IDENTIFICATION OF BIODEGRADATION PRODUCTS OF HIGHLY FLUORINATED PRODUCTS USING (TWO-DIMENSIONAL) LIQUID CHROMATOGRAPH COUPLED WITH HIGH-RESOLUTION MASS SPECTROMETER

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Introduction

Perfluorinated compounds (PFCs) have been considered an environmental problem, because PFCs were widely found in wildlife and in humans. PFCs had been applied to various scenes of our life and have taken many and varied forms. Perfluorinated polymers are one of the most frequent forms of PFCs. Perfluorinated polymers comprise not only high-molecular-weight compounds such as poly(tetrafluoroethylene) or perfluoroalcoxyalkane but also relatively small compounds with molar mass of approximately 2000. Environmental researchers have not discovered the full breadth of their structures. The elucidation of the degradation pathway of these polymers and determining whether these polymers are broken down to perfluorinated octanoic acid (PFOA) and perfluorinated octane sulfonic acid (PFOS) in the natural environment or not, are a challenging subject for environmental researchers[1,2]. However studies on the degradation of fluorinated polymers in the environment are still sparse due to the complexity of the polymers[3,4]. In the past few years, two or multidimensional liquid chromatography has been attractive for analyses of complex mixtures. Multidimensional chromatography coupled with mass spectrometry notably offers comprehensive analysis and is applied to the characterization of natural products and industrial materials such as polymers. Although the interpretation of data obtained from the measurement is often a laborious task, it is clear enough to extract the mass spectra of target materials from complicated mass spectra[5]. In the present study, we used a (2D) liquid chromatograph coupled with a high-resolution mass spectrometer (LC/HR-MS) operated in full-scan mode to identify biodegradation products of relatively small perfluorinated polymers.

Materials and methods

Fluorinated polymeric materials examined consisted of four fabric repellents. All of them were purchased in Japan. Detailed information of the samples is described in Table 1. Three of them were products sold before the switch by fluorochemical manufacturers. Liquid samples were diluted in water by 3000-fold. Biodegradability examination was conducted with reference to the Organization of Economic Co-operation and Development (OECD) guideline 301C. Briefly, activated sludge was added to the diluted samples at a concentration of 30ppm. Nutrient salts were also added to the samples. The samples were transferred in enclosed BOD meter, which were made to be able to supply oxygen for aerobic biodegradation. The stirred samples were examined for a period of 28 days in a darkened, enclosed BOD meter at 25 ± 1°C. No chemical-specific analyses are prescribed in the OECD guideline. Instead, 10-mL portions of samples were taken from each stirred sample once a week for chemical analyses. The portions passed through solid phase extraction cartridges, OASIS WAX (Waters), to extract PFCs. PFCs were eluted from the cartridges with 5 mL of 0.1% NH_4OH methanol. The eluates were analyzed by liquid chromatograph/mass spectrometers. An Ultimate 3000 (Dionex) HPLC system was applied to both 1D and off-line 2D chromatography. Off-line 2D chromatography was applied to especially complicated samples. An Exactive (ThermoFisher) mass spectrometer, which demonstrated a resolving power of 100 000, was used for acquisition of high resolution mass spectra. The samples were analyzed by electrospray ionization (ESI) running in both positive and negative ion mode. The scan range of *m/z* was from 200 to 3000. Two-dimensional LC was carried out with two different kinds of columns. A TSK-Gel ODS-100S (15cm × 2mm i.d. 5μm, TOSOH) and an Epic-FO column (15cm × 2mm i.d. 3μm, ES Industries) were used for each chromatography. Detailed description of chromatography can be found elsewhere[5]. In addition, an Acquity UPLC system and a Xevo TQ mass spectrometer (Waters) were used for quantitation of perfluoroalkyl

carboxylic acids (PFCAs) and prefluoroalkyl sulfonic acids (PFSAs) and acquisition of product ion spectra. The quantitation was conducted using PFCAs, PFSAs and their mass labeled standards, which were purchased from Wellington Laboratories.

Results and discussion

A typical 2D chromatogram of Sample A before biodegradation is shown in Figure 1, which was acquired by Exactive running in positive ion mode. Each peak in the 2D chromatogram had a series of ions in mass spectrum with an *m/z* interval of 44.026. This interval corresponded to ethoxy unit, C_2H_4O. Therefore it can be considered that Sample A consists of polymers which have many ethoxy units. In addition, these ethoxy series had other ethoxy series with an *m/z* interval of 99.994. Sample B also showed a series of ions with an *m/z* interval of 99.994. Sample C showed a series of ions with an *m/z* interval of 49.997. These intervals corresponded to CF_2 and C_2F_4, respectively. These samples showed polydispersity in their perfluoroalkyl chains. On the other hand, Sample D did not show such perfluoroalkyl chain polydispersity. To reveal their chemical structure, collision induced dissociation spectra were also acquired. In the spectrum of Sample A in negative ion mode, precursor ions having certain perfluoroalkyl chain length generated one prominent product ion. From the accurate mass, this ion was considered $[M\text{-}2HF\text{-}(C_2H_4O)_n]^-$. The elimination of hydrogen fluoride from alkyl chains that have both hydrogen and fluorine atoms is well-known[6]. It is, therefore, considered that the polymers included in Sample A are compounds that have polyfluoroalkyl chains with two hydrogen atoms. The chemical structure of this polymer was presumed to be $CF_3(CF_2)_mCH_2O(CH_2CH_2O)_nH$. This compound easily produces $[M+NH_4]^+$ in positive ESI. Extracted ion chromatograms with putative *m/z* values were shown in Figure 2. The existence of long perfluoroalkyl chain up to $C_{17}F_{35}$ and distribution from 0 to 20 of ethoxy unit number were revealed. In the same manner as Sample A, chemical structures of Samples B, C, and D were also examined. Sample B was presumed to be polyfluorinated carbonate. Samples C and D were presumed to be polyfluorinated urethane. There are studies that elucidate biodegradation pathways of precursor compounds that can turn to PFOS and PFOA. With respect to fluorinated telomer alcohols (FTOHs), thorough identification of a lot of biodegradation intermediates were conducted by synthesizing each compound[7,8]. The difference of chromatograms between before and after biodegradation was extracted from acquired data. As for Sample B, the most prominent ions that did not appear before biodegradation were 8:2 FTA $F(CF_2)_8CH_2COOH$ and 8:2 FTUA $F(CF_2)_7CF{=}CHCOOH$. It was difficult for the conventional ODS column to separate these degradation products although an HPLC column that had fluorinated stationary phase easily separated them. These structures were confirmed by corresponding authentic standard reagents. It is known that these degradation products generated from 8:2 FTOH $F(CF_2)_8CH_2CH_2OH$. Indeed, Sample B included 8:2 FTOH as impurities. The peak intensity of 8:2 FTOH rapidly diminished for a week but the peak intensities of 8:2 FTA and 8:2 FTUA continued to increase for four weeks. The existence of compounds other than 8:2 FTOH that generated 8:2 FTA and 8:2 FTUA was suggested. Wang et al. had reported that there were other degradation products. The existence of ions that had *m/z* values corresponded to 7:3 FTA and 3-OH 7:3 FTA was confirmed by accurate mass. However 2H-PFOA and 7:2 sFTOH could not be confirmed.

As to Sample C, the sample before biodegradation seldom included N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE). Nevertheless, the major degradation product was N-ethyl perfluorooctane sulfonamidoacetic acid (N-EtFOSAA). N-EtFOSE is known to be broken down to N-EtFOSAA[9]. Further degradation products such as prefluorooctane sulfonamide and PFOS, however, could not be found in the present study. Sample D, the replacement of Sample C, had short perfluorinated alkyl chain, C_4F_9, instead of C_8F_{17}. The major degradation product was N-methyl perfluorobutane sulfonamidoacetic acid. Further degradation products could not be found similar to Sample C. Both Samples C and D did not show a definitive increase of perfluoroalkanoic acid and perfluoroalkane sulfonic acid.

Despite the fact that identification of biodegradation products with an authentic standard should be carried out, two-dimensional LC/HR-MS provided accurate mass and fragmentation so that structural identification was sufficiently demonstrated.

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Table 1 Detailed information of samples.

Sample	Manufacturer	Product	Before voluntary phaseout	Note
A	Daikin	Unidyne	Yes	Industrial
B	Asahi Glass	Asahi Guard	Yes	Industrial
C	Sumitomo 3M	ScotchGard Protector	Yes	Household
D	Sumitomo 3M	ScotchGard Protector	No	Household

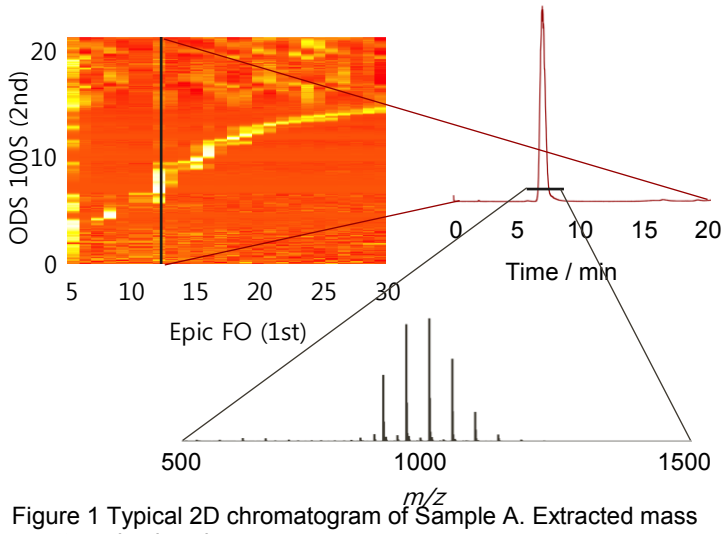

Figure 1 Typical 2D chromatogram of Sample A. Extracted mass spectrum is also shown.

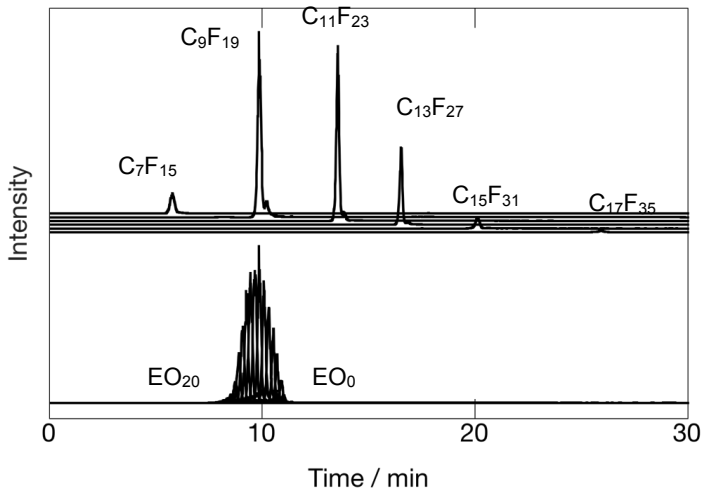

Figure 2 Extracted ion chromatograms of putative *m/z* values.