Photoacid generator formation for the selective enrichment of perfluoroalkyl sulfonates and their direct analysis by MALDI-TOF-MS†

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Triphenylsulfonium chloride (TPSC) was employed as a bifunctional agent for the selective enrichment of perfluoroalkyl sulfonates by triphenylsulfonium perfluorosulfonic acid (TPA, a photoacid generator) precipitation, and for the direct detection of perfluorosulfonic acid by MALDI-TOF-MS, using the triphenylsulfonium group of TPA as a matrix.

Introduction

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) has been widely used in the fields of proteomics, genomics, and biological imaging to characterize macromolecules (i.e., those molecules with molecular weight (M.W.) > 1000 Da, such as proteins and nucleic acids). For small molecules (M.W. < 1000 Da, such as fatty acids, organic amines), detection by MALDI-TOF-MS requires special matrices due to interference by the ions of conventional matrices (such as 2,5-dihydroxybenzoic acid (DHB) in the low mass region (m/z 0~1000 Th). However, MALDI-TOF-MS has not been widely used for the detection of organic pollutants (e.g., perfluoroalkyl sulfonates) in the environment.

Perfluoroalkyl sulfonates (PFSs), such as perfluorodecanesulfonate (PFDS), perfluorooctanesulfonate (PFOS), perfluorohexyl sulfonate (PFHxS), and perfluorobutylsulfonate (PFBS) are a typical class of environmental pollutants, and most of them are characterized by their persistence, long-distance migration, and bioaccumulation. Because of the potential hazards of PFSs to the environment and human health, a highly selective and sensitive method for their detection is needed. In recent years, high-pressure liquid chromatography/tandem mass spectrometry (LC-MS/MS) and high-pressure gas chromatography/mass spectrometry (GC-MS) methods have been widely developed to monitor PFSs. However, a low instrument blank is required for these analyses. Furthermore, stainless steel line fittings and tubing must be used to avoid contamination by the fluorinated compounds and this requirement greatly increases experimental costs. To the best of our knowledge, MALDI-TOF-MS is not subject to contamination by perfluorinated compounds. Thus, this highly sensitive instrumentation may serve as an alternative method to avoid the above-mentioned problem. However, to date, few reports of PFSs detection by MALDI-TOF-MS have appeared.

Triphenylsulfonium salts (such as triphenylsulfonium perfluorosulfonic acid (TPA)) are some of the best photoacid generators (PAGs) which have been widely used in microlithography as formulation components for chemically amplified resists. When PAGs are irradiated by the light of wavelengths ranging from λ = 250~440 nm, strong acids are generated in the resist which catalyze deblocking or crosslinking in the resist polymer. Meanwhile, several rearrangement products, such as phenylthiobiphenyls and biphenyl sulfide, are formed by free radical reactions. The photoinitiation mechanism for the photolysis of TPA was shown in Fig. 1.

![Fig. 1 Formation of triphenylsulfonium perfluorosulfonic acid and the photoinitiation mechanism for the photolysis of PAG.](image-url)
In this work, triphenylsulfonium perfluorosulfonic acid (TPA), a photocaged generator, was formed for the direct detection of PFS by MALDI-TOF-MS. Triphenylsulfonium chloride (TPSC), was employed as a bifunctional agent for the selective enrichment of perfluoralkyl sulfonates (PFSs) by TPA precipitation, and for the direct detection of perfluorosulfonic acid by MALDI-TOF-MS, using the triphenylsulfonium group of TPA as a matrix. Furthermore, a novel quantitative method for PFS analysis was established and applied in the detection of trace PFSs in real water samples.

Experimental

Chemicals

Perfluorooctanoic acid (PFOA, 95%) was bought from Alfa Aesar (Lancashire, England), potassium salts of perfluorobutane sulfonate (PFBS, ≥98%), perfluorohexane sulfonate (PFHxS, ≥98%), perfluorooctane sulfonate (PFOS, ≥98%), perfluorodecanesulfonate, (PFDS, ≥98%), and triphenylsulfonium chloride (TPSC, ≥99%) were obtained from Sigma-Aldrich (Steinheim, Germany). HPLC grade fluorooctane sulfonate (PFOS, 6 mL) was used as a sample pretreatment method to firstly enrich the completely react with TPSC. The triphenylsulfonium group of TPA is immediately. The resulting precipitate was dissolved in 20 mL of acetonitrile (ACN); 1 g of acetonitrile (ACN) was added to the water samples as an internal standard prior to the SPE elution and the eluent was collected in glass tubes containing 0.1% TFA were used as control matrices. The calibration curve was performed with the detection of PFOS by using 500 mL of ultra-pure water (no PFS contained) spiked with standard PFOS in the range of 0.1–10 ng L⁻¹. 1 ng of 13C₄-PFOS was added to the water samples as an internal standard prior to the SPE extraction and TPSC precipitation. The limit of detection (LOD), defined as the concentration that yielded an S/N ratio of higher than or equal to 3, was determined by the SPE extraction and TPSC precipitation of the spiked distilled water samples. Precision and accuracy were evaluated at two concentration levels equally distributed over the low and high linear range. Intraday precision was determined by analyzing five river samples, spiked with the standard PFOS and 13C₄-PFOS on the same day (n = 5). Interday precision was evaluated by determining five replicates per concentration level, on five consecutive days (n = 25). Accuracy was evaluated by comparing the mean recovery in the five or 25 analyses to the nominal concentration values.

MALDI-TOF-MS instrumentation

All analyses of PFSs were accomplished with an Autoflex III (Bruker Doltonics, Germany) MALDI-TOF-MS equipped with a pulsed nitrogen laser (337 nm) at a frequency of 20 Hz. An AnchorChip TM target plate with 384 spots was employed. Ions were desorbed from surfaces of the target plate with laser energies of about 72 μJ per pulse. The extraction delay time was optimized to 180 ns. The measurements were performed in the negative ionization reflection mode for quantitation analysis. Each mass spectrum was typically averaged over 2000 laser shots after being externally calibrated by using PEG-600 sulfate for a m/z range from 100 to 1200 Th in the negative ion mode. All mass spectra were analyzed by Flex Analysis software provided by Bruker Doltonics Corp. CHCA (5 mg mL⁻¹) and DHB (5 mg mL⁻¹) in 50% acetonitrile containing 0.1% TFA were used as control matrices.

Results and discussion

Scheme 1 shows a schematic diagram to illustrate the detection of PFS by MALDI-TOF-MS combined with the use of triphenylsulfonium chloride (TPSC) as a bifunctional agent. The theory of “photoacid generation” was introduced to illuminate our concept. Firstly, TPSC
selectively reacts with a PFS to form the corresponding TPA which is a photoacid generator and is insoluble in aqueous solution. By centrifugation and precipitation, the PFS could be selectively enriched. After dissolving the TPA in acetonitrile (ACN), it was analyzed by MALDI-TOF-MS. The perfluoroalkyl sulfonic acid was released easily from its TPA by the laser irradiation (λ = 337 nm) of MALDI-TOF-MS due to the photoacidic property of the TPA. At the same time, the triphenylsulfonium group of the TPA served as a matrix for absorbing energy from laser radiation and the energy was transferred to the perfluoroalkyl sulfonic acid to assist desorption/ionization. Therefore, the direct and sensitive detection of PFSs was achieved by MALDI-TOF-MS in the negative ion mode.

Several kinds of PFSs with different carbon chain lengths, such as PFDS, PFOS, PFHxS, and PFBS, were selected as target analytes and treated according to the same procedure in the Sample preparation section. All of the selected analytes could react with TPSIC immediately to form insoluble TPAs in aqueous solution. This suggested that the target analytes could be easily enriched by the precipitation of the TPAs. Carboxylic acid compounds, such as perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), were also employed to react with TPSIC, but no insoluble products were found (data was not shown). This indicates TPSIC selectively reacted with sulfonic acid groups of the PFSs to form precipitates. After precipitation and solubilization, the TPAs were spotted onto the MALDI target and analyzed by MALDI-TOF-MS. Fig. 2 shows the mass spectrum of each selected analyte. Clear signals for the acid anions were obtained for all the selected PFSs. Only deprotonated ions of PFDS at m/z 599.30, PFOS at m/z 499.24, PFHxS at m/z 399.40 and PFBS at m/z 299.20 were observed in the respective mass spectra, and no matrix ions were detected.

As a control experiment, the conventional matrices, α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB), were used to detect PFOS in the negative ion mode. As seen in Fig. 3, panels B and C, numerous matrix interference ions were observed in the mass spectra, and the ion signal of PFOS was suppressed by the matrix ions and was only found by amplifying the mass spectrum. However, in the mass spectrum of PFOS by TPA precipitation (Fig. 3A), only the peak of the deprotonated PFOS was observed without interference from any other matrix ions. The possible reason for the lack of interfering matrix ions was that only the acidic anion of PFS was released when TPA was irradiated by the laser, and the other products of TPA generated during irradiation were mainly positively charged ions which were not detected in the negative ion mode of MALDI-TOF-MS.

In order to demonstrate our assumption, TPA was also detected in the positive ion mode. The mass spectrum (Fig. S1, ESI†) showed a series of product cations from TPA. Hacker et al. reported that several rearrangement products, such as phenylthiobiphenyls (Ph3S+) and diphenyl sulfide, were formed by free radical reactions, where a PAG was irradiated and released acid." In our experiment, we found that the main peak in the mass spectrum was a cation at m/z 263.10, assigned to Ph3S+. We also detected other cations which were assigned to unknown compounds but were actually the products of TPA (Fig. S1B†). Confirmation of the structures of these products will require further study.

Furthermore, the triphenylsulfonium group has three phenyl rings which can absorb energy from laser radiation and transfer the energy to PFSs. So, the triphenylsulfonium group can be used as a matrix to assist PFS desorption/ionization. To demonstrate the matrix function of the triphenylsulfonium group, PFOS (20 pg) without any other matrix assistant, and with the corresponding TPA (containing 20 pg of PFOS), were separately spotted on the MALDI target and...
length of the PFS affects the detection limit. We assumed that even lower detection limit. Moreover, as is well known, the carbon chain facilitates the desorption/ionization process, could account for the ionization assistance of the triphenylsulfonium group, which could so far the most sensitive method for PFOS detection. We considered the potential application of TPSC as a bifunctional agent for PFS analysis in real environmental water samples was studied. PFOS was selected as a target analyte. In order to obtain a high enrichment efficiency and save time, SPE extraction was employed firstly to enrich PFOS in water samples, then TPSC precipitation was performed for further PFOS enrichment. The calibration curve was performed as discussed in the method validation section. The results showed that calibration equation was $y = 0.4371x + 0.0016$ with good linearity ($R^2 = 0.9989$, $n = 7$). The calibration curve showed a wide linear dynamic range of response (0.1–10 ng L$^{-1}$), which was over 2 orders of magnitude. The LOD of PFOS was evaluated as 0.021 ng L$^{-1}$ (calculated by using $S/N = 3$), which was lower than the LOD (0.036 ng L$^{-1}$) obtained by the LC-MS/MS method reported by Zhang et al. The precision and recovery data were summarized in Table S1. The result indicates that the intraday precision for PFOS was less than 11%, whereas the interday precision was less than 16%. The mean recovery for PFOS ranged from 88% to 109%. This data suggests that our described method can provide high reproducibility with excellent linearity and sensitivity for PFOS analysis.

Some real water samples from different districts of Beijing in China were collected, and pretreated according to the experimental section. Ultra-pure water and the collected water samples were spiked with 2.0 ng L$^{-1}$ of standard PFOS and 2.0 ng L$^{-1}$ of the internal standard $^{13}$C$_4$-PFOS, and extracted by a C$_{18}$-SPE cartridge, then precipitated by TPSC to investigate the matrix effect on the recovery method. The results were compared with each other (Table S2, ESI†). We found that the recoveries of PFOS in tap water, wastewater and river water samples were slightly lower than those obtained from the ultra-pure water samples. However, even for samples with a complex matrix, the recovery of PFOS were still higher than 88%, so we concluded that the matrix only had a slight influence on the method of recovery.

The concentrations of PFOS in the collected water samples were measured by MALDI-TOF-MS, and the obtained results were compared with the values of the same water samples detected by LC-MS/MS reported by Zhang et al. The chemical structure of PFOS was identified by the negative ion collision-induced dissociation (CID) spectra of PFOS performed by MALDI-TOF-MS. The concentrations of PFOS in different water samples were listed in Table S3, ESI†. The results indicated that the concentrations of PFOS in the Xiaoqinghe river water sample and the Gaobeidian wastewater sample were detected to be 1.08 ± 0.06 ng L$^{-1}$ and 3.30 ± 0.05, respectively, which are close to the concentrations of PFOS (0.96 ± 0.06 ng L$^{-1}$ in the Xiaoqinghe river, 3.22 ± 0.02 ng L$^{-1}$ in the Gaobeidian wastewater) detected by LC-MS/MS. Interestingly, PFOS in the tap water sample was not detected by LC-MS/MS but was detected by our described MALDI-TOF-MS method (0.38 ± 0.10 ng L$^{-1}$). This may be attributed to the higher sensitivity of our method.

Conclusions

In summary, the ability of TPSC to act as a bifunctional agent for PFSs selective enrichment and direct detection by MALDI-TOF-MS

Fig. 3 (A) The negative ion spectrum of PFOS (50 pg) obtained by MALDI-TOF-MS. PFOS was precipitated as its TPA following treatment with TPSC. (B, C) The negative ion spectra of PFOS (50 pg detected by MALDI-TOF-MS) combined with CHCA (B) and DHB (C) as matrices. Insets: partly amplified mass spectra of (B) and (C). (Peaks denoted by ‘‘*’’ and ‘‘#’’ represent the matrix ions of CHCA and DHB).
was demonstrated in this work. TPSC could selectively react with the sulfonic acid group of a PFSs to form a TPA precipitate, and therefore the PFSs could be easily enriched by precipitation. Furthermore, TPA, as a photoacid generator, could release PFS acids immediately upon laser irradiation, while the triphenylsulfonium group of TPA acts as the matrix to assist the desorption/ionization of PFSs. We demonstrated that as little as 0.2 fg of PFOS could be detected by MALDI-TOF-MS using TPSC as a bifunctional agent. The application of this method in the detection of trace PFOS in real environmental samples was carried out. The results indicate that our described MALDI-TOF-MS method is reliable and can be used as an alternative method for PFSs detection and quantification in environmental water samples.

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Notes and references