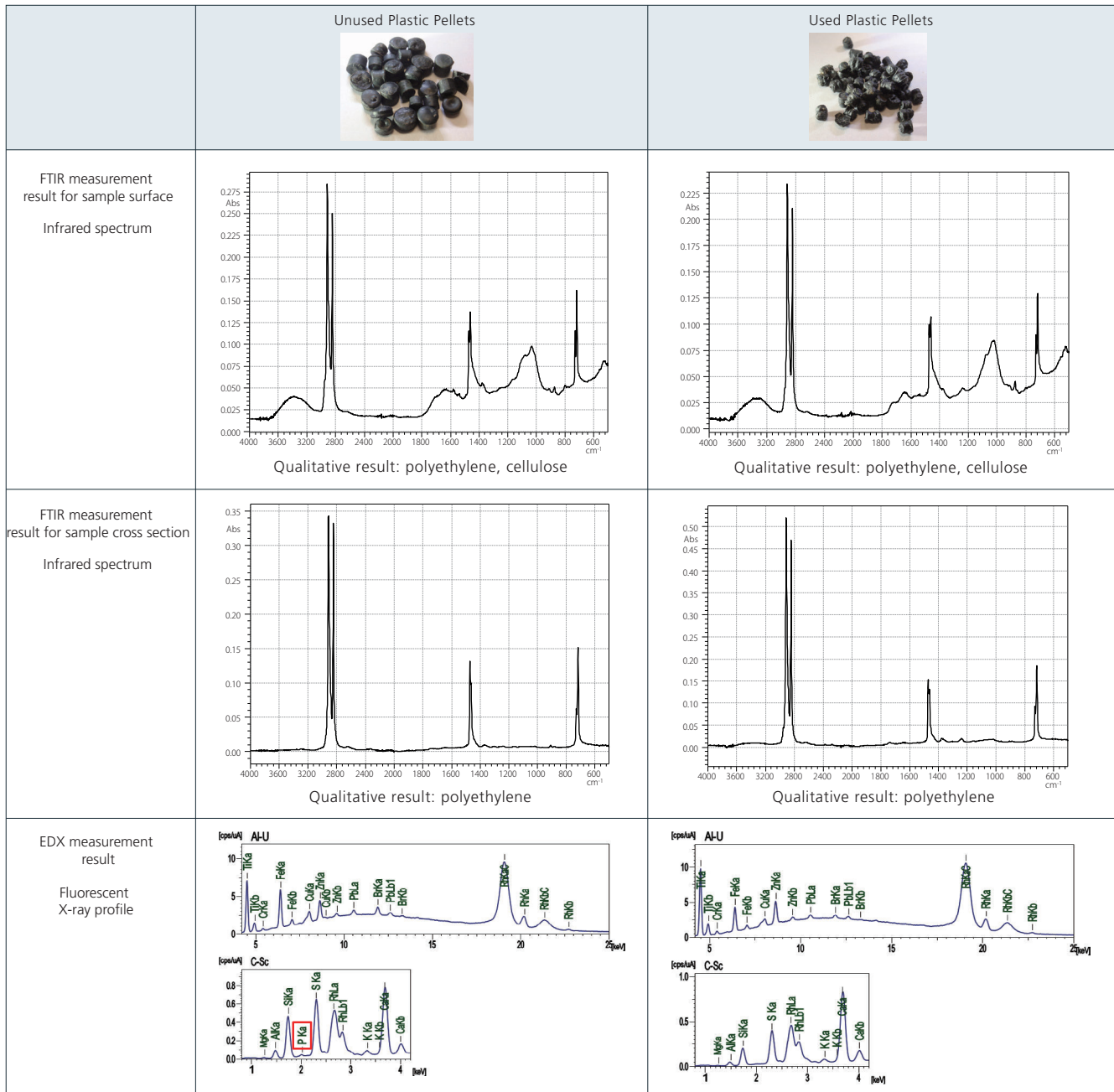


Fig. 3 Measurement Results

### Acknowledgments

We would like to thank Albert van Oyen (Carat GmbH, Bocholt, Germany), who is a collaborative researcher.

A dynamic particle image analysis system, which can automatically detect particles with sizes of 5 to 100  $\mu\text{m}$  and analyze their shape and particle count concentration in a short time, is suitable for analysis of the shape and particle count concentration (particles/mL) of microplastics dispersed in solutions. An infrared microscope, which excels in the analysis of organic compounds, is suitable for qualitative analysis of microplastics with sizes of 100  $\mu\text{m}$  or less that can be captured with filter paper.

Here we introduce an example of analysis of the shape and particle count concentration of particles contained in environmental water and their qualitative analysis with a dynamic particle image analysis system and an infrared microscope.

## Analysis of Shape and Particle Count Concentration of Particles

Environmental water containing microplastics was used as the sample, and the shape and particle count concentration of the particles contained in the sample were analyzed with an iSpect DIA-10. Fig. 1 shows some of the acquired particle images.

From Fig. 1, the shapes of particles with sizes of 100  $\mu\text{m}$  and less have been captured clearly, and various shapes, such as rodlike and fibrous shapes, can be confirmed.

Fig. 2 and Fig. 3 show the scattergram (scatter diagram) and histogram (frequency distribution graph), respectively (range shown on horizontal axis: 10 to 100  $\mu\text{m}$ ). Scattergrams and histograms can be prepared by selecting two desired measurement items (e.g., maximum length, aspect ratio, circularity). The results showed that the particle count concentration was 5,309 particles/mL. The average size was 24.315  $\mu\text{m}$ , and the largest number of particles were in the size range of 10 to 30  $\mu\text{m}$ , as shown by the red box in Fig. 3.



Fig. 1 Particle Images

### Measurement Conditions

Instrument	: iSpect DIA-10
Frame rate	: 8 fps
Analysis flow rate	: 0.1 mL/min
Total analyte	: 150 $\mu\text{L}$

### iSpect DIA-10 Dynamic Particle Image Analysis System

The iSpect DIA-10 measures particles by the microcell method, in which image acquisition efficiency is enhanced by passing the particles through a narrow imaging area. Because fewer particles pass outside the imaging area (to the right or left sides), blurring in the front and back directions is small in comparison with the conventional method, and virtually all particles are captured, the microcell method enables highly reliable particle detection. Moreover, this method supports the measurement of volumes as low as 50  $\mu\text{L}$ , which is useful in the case of samples that are small or difficult to obtain.



iSpect DIA-10

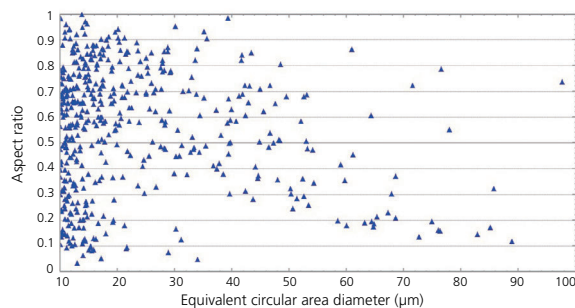


Fig. 2 Scattergram (Scatter Diagram)

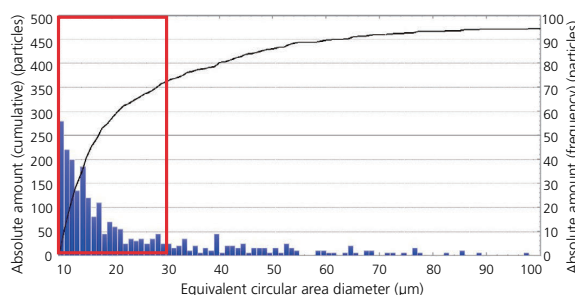


Fig. 3 Histogram (Frequency Distribution Graph)

## Qualitative Analysis of Microplastics

After the measurements with the iSpect DIA-10, the particles contained in the sample were captured with polytetrafluoroethylene (PTFE) filter paper and a mapping analysis was carried out with the AIM-9000.

Fig. 4 shows the visual observation image. As a result of the qualitative analysis of the infrared spectrum (Fig. 5) of the area in the red circle in Fig. 4, this particle was identified as polypropylene (PP).

Next, Fig. 6 shows a chemical image prepared using the corrected area value (area value of peak above the baseline) of the characteristic peak of PP in the range of 1,400 to 1,339  $\text{cm}^{-1}$  ( $\text{CH}_3$  bending vibration). Areas with high PP content are in red, and those with low PP content are in blue. This result confirmed that all of the rod-like microplastics that can be observed in the visual observation image are PP.

### Measurement Conditions

Instrument	: IRTracer-100, AIM-9000
Resolution	: 8 $\text{cm}^{-1}$
Accumulation time	: 40
Apodization function	: Sqr-Triangle
Aperture size	: 20 $\mu\text{m}$ $\times$ 20 $\mu\text{m}$
Mapping area	: 460 $\mu\text{m}$ $\times$ 1780 $\mu\text{m}$
Detector	: MCT

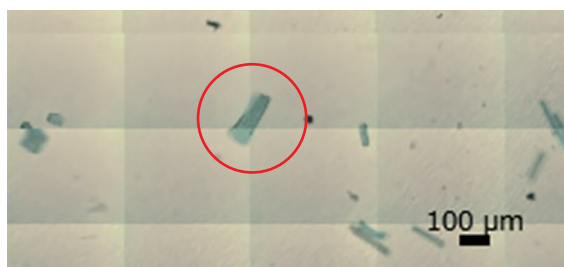


Fig. 4 Visual Observation Image

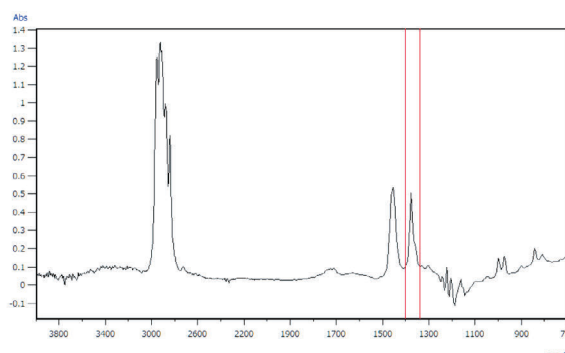


Fig. 5 Infrared Spectrum of Rod-like Microplastics  
(The red box shows the peak used in preparing the chemical image in Fig. 6.)

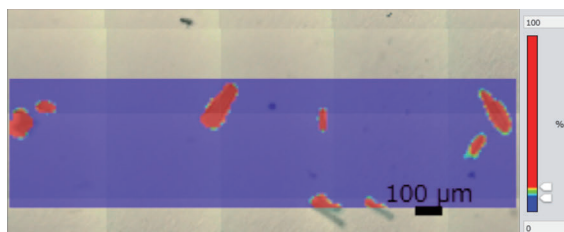


Fig. 6 Distribution of PP  
(Corrected Area Value of 1,400-1,339  $\text{cm}^{-1}$  Peak)

### AIM-9000 Infrared Microscope

The infrared microscope system makes it possible to acquire information on microscopic areas with high sensitivity by using the aperture to narrow the infrared light beam to the designated size. Visual observation of microplastics on filter paper is also easy using the digital zooming function of the Shimadzu proprietary wide-field camera (optional) and microscope camera (with magnification up to 330x).



IRTracer-100 Fourier Transform Infrared Spectrophotometer (Left)  
AIM-9000 Infrared Microscope (Right)

### Acknowledgments

We wish to express our deep gratitude to Associate Professor Yutaka Kameda of the Chiba Institute of Technology, who provided the samples used in these measurements and shared his knowledge of microplastics.

# Determination of Component Ratios for Blended Plastic Samples

Polymeric materials are often created by blending two or more types of polymers in order to obtain properties which cannot be obtained with a single type. Mechanically blended polymeric materials show the characteristics of each component, such as melting and crystallization, so multiple changes derived from each component can be observed through measurement with a DSC (differential scanning calorimeter).

If these materials are released to the environment, they become multiple types of plastic pollutants. In this case, the below measurement method can be used for the measurement of microplastics.

This article introduces the measurement results of blended plastic samples using low density polyethylene (LDPE), high density polyethylene (HDPE) and polypropylene.

## Methods Using Individual Heats of Fusion

### Using the Heat of Fusion of Individual Components

The content of LDPE and PP was determined from the individual heat of fusion of each component, and the heat of fusion of each component in the blend sample.

The blend sample data in Fig. 1 shows a sufficient temperature difference between the melting peaks of LDPE and PP to allow for separate detection. In this case, the content can be determined by simply dividing the heat of fusion (mJ) of the unknown sample by the known heat of fusion (J/g) of a component.

We prepared a sample with a known blend ratio of LDPE:PP = 80:20 and verified the component ratio using this method. A satisfactory result of 79.1 %:20.9 % was obtained with respect to the 80:20 ratio of LDPE and PP.

### Using an Approximated Heat of Fusion

The component ratio of a sample was determined from the measured melting peaks for HDPE and PP, and a blend of these two components.

Since the melting peaks of HDPE and PP are detected at very close temperatures, the peaks overlap and cannot be completely separated. In this case, the peaks are divided laterally as shown in Fig. 2 and the heat quantity (approximated) of each peak is calculated as the heat of fusion of each component. (An optional partial area analysis program is required to perform this calculation.)

Fig. 3 shows the data obtained using this method. The heat of fusion of 100 % HDPE and PP, and the approximated heat of fusion of each component in the blend sample were used for the same calculation as in the previous section. Measurement results that provide a roughly close ratio of HDPE:PE = 85.1 %:14.9 % with respect to the actual blend ratio of HDPE:PE = 79.7 %:20.3 % were obtained. Note that significant overlapping of the two peaks reduces accuracy.

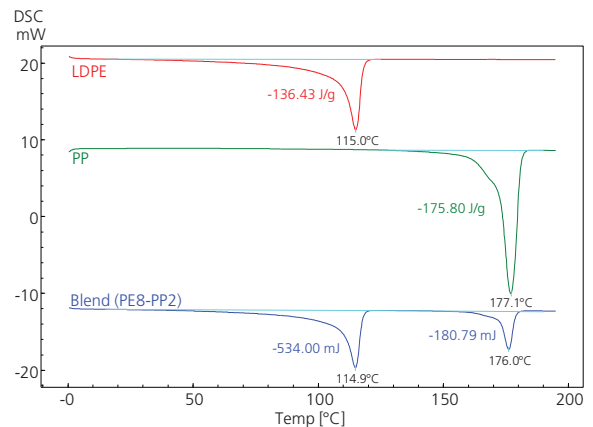


Fig. 1 DSC Curves of LDPE, PP, and Blend Sample

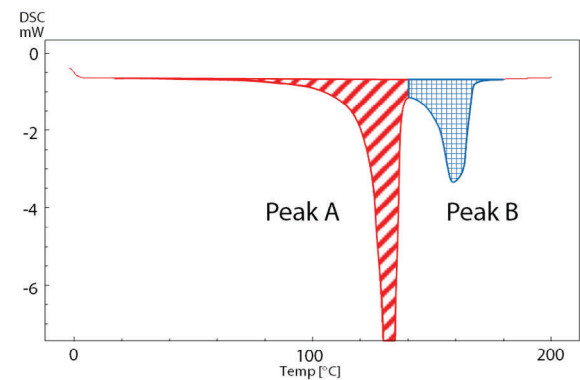


Fig. 2 Obtaining the Approximate Heat of Fusion

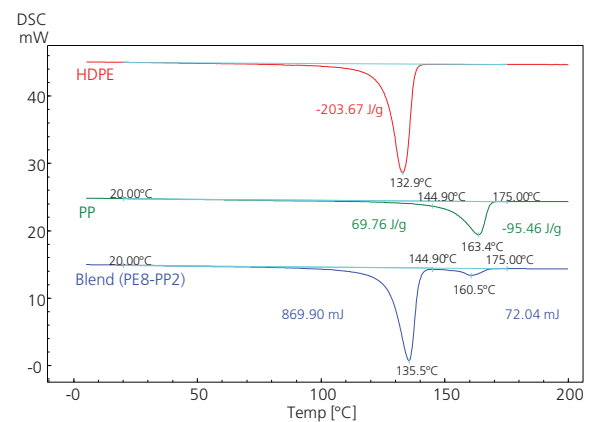


Fig. 3 DSC Curves of HDPE, PP, and Blend Sample

## Method Using the Total Heat of Fusion

In the case when peaks overlap, the content ratio can be determined from the total heat of fusion (value obtained through a single integration of the area of both peaks, as shown in Fig. 4) as an alternative to the method using an approximated heat of fusion as described in the previous section.

First, a calibration curve is created by plotting and joining the heats of fusion of the separate 100 % samples with a straight line, as shown in Fig. 5. This calibration curve expresses the relationship between the blend ratio and the total heat of fusion. The blend ratio can be determined from the measurement results for total heat of fusion by using this calibration curve.

For example, a total heat of fusion of 200.68 (J/g) corresponds to a component ratio of PE:PP = 77.4:22.6. Table 1 compiles the results of blend ratios determined from the total heat of fusion using this method with respect to samples A through E (which have known blend ratios).

This shows that an approximate blend ratio can be obtained from the total heat of fusion measured using a DSC and a calibration curve.

Table 1 Samples of Various Blend Ratios and Measurement Results

Sample	Blend Ratio		Total Heat of Fusion (J/g)	Result Determined from Calibration Curve	
	HDPE	PP		HDPE	PP
PP	0.00	100.00	97.07	0.00	100.00
A	19.72	80.28	122.11	18.70	81.30
B	40.64	59.36	151.95	40.99	59.01
C	49.90	50.10	159.98	46.99	53.01
D	60.16	39.84	174.53	57.86	42.14
E	79.88	20.12	200.68	77.40	22.60
HDPE	100.00	0.00	230.94	100.00	0.00

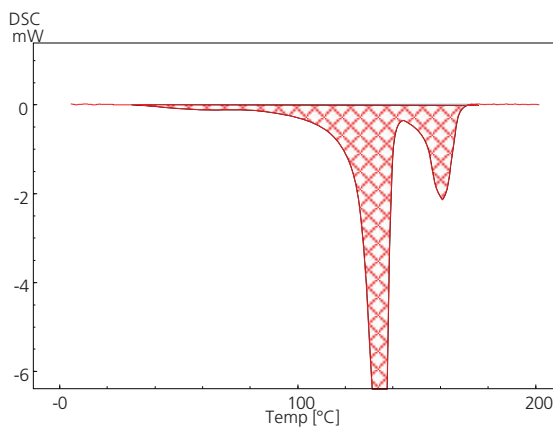


Fig. 4 Total Heat of Fusion of the Blend Sample

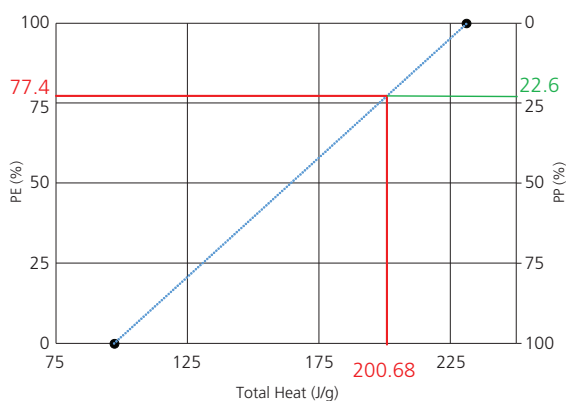


Fig. 5 Calibration Curve Determined from Separate HDPE and PP Samples

## DSC-60 Plus Series Differential Scanning Calorimeter

The temperature difference between a standard substance and a test sample is measured while applying constant heat. The endothermic reactions and exothermic reactions are measured, and can then be used for physical evaluation of polymer materials, metals, etc. When microplastics are measured, the types can be identified and component percentages obtained.



DSC-60 Plus

# Analysis of Toxic Chemical Substances Adsorbed on Microplastics

There is a possibility that toxic chemical substances adsorbed on microplastics (MPs) in the environment may impact the ecosystem by desorbing from the MPs, migrating to the bodies of living organisms, and becoming concentrated in those organisms.

The Shimadzu Group has been involved in evaluations of the adsorption characteristics of chemical substances on MPs as part of analysis of MPs. Here we introduce an example of an evaluation of the adsorption characteristics of polycyclic aromatic hydrocarbons (PAHs) and per- and polyfluoroalkyl substances (PFAS), which are known to have toxicity and bioaccumulation properties. Using commercially-available particulate polypropylene (PP), polystyrene (PS), and polyethylene (PE) as MP samples, the adsorption of PAHs and PFAS on the various plastics in water was evaluated quantitatively by GC-MS/MS and LC-MS/MS.

## MP Samples

The following three types of plastic particles were used as the MP samples.

- 1) Polypropylene (PP)
- 2) Polystyrene (PS)
- 3) Polyethylene (PE)

The major and minor axes of each type of particle were calculated from stereoscopic microscope images to confirm that the size of the materials used was less than 5 mm, which is the definition of MPs. The following instruments were used in the measurements.

- Microscope digital camera system : Moticam 1080
- Stereoscopic microscope : STZ-171-TP

## Test Method

First, an adsorption test of PAHs and PFAS (per- and polyfluoroalkyl substances\*) on the MP samples was conducted (Fig. 1). The MPs were immersed in water, to which PAHs or PFAS had been added, and the water was stirred gently for 24 h to promote adsorption. The amounts added to MPs were 100 ng of PAHs and 8 ng of PFAS in 300 mL of ultrapure water.

After the adsorption test, the MPs were removed from the test system and dried. With some of the samples, ultrasonic extraction by hexane was used as a pretreatment for the PAHs, and ultrasonic extraction by methanol was used as a pretreatment for the PFAS. The extracts obtained here were injected into the GC-MS/MS and LC-MS/MS, respectively, for quantitative analysis of the PAHs and PFAS.

\* For analogs, refer to EPA Method 537.1 and EPA Method 533.

Table 1 MP Samples

Material	Polypropylene (PP)	Polystyrene (PS)	Polyethylene (PE)	
Appearance				
Microscopic image				
Size [mm]	Major axis	4.899±0.151	3.191±0.149	4.331±0.194
	Minor axis	3.606±0.151	2.888±0.144	3.564±0.125

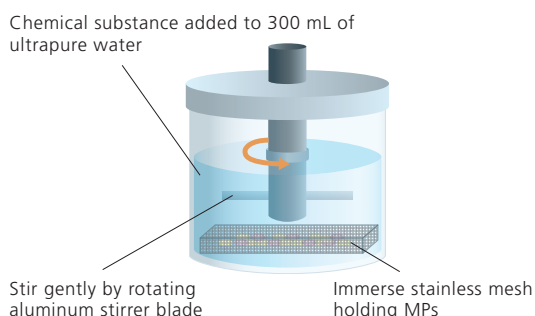


Fig. 1 Outline of Adsorption Test System

GCMS-TQ8040 NX  
Triple Quadrupole Gas Chromatograph  
Mass Spectrometer



LCMS-8060NX  
Triple Quadrupole Liquid Chromatograph  
Mass Spectrometer

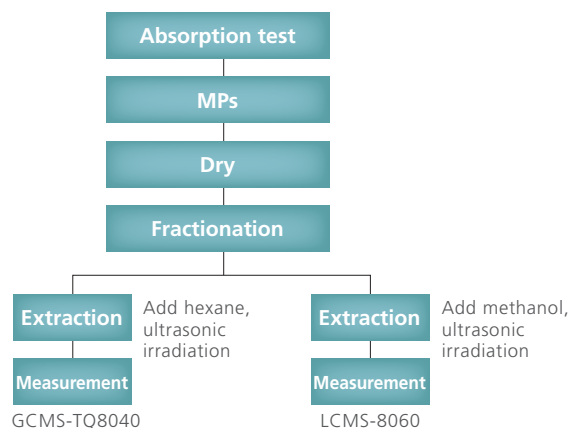


Fig. 2 Outline of Test Workflow



## Measurement of PAHs with GC-MS/MS

For measurement of the PAHs, a GCMS-TQ8040 triple quadrupole gas chromatograph mass spectrometer was used. The measurements were carried out in the high-selectivity MRM mode. Based on the calibration curve linearity and peak S/N ratio, the lower limit of quantitation of the compounds was assumed to be 0.5 to 2 ng/mL. Fig. 3 shows the MS chromatogram of the PAHs standard solution (2 ng/mL each). Quantitative calculations were performed by the internal standard method.

### Measurement Conditions

#### GC

Column : DB-5ms (30 m x 0.25 mm (id), 0.25  $\mu$ m)  
 Column oven temp. program : 60 °C (1 min) - 15 °C/min - 200 °C (0 min)  
 - 8 °C/min - 320 °C (10 min)  
 Injection mode : Splitless  
 Vaporizing chamber temp. : 300 °C  
 Injection volume : 2  $\mu$ L  
 Carrier gas : Helium (1.0 mL/min)

#### MS

Ionization method : EI  
 Ionization voltage : 70 V  
 Interface temp. : 300 °C  
 Measurement mode : MRM

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benzo[a]anthracene	Chrysene	Benzo[b]fluoranthene	Benzo[k]fluoranthene	Benzo[a]pyrene	Indeno[1,2,3-cd]pyrene	Dibenzo[a,h]anthracene	Benzo[ghi]perylene

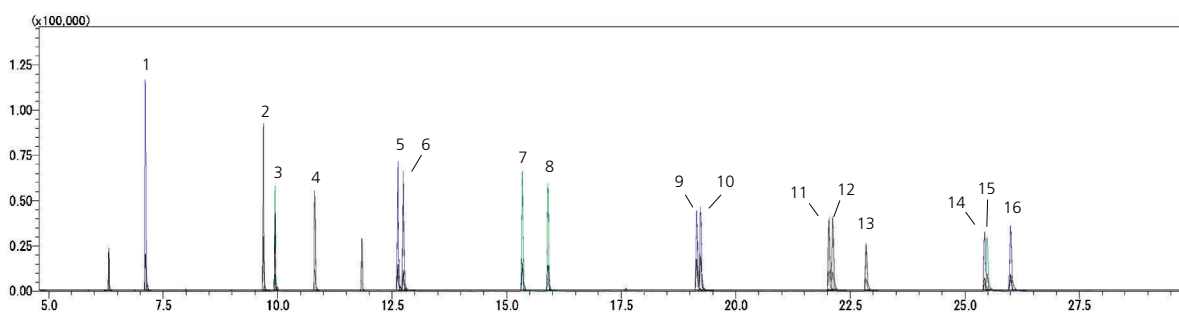


Fig. 3 MRM Chromatogram of PAHs Standard Solution (2 ng/mL each) (only target substances)

## Measurement of PFAS with LC-MS/MS

For measurement of the PFAS, a LCMS-8060 triple quadrupole liquid chromatograph mass spectrometer was used. The measurements were carried out in the high selectivity MRM mode. Based on the calibration curve linearity and peak S/N ratio, the lower limit of quantitation of the compounds was assumed to be 0.01 to 0.5 ng/mL. Fig. 4 shows the MS chromatogram of the PFAS standard solution (0.5 ng/mL each). Quantitative calculations were performed by the internal standard method.

### Measurement Conditions

#### LC

Column : Inertsil ODS-SP (150 mm x 2.1 mm (id), 3  $\mu$ m)  
 Column temp : 40 °C  
 Injection volume : 10  $\mu$ L  
 Mobile phase A : 10 mmol/L ammonium acetate aqueous solution  
 Mobile phase B : Acetonitrile  
 Mobile phase flow rate : 0.2 mL/min

#### MS

Ionization method : ESI  
 Polarity : Negative Measurement mode  
 Measurement mode : MRM

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	PFHxA	PFBS	PFHpA	PFPeS	PFOA	PFHxS	PFNA	PFHpS	PFDA	PFOS	PFUnDA	PFNS	PFDODA	PFDS	PFTeDA	PFDoS	PFHxDA	PFocDA	

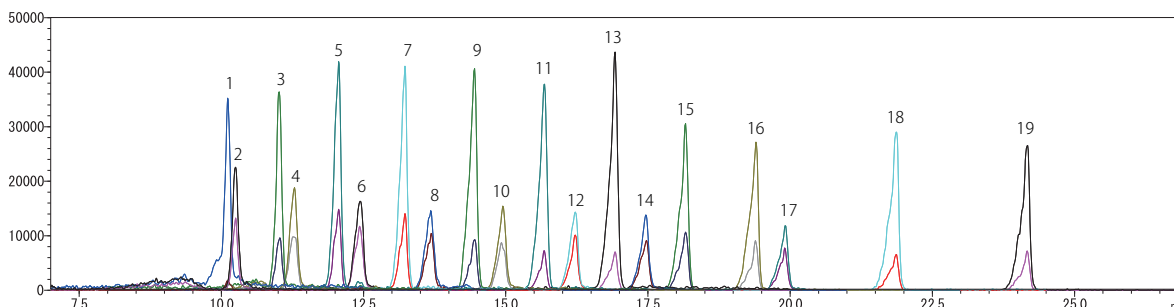


Fig. 4 MRM Chromatogram of PFAS Standard Solution (0.5 ng/mL each) (only target substances)

## Analysis Results

Fig. 5 shows the results of the analysis of PAHs with GC-MS/MS, and Fig. 6 shows the results of the analysis of the PFAS by LC-MS/MS. Adsorption on the MPs was confirmed for all PAH substances and for some PFAS substances. Adsorption of the PAHs on PP and PE tended to be large. However, the adsorption of the PFAS tended to differ for each chemical substance. Because the adsorption characteristics on MPs differed depending on the chemical substance, it is thought that some are easily affected by the type of MP, that is, its molecular structure, while others are not significantly affected.

Adsorption of chemical substances on MPs is considered to depend mainly on their hydrophobic interaction. Therefore, we examined the correlation between Log Kow<sup>(\*)</sup> and Log D<sup>(\*\*)</sup>, which show the hydrophobicity of chemical substances, and chemical substances transfer ratio to MPs. The transfer ratio to MPs was defined as the value obtained by dividing the amount of PHAs or PFAS adsorbed on the MPs by the amount of those substances added to water. Fig. 7 shows the relationship between the hydrophobicity of the chemical substances and the transfer ratio to the MPs. From top down, this figure shows the transfer ratios to PP, PS, and PE. In these results, the transfer ratio showed a tendency to increase with higher values of Log Kow and Log D, independent of the type of MPs.

(\*1) Log Kow : octanol/water partition coefficient

(\*2) Log D : Log Kow is the partition coefficient of a chemical substance considering the influence of electrolytic dissociation (ionization) in water. Here, this coefficient was calculated for water with pH 6.4 at the time of the test.

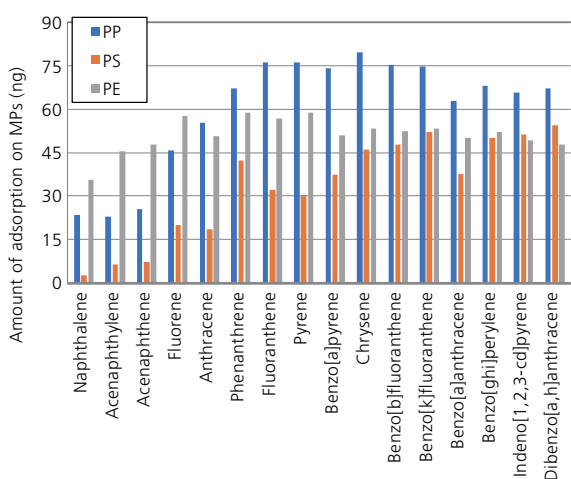


Fig. 5 GC-MS/MS Analysis Results: PAHs

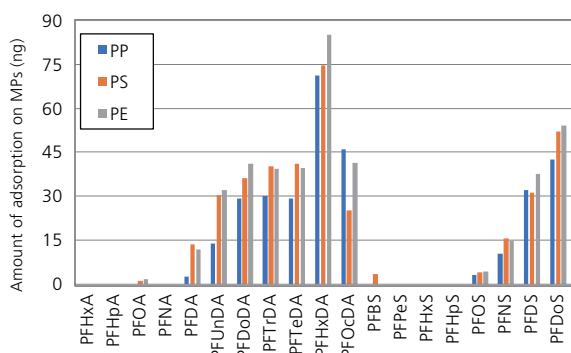


Fig. 6 LC-MS/MS Analysis Results: PFAS

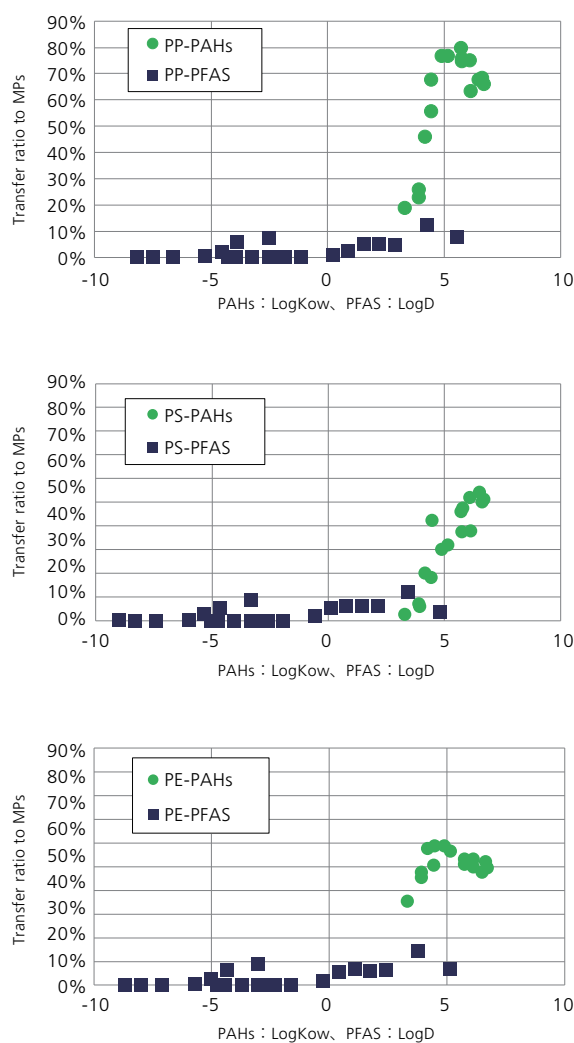


Fig. 7 Hydrophobicity of Chemical Substances and Transfer Ratios to MPs (Top: PP, Middle: PS, Bottom: PE)

## References

- (1) Makoto Yasojima, Haruna Mizuka, Takaki Mine, Hiroaki Takemori, Shinji Takeuchi, Yoshihide Yasui, "Adsorption Characteristics of Chemical Substances on Microplastics," Proceedings of the 22nd Symposium of the Japan Society on Water Environment (2019)
- (2) Makoto Yasojima, Haruna Mizuka, Takaki Mine, Hiroaki Takemori, "Existence of Unknown Chemical Substances Adsorbed on Microplastics Immersed in Rivers and Adsorption Characteristics of Chemical Substances on Microplastics," Proceedings of the 56th Environmental Engineering Research Forum (2019)

## Measurement of the impact of microplastics in the ocean

Dr. Alan Jamieson

Marine Science – School of Natural and Environmental Sciences,  
Newcastle University



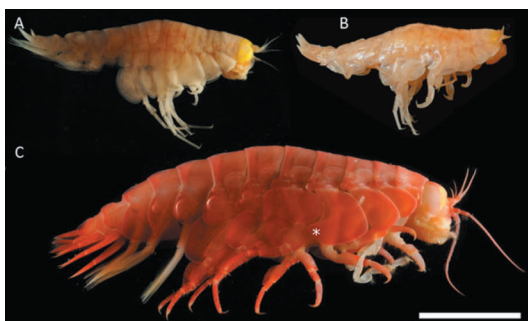
### Research and Achievement

My main research focus is the exploration of the deepest parts of the ocean, namely the 'hadal zone' which means anywhere deeper than 6,000 metres. There are usually large ultra-deep trenches that until recently we knew very little about. Our main goals are to study the ecology, habitats and connectivity between different trench communities and we have been really successful in having studied nine of these deep trenches, including the deepest place on Earth. In the process we have amassed a large sample archive, particularly samples of the crustacea amphipoda (hoppers). A couple of years ago we thought it would be interesting to investigate anthropogenic impacts at the greatest ocean depths. We did a study showing extraordinary high levels of persistent organic pollutants (PCBs and PBDEs) in the deepest samples. This was shocking and gained a huge international interest in the media. During that time many people were asking if these hadal animals showed any signs of having ingested plastic, which is of course a very concerning and hot topic at the moment. Having worked with Shimadzu we established that a saddening high level of these animals from 6 of the deepest trenches in world had indeed ingested plastics and through collaboration with Shimadzu in Milton Keynes, UK, we were able to identify the materials as well.

### Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth

#### Abstract

Whilst there is now an established recognition of microplastic pollution in the oceans, and the detrimental effects this may have on marine animals, the ocean depth at which such contamination is ingested by organisms has still not been established. Here we detect the presence of ingested microplastics in the hindguts of Lysianassoidea amphipod populations, in six deep ocean trenches from around the Pacific Rim (Japan, Izu-Bonin, Mariana, Kermadec, New Hebrides and the Peru-Chile trenches), at depths ranging from 7,000 m to 10,890 m. This illustrates that microplastic contaminants occur in the very deepest reaches of the oceans. Over 72% of individuals examined (65 of 90) contained at least one microparticle. The number of microparticles ingested per individual across all trenches ranged from 1 to 8. The mean and standard error of microparticles varied per trench, from  $0.9 \pm 0.4$  (New Hebrides Trench) to  $3.3 \pm 0.7$  (Mariana Trench). A subsample of microfibrils and fragments analysed using FTIR were found to be a collection of plastic and synthetic materials (Nylon, polyethylene, polyamide, polyvinyl alcohol, polyvinylchloride, often with inorganic filler material), semi synthetic (rayon and lyocell) and natural fibre (ramie). Notwithstanding, this study reports the deepest record of microplastic ingestion, indicating that anthropogenic debris is bioavailable to organisms at some of the deepest locations in the Earth's oceans.



The 3 species of Lysianassoidea amphipods collected from six hadal trenches around the Pacific rim.  
(A) *Hirondellea gigas*, (B) *Hirondellea dubia* & (C) *Eurythenes gryllus*.

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[https://www.shimadzu.com/an/journal/content\\_vol7\\_iss1.html](https://www.shimadzu.com/an/journal/content_vol7_iss1.html)

## Plastics Analysis and Evaluation Techniques

Shimadzu provides a wide range of solutions of analysis and evaluation for plastic materials.

Test Evaluation Items	Materials Characteristics	Instruments/Products
Observation and Analysis	Observation	Scanning Probe Microscope / SPM-9700HT, SPM-8100FM
	Nondestructive internal observation	X-Ray Inspection System / SMX-6000, X-Ray CT System / SMX-CT series
	Elemental analysis	Energy Dispersive X-ray Fluorescence Spectrometer / EDX series
		Sequential X-Ray Fluorescence Spectrometer / XRF-1800
	Observation and elemental analysis	Electron Probe Microanalyzer / EPMA series
	Observation and component analysis	Imaging Mass Microscope / iMScope QT
Material Properties (Research and Development, Quality Control)	Synthesis reaction analysis	Imaging X-Ray Photoelectron Spectrometer / KRATOS ULTRA2 (AXIS Supra)
		Probe Electrospray Ionization Mass Spectrometer / DPiMS-2020, DPiMS-8060
	High separation purification	Fourier Transform Infrared Spectrophotometer / IRTracer-100, IRAffinity-1S, IRSpirit
		Preparative Purification Liquid Chromatograph / Nexera Prep
		Supercritical Fluid Extraction/Chromatograph System / Nexera UC
	Molecular weight distribution, molecular weight measurement	Recycling Preparative HPLC System / Recycle-Assist
	Determination of material properties	High Performance Liquid Chromatograph / Nexera series, Prominence-i GPC system
		MALDI Mass Spectrometer / AXIMA series, MALDI-7090, MALDI-8020, MALDImini-1
Crystallinity	Fourier Transform Infrared Spectrophotometer / IRTracer-100, IRAffinity-1S, IRSpirit	
Color measurements and optical characteristics	X-Ray Diffractometer / XRD-6100/7000 OneSight	
Additives and Harmful Substances	Foreign odors and gases produced	UV-VIS-NIR Spectrophotometers / UV series, SolidSpec-3700i
	Heavy metals and trace elements	GC-MS Off-Flavor Analyzer, Thermal Desorption System / TD-30 series
		ICP Emission Spectrometer / ICP series, Inductively Coupled Plasma Mass Spectrometer / ICPMS-2030
		Atomic Absorption Spectrophotometer / AA-7000
	Identification and quantitation of additives	Ion Chromatograph / HIC-ESP
		High Performance Liquid Chromatograph / Nexera series, i-Series Plus
Residual solvent	Liquid Chromatograph Triple Quadrupole Mass Spectrometer / LCMS-8060NX, LCMS-IT-TOF	
Thermal Characteristics	Endothermic/exothermic reaction and reaction rates	GC-MS Pyrolysis System
	Relative heat capacity	GC-MS Headspace Analysis System
	Evaporation, decomposition, gas adsorption, moisture content, and thermostability	Differential Scanning Calorimeter / DSC-60 Plus, TG-DTA Simultaneous Measuring Instrument / DTG-60
	Thermal expansion, contraction percentage, and softening point	Differential Scanning Calorimeter / DSC-60 Plus
Physical Characteristics	Particle size distribution	Thermomechanical Analyzer / TMA-60
		Particle Size Analyzer / SALD series
Mechanical Performance	Tensile, compression, bending	Dynamic Particle Image Analysis System / iSpect DIA-10
		Precision Universal Tester / Autograph AGX-V series, AGS-X series
	Hardness	Micro Strength Evaluation Testing Machine / Micro Autograph MST-I
		Micro Vickers Hardness Tester / HMV-G series, Dynamic Ultra Micro Hardness Tester / DUH-211 series
	Friction force (tribology)	Scanning Probe Microscope / SPM-9700HT, SPM-8100FM
	Fatigue strain test	Fatigue and Endurance Testing Machine / Servopulser
High speed tensile and high speed punching	Electromagnetic Force Micro Tester / Micro-Servo MMT series	
Rheological Characteristics	Particle strength	High-Speed Impact Testing Machines / HITS-X series
	Viscosity	Micro Compression Tester / MCT series
Mass	Viscoelasticity evaluation	Capillary Rheometer Flowtaster / CFT-500EX/100EX
	Specific gravity	Mooney Viscometer / SMV-301/301RT
	Mass	Specific Gravity Measurement Balances / AP/AU/UP series
	Moisture content	Analytical Balances / AP/AU/AT-R series, Electronic Balances, Precision Platform Balances
		Moisture Analyzer / MOC63u



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