Concentration of perfluorochemicals in bivalves in Japan by alkaline digestion method

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Introduction

Recently, perfluorinated chemicals (PFCs) have attracted much attention because of their persistent, bioaccumulative and toxic properties. Major applications of PFCs include coating of paper, food packaging, carpet and fabric to add repelling properties against oil and water. Now these chemicals are distributed widely in the environment, and PFOS and PFOA as well as other PFCs have been detected in wildlife.

The use of mussel or other bivalves for PFCs monitoring, however, has been scarce because of lower concentrations of PFCs in lower trophic level organisms.[1] The purpose of the present study is to develop analytical method based on LCMSMS for the use of mussels for PFCs monitoring in coastal environment. Contaminations during pretreatment processes have to be decreased to sufficiently low levels so that the concentrations in mussels in many places will be accurately determined. Effect of alkaline digestion was examined in order to improve efficiencies of extraction and clean up procedures.

Materials and Method

Mussel samples (*Mytilus galloprovincialis* and *Septifer virgatus)* were collected from various locations along coastline of Japan. The bivalves were deshelled and frozen by liquid nitrogen on site, transported and stored lower than -80 °C, and cryo-homogenized in two steps (a stamp mill made of titanium (TAMP-1X,Tokyo Atomizer Manufacturing) and a planetary ball mill made of zirconium (P-5/4,Fritsch)) with median diameter less than 100 micrometer. Bivalve samples collected in 1990 to 1999 were stored at -20 °C in environmental specimen bank at the National Institute for Environmental Studies.

After half-thawed, the samples were deshelled and homogenized by a Polytron homogenizer in a cleaned plastic tube with a generator shaft specifically made to eliminate use of fluoropolymer within the shaft.

Sample treatment was either alkaline digestion (90 °C for 3hr in 2N NaOH) followed by ion-pair extraction (tetrabutyl ammonium as ion-pair reagent) and hexane acetonitrile partition (Chem Elut, Varian), or directly extracted with ion-pair reagent followed by ion-pair extraction and SPE clean up. (Chem Elut, HLB and MCX, Waters). Some samples were first extracted by pressurized fluid extraction (ASE 200, DIONEX) and treated with either of the above two methods.

Analysis of 6 PFCs, *i.e.* perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA),

perfluoroundecanoic acid (PFuDA), perfluorododecanoic acid (PFdDA), perfluorotetradecanoic acid (PFteDA) and perfluorooctane sulfonic acid (PFOS), was performed by high performance liquid chromatography and tandem mass spectrometry (LCMSMS, API4000 Qtrap, Applied Biosystems). Parent and daughter ion mass selections were according to the literature[3]. $^{13}C_4$ labeled PFOA and $^{13}C_4$ labeled PFOS (Wellinton. Lab. Inc.) were used as surrogate.

Results and Discussion

The concentrations of PFCs in bivalves were generally low, in the range of ng g^{-1} wet tissue or lower, with BCF values of less than 1,000 for PFOS and around unity for PFOA.[1] Therefore, much effort had to be paid for the removal of contamination during sample preparation in order to attain low enough blank levels for the analysis of PFCs, especially PFOA. After careful examination of source of contamination, following procedures were found to be effective to reduce blank levels; to eliminate fluoropolymers, careful wash of glass and plastic wares with methanol before use, precleaning of reagent and buffer for ion-pair extraction by *t*-butylmethylether, and precleaning and conditioning of SPE cartridges by acetonitrile and water. After repeated use of glassware, blank levels tended to be higher significantly. Therefore it was decided that all the glassware was soaked into concentrated sulfuric acid for 24 hrs and washed with purified water and methanol before use. These efforts made it possible to reduce typical blank levels of PFOS, PFOA, PFDA, PFuDA, PFdDA and PFteDA to 0.01, 0.05, 0.01, 0.01, 0.03 and 0.04 ng g^{-1} per sample (provided that 2 g of wet tissue was analyzed).

Recovery of target compounds during pretreatment procedure can be properly assessed by the internal standard by using stable isotope labeled compounds (surrogate). However, extraction efficiency itself can not be corrected by surrogate. Previously, extraction was performed by fairly mild technique, such as ion-pair extraction of the tissue homogenate[1] and dilute alkaline treatment at room temperature[4]. In the present study, a well established extraction technique for persistent organic compounds, alkaline digestion, was selected as the first pretreatment step, and its additional effect to the conventional ion pair extraction was evaluated.

Both tissues and tissue extracts by pressurized fluid extraction (PFE) were treated with ion-pair extraction method with or without alkaline digestion (2N NaOH at 90 °C for 3hrs). The final SPE clean up procedures with HLB and MCX, both of which raised blank level of PFOA, could be omitted in the pretreatment procedures including alkaline digestion. Recoveries of the surrogates were found to be good in all the procedures.

However, concentration of PFOS and PFOA were considerably different among them. Large differences, about 8 fold in PFOA and 4 fold in PFOS, between (1) and (2) in Table 1 suggest either insufficient extraction efficiency from tissues without alkaline digestion, or presence of PFOS/PFOA derivatives which liberate free PFOS/PFOA with alkaline digestion. In the case of PFOS, the effect of alkaline digestion on already extracted sample ((3) and (4)) is identical with the effect on tissues, indicating that the latter hypothesis is reasonable. Similar tendency was also observed in the case of PFOA, again supporting the latter hypothesis of the presence

of PFOA derivative, although extraction efficiency may also be increased by alkaline digestion as suggested by the difference between (1) and (3).

Difference between (2) and (4) suggests that part of the PFOA derivative maybe decomposed to free PFOA during PFE procedure. This trend was PFOA specific, not observed in PFOS. PFOA is structurally similar to fatty acids, which are esterified to hydroxyl groups on biomolecules. Ester groups are chemically not so stable and tend to be hydrolyzed under high pressure and hot temperature as well as in alkaline solution. The above results may reasonably be interpreted to show the presence of esterified PFOA derivative within biological tissue.

Table.1 Effect of alkaline digestion procedure

	PFOA (n=3)		PFOS (n=3)	
	Recovery (%)	Concentration ng g^{-1}-wet	Recovery (%)	Concentration ng g^{-1}-wet
(1) Alkaline + ion-pair	101 ± 2	10.5 ± 0.2	86 ± 1	0.63 ± 0.01
(2) ion-pair	94 ± 19	1.3 ± 0.1	119 ± 1	0.16 ± 0.00
(3) PFE + Alkaline + ion-pair	86 ± 3	5.8 ± 0.4	71 ± 40	0.68 ± 0.02
(4) PFE + ion-pair	71 ± 16	2.6 ± 0.1	87 ± 4	0.17 ± 0.01

Based on the result summarized in Table 1 alkaline digestion followed by ion-pair extraction (1) was selected as most suitable method to reveal total PFCs concentration within biological tissues. PFCs in bivalves collected from Japanese coastline were analyzed by the method. The result was summarized in Figure 1.

PFOS and PFOA levels were corrected by surrogate while the other PFCs levels were uncorrected because of the unavailability of isotope labeled compounds. The concentration ranges of PFCs were as follows; PFOA: <0.05 to 10.5, PFDA: 0.013 to 6.0, PFuDA: 0.07 to 4.5, PFdDA: <0.03 to 11.9, PFteDA: <0.04 to 3.8 and PFOS: <0.01 to 1.5 ng g^{-1}-wet, respectively. Generally speaking PFOS levels were relatively high followed by PFdDA, PFuDA, PFteDA, PFOA and PFDA.

Bivalves collected in densely populated area such as Tokyo Bay (Sarushima and Dainikaiho), Ise Bay (Oyodo) and Osaka Bay (Kaizuka) show sub ppb to 1ppb of PFdDA, PFuDA, PFteDA and PFOS. These levels do not seem to be significantly different from those in decade ago (Tokyo Bay (‘93) and Osaka Bay (’93)). Bivalves collected in Funka Bay, Hokkaido, contained lowest level of all PFCs. On the other hand, there are a few hot spots, such as Isohara in Ibaraki prefecture and Katano in Ishikawa prefecture. More than 10ppb of PFOA and PFdDA, 6ppb of PFDA and 4ppb of PFteDA were detected in Isohara samples. There is a small industrial park near the sampling site of Isohara and some company in this park might release PFCs.

So et al[4] reported some PFCs levels in oyster in Tokyo Bay; PFOS: 0.59, PFOA: 0.66, PFDA: 0.12 and PFdDA: 0.20ng g^{-1}-wet, respectively. The present study shows a similar level of PFCs in mussels collected in

Tokyo Bay; PFOS: 1.0 and 1.5, PFOA: 0.21and 0.12, PFDA: 0.07 and 0.04, and PFdDA: 0.33 and 0.01 ng g^{-1}-wet, respectively. Furthermore additional information was obtained on PFuDA (0.57 and 0. 3) and PFteDA (0.25 and 0.03 ng g^{-1}-wet).

In summary, a new preparation method based on strong alkaline digestion was established and concentration of perfluoroalkyl carboxylic acids (C8 to C14) and PFOS in bivalves along coastline of Japan were revealed. The presence of unknown derivatives of PFOS and PFOA within bivalve tissues was shown. Further research is needed to elucidate their chemical structure.

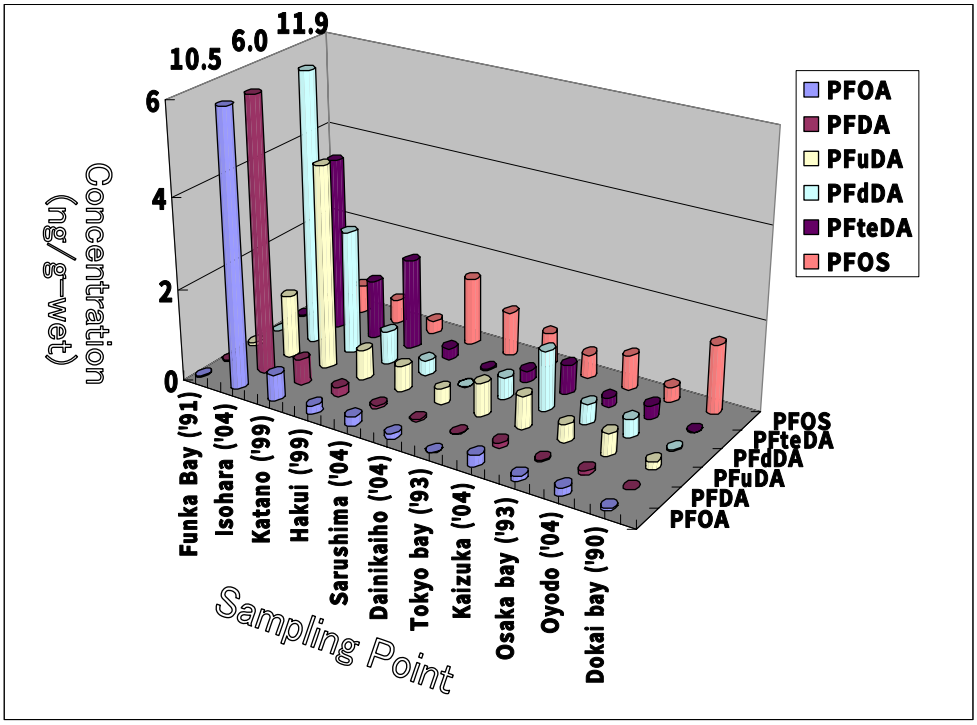

Fig 1 PFCs concentration in bivalves along coastline of Japan

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