Dimeric ions of emerging and branched PFASs in ion mobility spectrometry: Artifacts to be minimized or valuable features for non-targeted analyses?

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1 Introduction

Per-and polyfluorinated substances (PFASs) possess remarkable properties, such as thermal and chemical stabilities and an amphiphilic nature, which render them ubiquitous in our everyday consumer products and in industrial applications developed over the last few decades[1]. Due to their intrinsic properties, these fluorinated polymers are omnipresent in the environment, water and soil, but they also enter the food chain, raising concerns about exposure of all age groups of the population worldwide[2]. As a result, the production and use of certain PFASs have been restricted or even banned by the Stockholm convention because of their known health effects (e.g. thyroid disorders and cancer[2]). In response, the chemical industry has synthetized alternative PFASs, and over 10,000 compounds have been inventoried to date[3]. There is therefore a great need for non-targeted analytical methods that can detect, identify, and quantify as many of these compounds as possible. Today, the state-of-the-art shows that most of these analyses are performed using liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS)[4]. However, these approaches may have some limitations, particularly in the separation and identification of isobars or branched isomers[4,5]. The coupling of Ion Mobility Spectrometry (IMS) to conventional LC-HRMS setups has been introduced to offer new perspectives to the above-mentioned limitations[5]. Indeed, IMS brings an additional separation dimension to LC-HRMS, and the collision cross sections (CCSs) can be seen as a descriptor related to the bulk apparent density of gas-phase ions, providing an additional point of identification[5,6]. In addition, in the case of PFASs, CCS-*m/z* trendlines are observed for homologous series and can therefore increase the confidence in the identification of homologous PFASs[5,7]. However, when LC-IMS-MS experiments are carried out in negative ionization mode, some PFASs, such as perfluorocarboxylic acids (PFCAs) form or produce dimeric ions ($[2M-H]^-$) in source, in addition to the expected deprotonated ion ($[M-H]^-$). These dimeric ions have been observed in both drift-tube IMS[5,8] (DTIMS) and traveling-wave IMS (TWIMS)[9]. In our study, these dimers were also identified using a third type of IMS, namely trapped IMS (TIMS). In fact, some of these dimeric ions may dissociate after mobility separation into the corresponding deprotonated ion ($[M-H]^-$). As a result, the mobilogram of the deprotonated shows several peaks for a single *m/z*: one for the $[M-H]^-$ ion in its monomeric form and the other for the $[M-H]^-$ ion resulting from post-IMS dissociation of the $[2M-H]^-$ ion. Consequently, these dimers can have a negative impact on the sensitivity of the method and hamper the identification process, as the mobility peak of the pseudo-parent ion is split in several peaks. However, from another point of view, these dimeric ions can also serve as an additional identification point that can be valuable if there are interfering ions with the $[M-H]^-$ ion or if the latter shows a strong tendency to fragmentation within the source. The aim of this study is therefore to analyze various carboxylated PFASs in TIMS and determine whether monitoring their dimeric ions is worthful or whether analytical and instrumental parameters need to be optimized to minimize their formation. To this end, two cases will be studied. Firstly, compounds which have a strong tendency to fragmentation in the source, but which could form dimeric ions, will be investigated. This is the case for perfluoroalkyl ether carboxylic acids (PFECAs), for which no CCS value could be reported for their $[M-H]^-$ ion in DTIMS[5]. However, one paper reported that some PFECAs exhibited dimeric ions in LC-MS, which were much more prominent than the $[M-H]^-$ ion[10]. Some PFECAs will therefore be analyzed in TIMS, to verify whether dimers can also be observed in IMS, whose CCS could be used as an identification point for these compounds. These compounds are also of major interest for environmental studies, as some PFECAs, such as Gen-X, may be no safer than the legacy PFCAs they replace[4]. The second aspect that will be studied is whether dimeric ions can help in the separation of branched isomers. Indeed, it could be that the difference in CCS between dimeric ions is higher than the difference in CCS between the monomeric ions, thus favoring isomer separation. This will be considered using four isomers of perfluorononanoic acid (PFNA). This is important from an analytical point of view, as it is still difficult to separate and identify branched and ether PFCA isomers using conventional LC-MS/MS methods[5].

2 Materials and Methods

All native standards were purchased form Wellington Laboratories, Inc (Ontario, Canada). The three PFECAs standards used were perfluoro-4-oxapentanoic acid (PF4OPeA, CAS 377-73-1), perfluoro-5-oxahexanoic acid (PF5OHxA, CAS 863090-89-5), and 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoroproproxy)propanoic acid

(HFPO-DA (Gen-X), CAS 13252-13-6). These three compounds were analyzed as a mixture of 100 ng/mL in MeOH. The three PFNA branched isomers studied were perfluoro-4-methyloctanoic acid (P4MOA), perfluoro-7-methyloctanoic acid (ipPFNA, CAS 15899-31-7), and perfluoro-3,5,5-trimethylhexanoic acid (P355TMHxA, CAS 238403-51-5). Each isomer was analyzed as an individual solution of 100 ng/mL in MeOH. The linear PFNA isomer was part of a mixture of 100 ng/mL of native standards (PFAC-MXC), composed of C_4-C_{14}, C_{16}, and C_{18} perfluoroalkyl carboxylic acids (PFCAs), and C_4-C_{10} and C_{12} perfluoroalkane sulfonic acids (PFSAs). The mixtures and individual solution were analyzed in LC-TIMS-TOFMS. Chromatographic separation was performed on an Acquity I-Class UPLC system (Waters, Milford, MA, USA) using a Acquity BEH C_{18} column heated to 45 °C (2.1 × 150 mm × 1.7 µm particles) (Waters, Milford, MA, USA). Chromatographic separation was conducted on an injected volume of 5 µL for each solution. The flow rate was 0.2 mL/min with a binary mobile phase gradient of solvent A (water + 0.1% formic acid) and solvent B (acetonitrile). The gradient was adapted from the literature[11] and started at 20% B and increased linearly from 20% to 40% (0-0.5 min); remained constant for 1 min (0.5-1.5 min); increased linearly from 40% to 100% for 10 min (1.5-11.5 min); remained constant at 100% until 19.5 min; decreased from 100% to 20% (19.5-20 min) and was kept constant at 20% during 9 min to recondition the column for the next analysis. The LC was coupled to a commercial timsTOF Pro II mass spectrometer (Bruker, Bremen). The electrospray source was operated in negative mode with a dry temperature of 180°C and a nebulizer pressure of 1.5 bar. The end plate offset and capillary voltages were set at 500 V and 4000 V, respectively and the dry gas flow rate applied was 4 L/min. The mass range analyzed was 50 to 2500 m/z and the inverse reduced ion mobility range scanned was from 1/K0 = 0.4 to 1.30, which is a range encompassing the ion mobilities of the $[M\text{-}H]^-$ ions of the C_4 to C_{18} PFCAs. Ions were accumulated for 100 ms before being separated in the TIMS cell with a ramp time of 300 ms, leading to a 33% duty cycle. High-purity nitrogen was used as the buffer gas in the TIMS cell and the pressure at the TIMS cell entrance was 2.74 mbar and 0.89 mbar at the mobility cell exit. The TIMS RF voltage was 250 Vpp, and the deflection voltages were 20V (Δ1), 120V (Δ2), -50V (Δ3), -20V (Δ4), -0V (Δ5), and -20V (Δ6). The qTOF parameters were the following: 250 Vpp (Funnel 2 RF and Multipole RF), 1000 Vpp (Collision RF), 8 eV (Quadrupole energy and Collision cell energy), -200 V (Collision cell in), 60 µs (Transfer time), and 5 µs (Pre-pulse storage). In TIMS, the relation between the elution voltage and inverse reduced ion mobility (1/K0) is virtually linear. Therefore, ion mobility values were calibrated using the ESI low concentration tune mixture (Agilent Technologies, Santa Clara, USA) as a reference standard, using the same TIMS parameters as for the compounds of interest. Measured mobility values were converted into CCS values during data processing, which was performed using DataAnalysis v4.0. The reported values are the averaged $^{TIMS}CCS_{N2}$ value obtained from five injections, and the standard and relative standard deviations were calculated.

3 Results

1. PFECAs

No CCS value could be assigned to the monomeric $[M\text{-}H]^-$ ion of the three PFECAs analyzed (see Figure 1 for the structures). However, a dimeric ion was observed for all three compounds. Calculated CCS values were 170.28 (± 0.28) $Å^2$, 184.68 (± 0.24) $Å^2$ and 198.83 (± 0.24) $Å^2$, for the dimeric ions of PF4OPeA, PFO5HxA and HFPO-DA, resepctively.

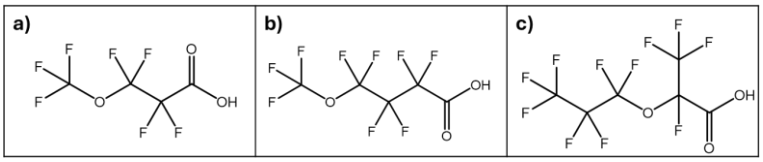

Figure 1: Structures of the PFECAs analyzed: a) PF4OPeA, b) PFO5HxA, and c) HFPO-DA.

Interestingly, in the mass spectra of PF4OPeA and PFO5HxA, the *m/z* of the $[M\text{-}H]^-$ ion is nevertheless present at a higher intensity than the *m/z* of the $[2M\text{-}H]^-$ ion, as shown in Figure 2a for PF4OPeA. However, by examining the mobilograms of these two ions (Figure 2b), it is clear that most of the $[M\text{-}H]^-$ ion intensity results from post-IMS dissociation of the corresponding $[2M\text{-}H]^-$ ion, as their inverse reduced mobilities ($1/K_0$) are aligned. This Figure 2b also shows that a peak with a lower $1/K_0$ (i.e. a lower CCS) is not observed for the $[M\text{-}H]^-$ ion, meaning that the $[M\text{-}H]^-$ ion in its monomeric form is not observed for these compounds. Similar behavior was observed for HFPO-DA, except that a fragment ion resulting from the loss of the CO_2 moity from the $[M\text{-}H]^-$ ion, was also identified. This fragmentation occurred before (*or during*) the mobility separation, and thus a CCS value could be assigned to the $[M\text{-}H\text{-}COO]^-$ fragment ion, which was 125.78 (± 0.30) $Å^2$. As CCS values could not be obtained for the monomeric deprotonated ions, the CCS values of the dimeric ions of the PFECAs studied are compared with those of the legacy PFCAs they replace, in Figure 3. PF4OPeA, which is similar to perfluoropentanoic acid (C_5 PFCA), but with an oxygen atom replacing a CF_2 unit, lies between C_4 PFCA and C_5 PFCA in CCS-retention time space, which is consistent with the difference in "volume occupancy" between an O atom and a CF_2 unit.

Similarly, PFO5HxA is located between C_5 PFCA and C_6 PFCA and HFPO-DA is located between C_6 PFCA and C_7 PFCA. These trendlines comparison can be helpful for identification purposes.

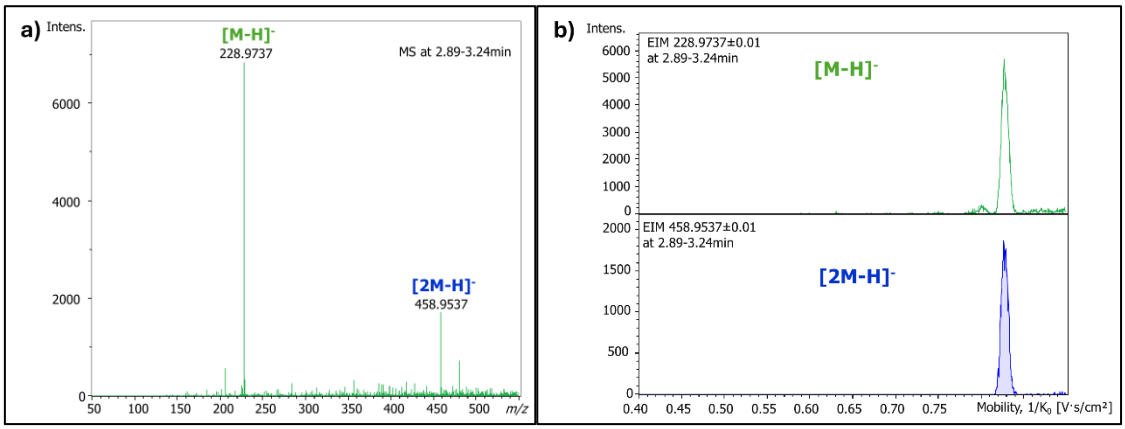

Figure 2: a) Extracted mass spectrum at the retention time of PF4OPeA, b) extracted mobilograms of the deprotonated PF4OPeA ion, in green, and of the dimeric PF4OPeA ion, in blue.

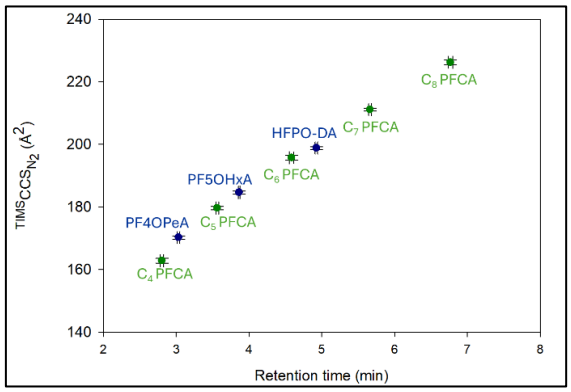

Figure 3: $^{TIMS}CCS_{N2}$ values versus retention times of the $[2M-H]^-$ ions of legacy C_4-C_8 PFCAs (green dots) and of the $[2M-H]^-$ ions of the PFECAs studied (blue dots). Error bars represent twice the standard deviations.

2. PFNA isomers

The CCS values could be attributed to the monomeric $[M-H]^-$ ion of three of the four PFNA isomers studied. P355TMHxA displayed no monomeric $[M-H]^-$ ion, as it either dimerized or fragmented into a stable $C_4F_9^-$ fragment. However, a CCS value could be assigned to dimeric $[2M-H]^-$ ions for all four isomers.

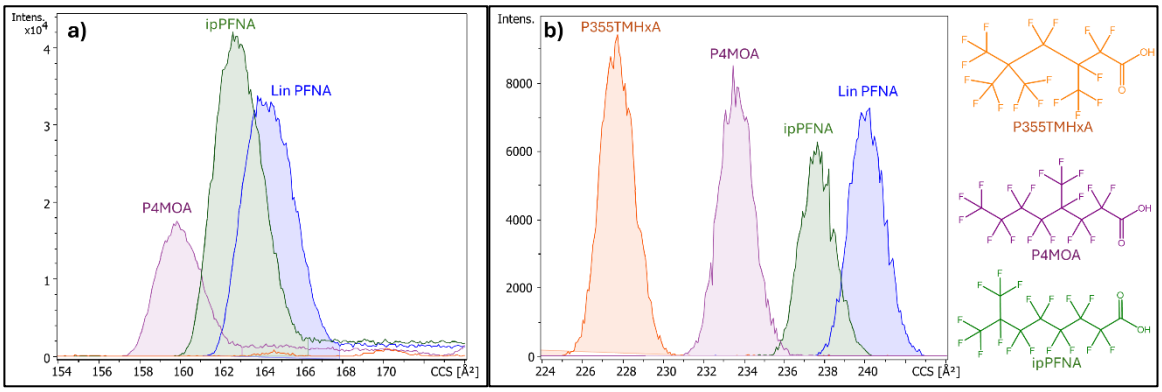

Figure 4: Ion mobility spectra of $[M-H]^-$ ions (a) and $[2M-H]^-$ ions (b) of the PFNA isomers studied, with the structures of branched isomers on the right.

Superimposing the mobilogram of monomeric $[M-H]^-$ ions (Figure 4a) and dimeric $[2M-H]^-$ ions (Figure 4b), shows that dimers are better separated than monomers. Indeed, the difference in CCS bewteen the dimeric ions of two isomers is greater than the difference in CCS between their corresponding monomeric ions. In addition, the full width at half maximum (FWHM) of the mobility peaks for dimeric ions is smaller than for monomeric ions.

However, in this case, a complete separation could have been achieved using the chromatographic information, as the retention time for P355TMHxA, P4MOA, ipPFNa, and linear PFNA are 6.85 (± 0.01) min, 7.43 (± 0.02) min, 7.54 (± 0.02) min, and 7.87 (± 0.01) min, respectively.

4 Discussion

1. PFECAs

The failure of determining a CCS value for the $[M-H]^-$ ion of the PFECAs analyzed is consistent with the DTIMS experiments performed in the literature[5]. In the same article, $[M-H-CO_2]^-$ ions were only observed for PFECAs with CF_3 branching on the alpha carbon of the carboxylic acid, such as HFPO-DA. The reported $^{TIMS}CCS_{N2}$ value for this $[M-H-CO_2]^-$ ion (125.78 (± 0.30) Å^2) is consistent with their $^{DTIMS}CCS_{N2}$ value (127.5 Å^2), within the commonly accepted CCS deviation of 2%[12]. It is also consistent with the $^{DTIMS}CCS_{N2}$ value (126.89 (± 0.09) Å^2) reported by Belova et al.[8]. These authors also determined the CCS value of the HFPO-DA dimer (198.61 (± 0.09) Å^2), which is consistent with our reported $^{TIMS}CCS_{N2}$ value (198.83 (± 0.24) Å^2). The results obtained in the case of PFECAs show that it can be interesting to take dimeric ions into account in LC-IMS-MS, as they are the only ions for which a CCS value can be reported with confidence. Therefore, the CCS value of these dimeric ions could be used as an identification point, in addition to the LC retention time, and these two identifiers could be compared with those of the legacy PFCAs they replace, as shown in Figure 3. These PFECAs thus illustrate that the absence of CCS values for the pseudo-parent monomeric ions can be circumvented based on a comparison of the trendlines of dimeric ions of common PFCAs and emerging PFECAs.

2. PFNA isomers

The influence of the number of branching on the LC retention times and CCS evolution is similar to the observation made by Dodds et al.[5] in DTIMS on perfluorooctanoic acid (PFOA) isomers. In other words, compounds with a greater number of branching have a lower retention time and CCS value, which is coherent, as they should be more compact than a corresponding linear isomer. Secondly, the effect of branching position on CCS and retention time is more subtle, as observed here between ipPFNA and P4MOA. However, in this paper[5], the possibility of studying their dimeric ions is not mentioned. In our study, we showed that dimeric ions might be better resolved, as they FWHM is lower than for monomeric ions. For example, the calculated resolving power is around 119 for the dimeric ion of PF4MOA versus 62 for its monomeric ion. This could be linked to the fact that dimers have a lower intensity than monomers, resulting in fewer space charge effects[13]. This could be the main explanation, because although the net difference in CCS between the different dimeric ion is higher than between their corresponding monomeric ions, the relative differences in CCS (i.e. the difference in CCS normalized with respect to the mean CCS value) are similar between dimers and between monomers. However, it is the relative difference in CCS that reflects the resolving power necessary to separate the ions[14]. Nevertheless, dimers can also be useful in cases where the $[M-H]^-$ is not sufficiently stable for certain isomers such as P355TMHxA.

5 Conclusions

Carboxylated PFASs form dimeric ions when analyzed by LC-IMS-MS, in TIMS, in our study, and in DTIMS and TWIMS, in the literature. Therefore, dimerization seems to be an effect more related to the chemistry of these compounds, rather than one associated to instrumentation or ionization parameters. In our study, we set out to determine whether these dimeric ions could be of useful for non-targeted screening, or whether they should be avoided as much as possible. The study was based on example compound families: PFECAs and branched isomers of PFNAs. It demonstrated that dimers can indeed be of valuable use, particularly when the monomeric pseudo-parent ion is susceptible to fragmentation (e.g. HFPO-DA and P355TMHxA) or cannot be detected (e.g. PF4OPeA and PFO5HxA). Moreover, in some cases, they could be useful to better separate different branched isomers of the same compound. However, in this study, we only injected four relatively different isomers separately. It might be useful to investigate this further on a more complex mixture of PFCA isomers, in a technical mixture or in a real sample, for instance. Then, it could also be relevant to see whether these dimers also form with other ionization sources such as VIP-HESI, developed by Bruker, which is claimed to significantly increases ion yield, thanks to its heated electrospray technology.

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