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Characterisation of fluorinated copolymers using liquid chromatography coupled on-line to mass spectrometry, with automated data interpretation

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Abstract

A perfluorinated co-polyether was characterised in terms of the number and type of functional end groups present on the molecule. The polymer was separated chromatographically according to the polarity of the polymer end groups and the separation was coupled on-line to an electrospray ionisation time-of-flight mass spectrometer. Negative-mode electrospray ionisation of the relatively non-polar polymer was achieved by post-column addition of a polar constituent to the mobile phase. LC–MS analysis of polydisperse analytes is a highly data intensive technique and manual interpretation of the resulting data can be extremely complicated, especially for the characterisation of copolymers or polymers with end-group distributions. In order to overcome this problem, an automated data-analysis program was developed that allows the user to quickly determine the probability of the presence of a certain molecular compound. The program evaluated data in terms of the possible combinations of monomeric units and end groups that could be combined to make up the mass values present in the mass spectra. Using the program, the polymer can be characterised according to its molar-mass, chemical-composition and functionality-type distributions. A graphical representation of the LC–MS analyses is presented to give a clear overview of the two-dimensional separation. The identification of various end groups on the polymer is also presented graphically, as (a) a histogram (frequency of matches versus time), (b) a two-dimensional plot (masses that match the particular end group combination versus LC retention time) and (c) a plot of average chemical composition versus LC retention time.

Keywords: Data interpretation, automated; Automation; Organofluorine compounds; Polymers

1. Introduction

1.1. Characterisation of polymeric distributions

Synthetic polymers exhibit multi-dimensional distributions. Even in the case of the simplest homopolymer, a molar-mass distribution (MMD) is present. When the sample becomes more complex, for instance a copolymer or a functionalised polymer, other distributions, such as a chemical-composition distribution (CCD) or a functionality-type distribution (FTD) will also be present. These distributions all influence the physical and chemical characteristics of the final polymer and thus need to be characterised for a fuller understanding of the relationships between molecular structure and the properties of the polymer [1].

1.2. Perfluoropolyethers

Perfluoropolyethers (PFPEs) are low-molar-mass polymers that are liquid over a broad range of temperatures (−100 °C to +400 °C). They exhibit low volatility and their viscosity shows very little dependence on temperature, making them excellent high-performance lubricants [2]. In addition, PFPEs are highly stable in aggressive environments (e.g. in the presence of oxidisers), making them useful for applications under extreme conditions. They have found a

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wide range of uses, including high-performance lubricants for space equipment, jet engines and magnetic-recording disks [3] and as anticorrosive coatings [4]. When the polymer is functionalised, it can be cross-linked to form durable coatings or used as an intermediate in polymer synthesis to impart specific properties, such as flexibility at low temperatures and improved fracture and wear resistance [5].

PFPE polymers can have various chemical structures depending on the polymerisation technique used. Possible monomeric units include CF_2O, CF_2CF_2O, $CF_2CF_2CF_2O$ and $CF(CF_3)CF_2O$. In the present case, the polymer is a copolymer consisting of CF_2O and CF_2CF_2O repeat units, that are combined to form a random copolymer. It is synthesised in a stepwise reaction, first to perfluoropolyperoxide and then from an esterified to a hydoxylated polymer [2].

The polymer can be capped by a number of different end groups (functional or non-functional), depending on the method and conditions of synthesis and on the required final application [6]. In the present application, two hydroxyl end groups are required, so that the PFPE can be further used as a cross-linking reagent. However, due to the nature of the PFPE synthesis reaction, a number of other end groups are also present and can affect the cross-linking ability of the PFPE. These end groups can be identified by ^{13}C and ^{19}F NMR analysis.

In order to fully predict and control the cross-linking efficacy of the polymer (and therefore the physical and chemical properties of the final polymer product), it was necessary to characterise the end-group distributions of the various batches of the polymer, especially the ratio of the polymer fractions containing one and two hydroxyl end groups. Other functional end groups present in the distribution (e.g. carboxylic and ester groups) also need to be quantified in terms of their distribution. Non-functional polymer, i.e. PFPE with no functional end groups, is known to be present, but since this can be selectively removed, it was not considered relevant in this characterisation.

The average molar mass of the polymer has been found by size-exclusion chromatographic analysis to be approximately 3000 Da and the average chemical composition (from NMR) is approximately *m*:*n* 10:10 (where *m* and *n* are the numbers of CF_2O and CF_2CF_2O monomers, respectively). Table 1 lists the end groups that have been identified by NMR analysis. However, since NMR can only indicate distribution *averages* and does not provide any indication of the underlying distribution, a separation technique is needed to address this problem.

Table 1
End groups present in the PFPE polymer samples

Possible end groups	Molar mass
–H	1.00783
–OH	17.00275
$–CF_2–CH_2–OH$	81.0152
$–CF_3$	68.9952
$–CF_2–Cl$	84.96565
$–CF_2–CF_2–Cl$	134.9624
$–CF_2–H$	51.00463
$–CF_2–CF_2–H$	101.0014
$–C(=O)–OCH_3$	59.01332
$–C(=O)–OC_2H_5$	73.02897

1.3. LC of PFPEs

Liquid chromatography can separate polymers on the basis of their size (e.g. size-exclusion chromatography) [7] or on the basis of their molecular interactions with the mobile and stationary phases (interactive LC, e.g. normal-phase and reversed-phase LC) [8,9]. Coupling the separation to an informative detector, such as a photodiode-array UV detector or an infrared detector, or to a second (ideally orthogonal) LC technique or a mass spectrometer, can add a considerable amount of additional information [10]. For these samples, a separation based on the functional end groups was required and so, the influence of the polymeric backbone on the separation needed to be minimised. By combining a polar stationary phase, e.g. a silica column, with a properly tuned mobile-phase system, (i.e. a thermodynamically good solvent for the polymer, that was not a strong (polar) eluent), retention on the stationary phase can be controlled by interactions between the polar end groups of the polymer and the silanol groups of the stationary phase.

1.4. Mass spectrometry of polymers

Mass spectrometry is a powerful analytical technique and is widely used for the analysis of chemical and biological samples. In the case of synthetic polymers, the mass spectrum will consist of a distribution of mass/charge values. Provided that a very soft ionisation technique is used (so that fragmentation of molecular ions is negligible) and the polymer sample is sufficiently narrow in its polydispersity, the observed mass/charge ratio can be related to the molar-mass distribution, so that the average molar mass and the polydispersity of the sample can be calculated [11]. Although it has been suggested that MS can be used as a ‘stand alone’ technique for polymer characterisation, it is now generally accepted that some form of separation is essential prior to MS analysis of polydisperse samples because mass discrimination and ion suppression can lead to inaccurate mass spectral information [12,13]. By separating the polymer according to molecular differences, the molecules entering the ionising interface at any given time will be quite similar, so that loss of information due to ion suppression can be avoided or, at the very least, minimised [14].

1.5. Mass spectrometery of PFPEs

The polymeric backbone of PFPE is not polar and will not ionise readily in the non-polar solvents that are required for elution in L. Latourte et al. [15] studied various matrix-assisted laser desorption ionisation (MALDI) and

electrospray ionisation (ESI) approaches for the characterisation of fluorinated polymers. With ESI, they found that the addition of an aqueous modifier (water and methanol) to the solvent vastly improved the observed signal intensities in the MS. However, they suggested that excessively high aqueous contents disfavoured ionisation of longer-chain fluorocarbons due to preferential intermolecular aggregation. Protonation (or deprotonation) was found to be the main mechanism for ionisation. For MALDI-MS, detection of ions related to the intact polymer was possible. However, incompatibility of the polymer solvent with the matrix was sometimes a problem and could lead to preferential ionisation. Fluorinated matrices have been shown to improve the ionisation efficiency in MALDI [16]. Time-of-flight (TOF) secondary ion mass spectrometry (SIMS) has been used for the characterisation of PFPEs deposited on a solid surface. For non-functionalised PFPE, Spool and Kasai [17] found that the negative-ion spectra showed simple patterns, with mass values that exceeded the average molecular weight. Spectra in positive-ion mode were more complex and were skewed towards the lower molar-mass region. When hydroxyl end groups were present on the polymer [18], ions were observed in both ionisation modes. A fragmentation pattern associated with the molar-mass distribution was seen in the positive-ion mode. In the negative-ion mode, no pattern due to R–O– ions was seen. It was therefore concluded that ionisation of the polymer occurred at the hydroxyl sector. Marie et al. [19] characterised polyvinylidene fluoride by collision-induced dissociation in an ESI-ion-trap–MS. Information on the repeating unit and end groups could be obtained. End-group substituents were found to influence the orientation of the fragmentation pathways. Backbone cleavage did not take place.

2. Experimental

2.1. LC separation

A Waters 2690 gradient-LC system (Waters, Milford, MA, USA) was coupled to a SEDEX 55 (Sedre s.a., Alfortville, France) evaporative light-scattering detection (ELSD) system. The mobile phase was a mixture of either methyl *tert*-butyl ether (MTBE) or acetone and 1,1,2-trichlorotrifluoroethane (Freon 113). All solvents were HPLC grade and were supplied by Merck (Darmstadt, Germany). The flow rate was 0.4 mL/min. A Nucleosil silica stationary phase (Machery–Nagel, Easton, PA, USA) (150 mm × 2.1 mm), was used at a temperature of 35 °C. Three PFPE sample oils were studied, namely HF2000, BL0420 and 1603RG. These were supplied by Ausimont (Bollate, Milan, Italy). The samples were taken from different batches of the same product. One of the samples (HF2000) was a purified batch, which had a significantly lower concentration of non-functional and di-functional polymer. Sample concentration was approximately 10 mg/ml in each case and 7 μL were injected onto the column. Mixtures of HPLC-grade isopropanol and water were added post-column at 20 μL/min using a Shimadzu LC-10ADVP micro-plunger pump.

2.2. Mass spectrometry

MS analysis was carried out on a Micromass ESI–TOF mass spectrometer with an orthogonal interface. The MS was operated in the negative-ion mode. The eluent from the LC was split after the column in a 90:10 ratio (ELSD:MS). The capillary voltage was 3 kV, the desolvation temperature was 350 °C and the source temperature was 120 °C. The cone-gas flow rate was 30 L/h and the desolvation gas flow was 350 L/h. The MS scans were collected in the continuous mode.

For data-analysis purposes, the mass spectra were calibrated using an external NaCsI calibration solution. The spectra were then combined into files that each covered ten scans of consecutive MS data (corresponding to ten seconds of chromatographic separation). The combined spectra were smoothed (twice, over two channels using the Savitzky–Golay method) and then centred (according to area, using a minimum peak width of three channels and a centroid top of 70%). The relevant data in the centred and calibrated spectra, i.e. mass values and corresponding intensities were then converted to text files for export to a dedicated data-analysis program.

3. Results and discussion

3.1. LC separation

The PFPE samples are soluble in a limited number of unconventional HPLC solvents (e.g. freon, hexafluoroisopropanol and hexafluoroxylene). In this work, freon 113 was chosen as the good solvent and the retention of the polymer was investigated in a freon–acetone mobile phase system and in a freon–MTBE mobile phase system. The PFPEs are insoluble in both acetone and MTBE and so care was needed to ensure that the mobile phase was polar enough to displace the polymer from the polar silanol groups of the stationary phase, but not so polar as to induce precipitation of the polymer onto the stationary phase.

The LC separation was based on interactions between the polymer end groups and the silanol groups of a silica stationary phase. The PFPE samples were all fully retained in a 100% freon mobile phase, indicating that retention is based on interactions between the polar end groups of the polymer and the stationary phase. Thus, Freon is a *good* solvent, but not in this case a *strong* eluent [20]. In a freon–acetone (97:3) mobile phase, an isocratic separation resulted in three discernible peaks. However, resolution was poor (Fig. 1). MTBE is less polar than acetone and using this solvent, the resolution was significantly improved. A gradient from 0.1

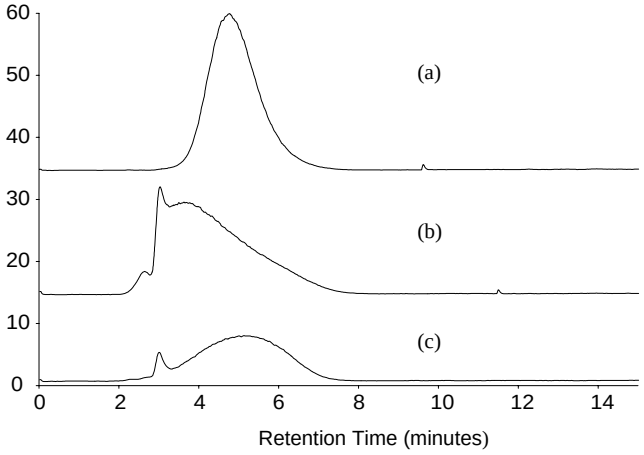


Fig. 1. Chromatographic separation of the three PFPE samples (a) HF2000, (b) BL0420 (c) 1603RG. Stationary phase: Nucleosil silica 5 μm, 150 mm × 2.1 mm. Mobile phase: 3% acetone in 1,1,2-trichlorotrifluoroethane. Injection volume 5 μL. Column temperature 35 °C.

to 20% MTBE in freon in 15 min was found to be optimal. Typical ELSD chromatograms for each of the three sample PFPE oils are shown in Fig. 2a–c. Only one peak was seen for the HF2000 sample, because this is a refined sample that contains almost exclusively diol end groups. For the other two PFPE samples (BL0420 and 1603RG), three peaks are seen in the ELSD trace. It was tentatively assumed that the peaks corresponded to the non-functional (ca. 1.6 min), the mono-functional (ca. 7 min) and the di-functional polymer (ca. 10–12 min). However, this could not be proven using ELSD alone.

3.2. Flow-injection MS

The MS system was operated in the negative-ion mode. No ionisation of the polymer was possible, unless a polar solvent was added post-column to the mobile phase.

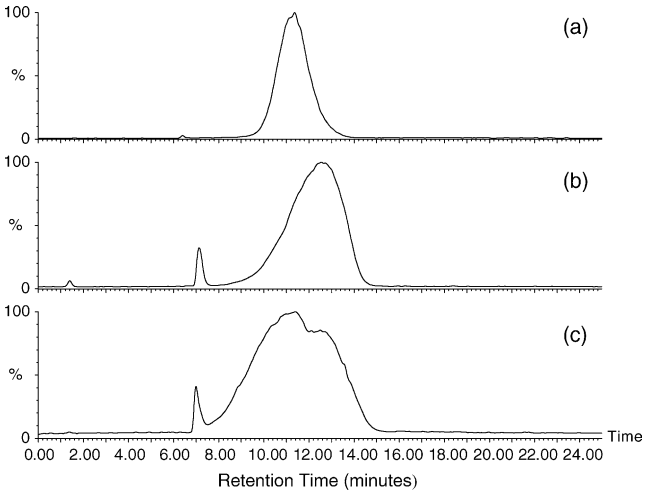


Fig. 2. Chromatographic separation of the three PFPE samples (a) HF2000, (b) BL0420 (c) 1603RG. Mobile phase: 0.1–20% of methyl *tert*-butyl ether (MTBE) in 15 min. Other conditions as in Fig. 1.

It was found that a mixture of isopropanol–water (50:50) (20 μL/min), added to the mobile phase via a T-piece just before the ESI interface, gave a reasonable ionisation of the samples, as observed by the total-ion-current (TIC) signal. It is interesting to note that the post-column mixture is imiscible with the non-polar freon–ether mobile phase. Since no proper mixing can take place when the solvents are in the condensed phase, this observation seems to suggest that ion-transfer from the solvent to the analyte molecules takes place in the gas phase rather than in the condensed phase. No multiple charging was observed in the mass spectra. This may be due to the non-polar nature of the solvent, which is reported to diminish multiple-charge states [21]. To date, no ionisation of the polymer has been possible in the positive-ion mode. However, this will be the subject of further research.

The effect of the cone voltage of the (negative-mode) ESI on the observed molar-mass pattern was also optimised to give the highest observed intensity. The cone voltage is a particularly important parameter for mass spectrometry of polydisperse samples, because it can affect the observed distribution over a given *m*/*z* range. The effect of cone voltage on the observed ion intensities is not fully understood, although it has been suggested that it is related to an ion-optical effect arising indirectly from gas dynamics [22]. Fig. 3 shows the mass spectra obtained for the same PFPE sample at different cone voltages. The apparent distribution changes significantly with cone voltage, although for polymers with low polydispersities (or polymers that have been separated according to molecular weight) the effect of the cone voltage has been reported to be less pronounced [22]. A cone voltage of 50 V was used in further experiments, since it gave the highest intensities over the relevant mass range.

3.3. Coupling LC–MS

On-line coupling of a liquid separation to a mass spectrometer is relatively straightforward using an ESI interface [23]. The LC flow rate (0.4 mL/min) was split so that only 10% of the volume was pumped to the MS. The samples were separated chromatographically according to the polarity of their end groups (and to a lesser extent their molar mass). Assuming that it is the polar end groups of the polymer rather than its non-polar backbone that is ionised (see discussion below), ion suppression due to molecules with chemically different end groups entering the ionisation interface simultaneously should be minimised by the LC separation. Care was also taken to ensure that tubing between the LC and MS was compatible with the LC solvents. In particular, polyether ether ketone (PEEK) tubing was replaced with stainless-steel and fused-silica capillaries.

3.4. Data interpretation of LC–MS data

Coupling LC–MS is a particularly powerful combination for polymer analysis. The multi-dimensional nature of

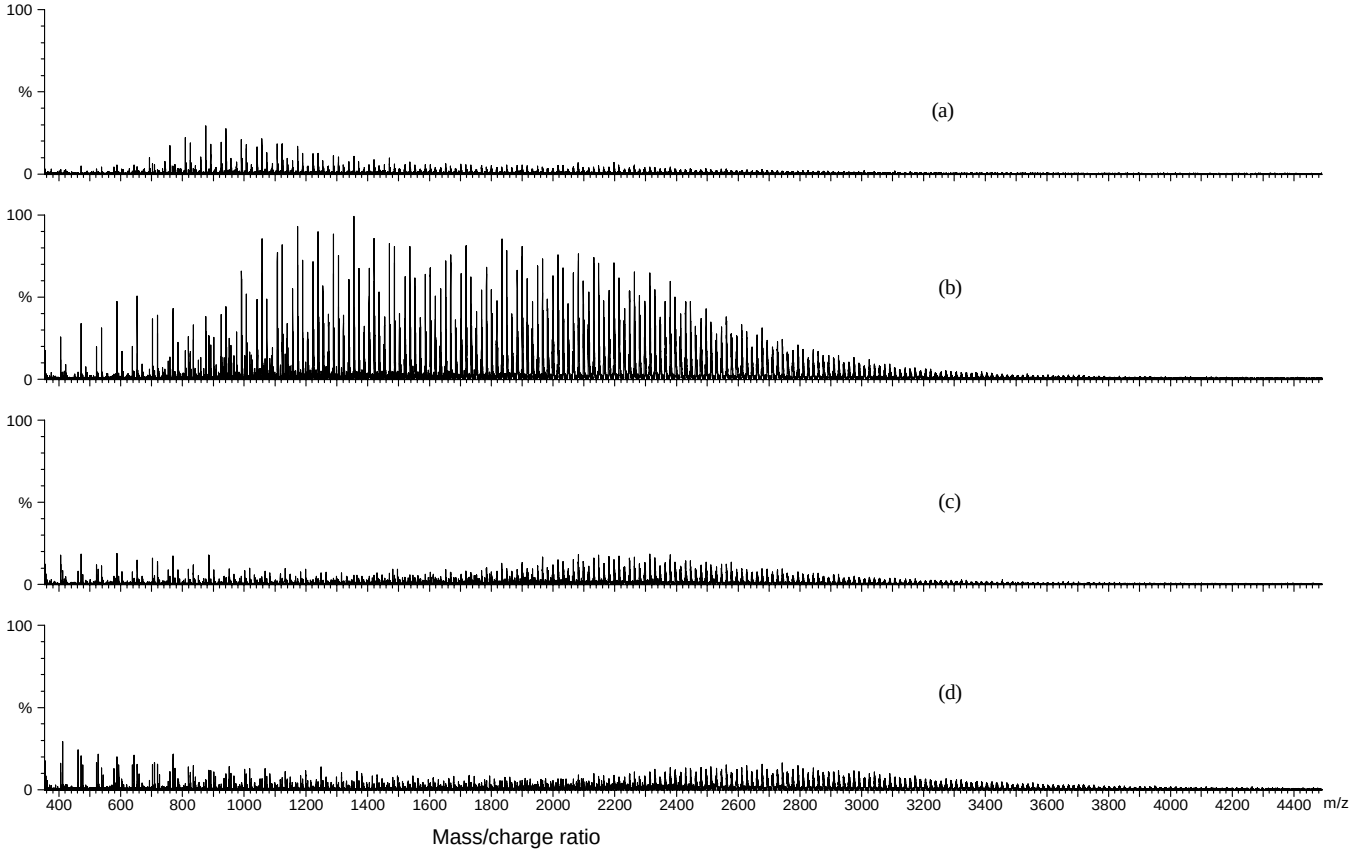


Fig. 3. Observed MS intensities for sample BL0420 at four ESI cone voltages: (a) −30 V; (b) −50 V; (c) −70 V and (d) −100 V. 100% intensity corresponds to 400 counts in each case. A cone voltage of −50 V was chosen for further experiments.

polymers implies that the molar mass (and other structural properties) will vary across the chromatographic peak. This can be clearly seen if the MS spectra of a series of consecutive scans taken from one chromatographic peak are compared. In Fig. 4, four combined spectra, each consisting of 10 s of MS data are shown. The molar-mass distribution in each of the spectra is different and the average mass is seen to decrease with increasing time. When retention times are greater than the dead time of the column (t_0) (i.e. when the retention mechanism is not based on exclusion), this is indicative of a separation based on functional end groups rather than on the monomeric units.

Data interpretation of LC–MS analyses of polymers can be very complicated. The chromatographic peaks obtained in polymer separations are not unimolecular, but instead contain a large number of molecules that differ not only in molar mass, but often also in chemical structure. For example, the monomeric ratio of a copolymer or the end groups of a functionalised (co)polymer may vary. Even when there are no fragments or multiply charged ions formed in the ionisation process, a huge number of masses can be observed and need to be assigned to particular structures.

In the simplest case (i.e. a homopolymer), the mass of the end groups on the polymer can be determined by extrapolating back to zero number of monomeric units [or by further ionising the polymer so that it fragments and the masses of the individual end groups can be discerned (MS–MS)]. When the polymer is more complicated, for example for copolymers, data interpretetaion becomes much more complicated. For the PFPE copolymers, each observed ionic mass is made up of *m* CF_2O monomeric units and *n* CF_2CF_2O monomeric units plus the masses of both end groups (E), minus the mass of the abstracted ion (X). Depending on the differences between the masses of the monomeric units and the end groups, a large number of theoretical masses can be expected in the MS spectra and each mass needs to be assigned to a particular combination of *m and n* monomers plus the mass of E–X.

Manual data interpretation of these types of MS spectra can be extremely difficult. The number of peaks that are present and the number of possible combinations of monomeric units and end groups that each mass can be made up from, make manual peak interpretation excessively time consuming and complicated. Using an automated peak-matching program, the task of assigning each observed peak in the mass spectrum to a given polymeric structure becomes less tedious. For each peak, it can be automatically determined which structure(s) fit the corresponding mass.

For the analysis of the PFPEs, the primary aim was to determine what the end groups of the polymer were. To achieve

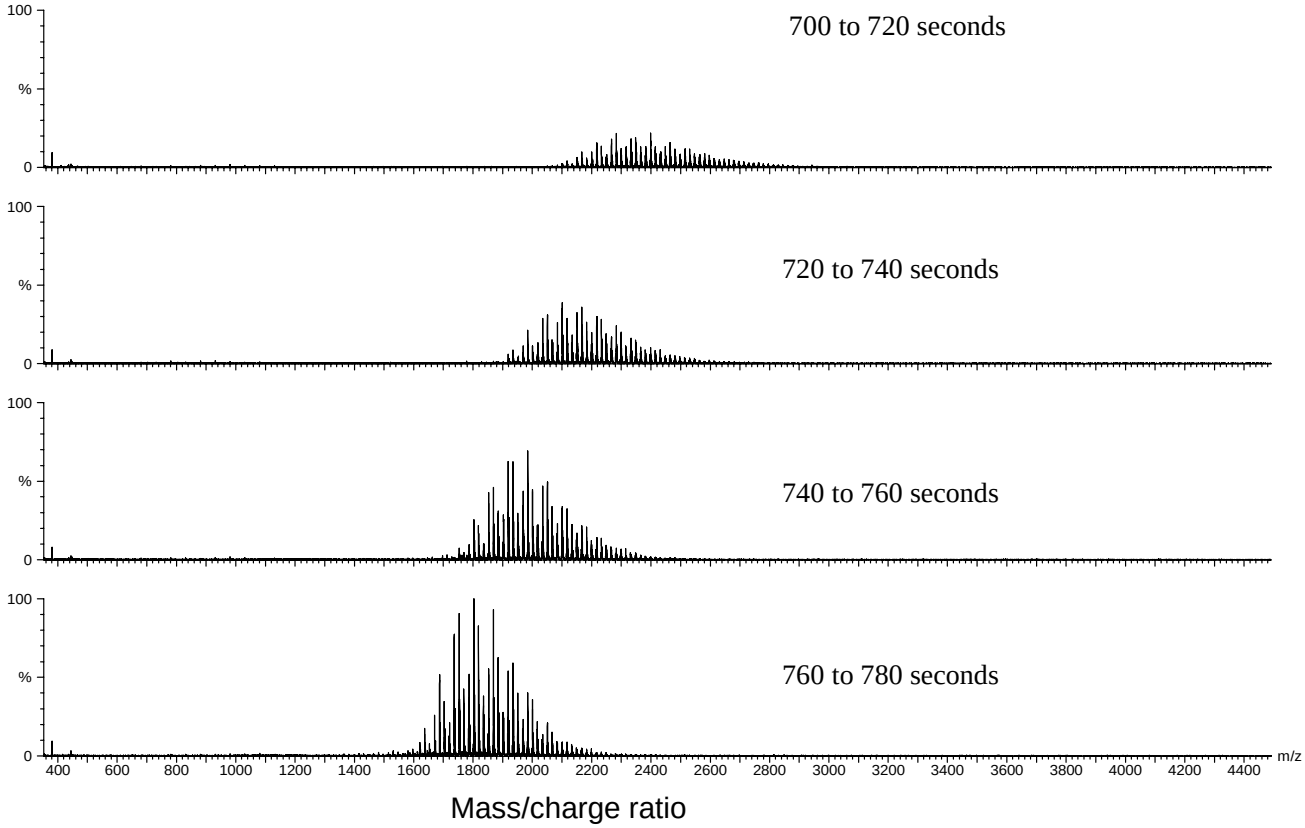
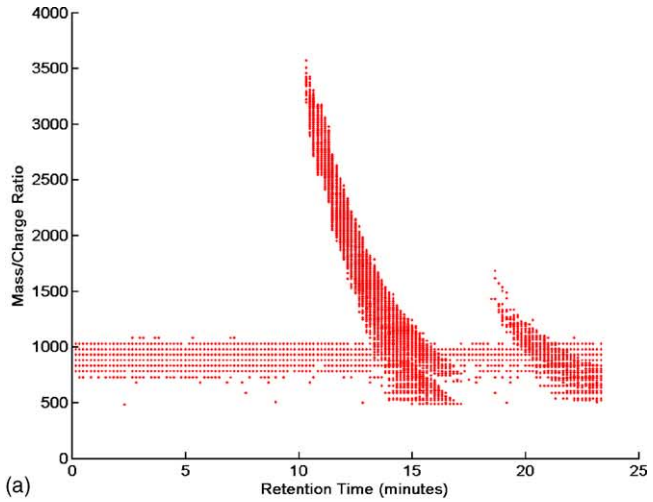


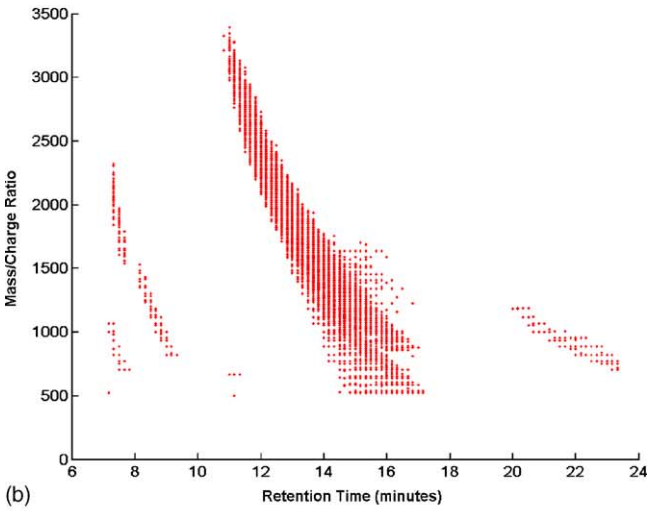
Fig. 4. Four combined mass spectra from sample BL0420. Each spectrum combines 20 scans, i.e. 20 s of MS data (from 700 to 780 s).

this, each observed mass/charge value was automatically examined to see if it could be made up by a given combination of end groups and monomeric units (taking the mass of the abstracted ion into account). By repeating this for every peak in each scan over the chromatographic separation, a clear indication of the end groups present on the PFPE polymer eluting across the chromatographic peaks could be obtained.

Within the program, masses were selected, either on the basis of a noise threshold (set by the user), or on a fixed number of the most intense peaks in each scan (for example, the 10 highest peaks). An overview of the separation was obtained by plotting the chromatographic and mass-spectrometric separations in a planar two-dimensional format. This type of plot shows quite clearly how the mass values and mass ranges of the PFPE copolymer vary across each of the chromatographic peaks. It is a particularly useful representation of the data, because it clearly illustrates 'group type' patterns that may not always be obvious from chromatograms. Some other studies have also used plots such as these for the structural elucidation of complex polymers [24], as well as for peptide mapping [25]. Fig. 5a–c are examples of two-dimensional (2D) plots for each of the three sample oils, HF2000, BL0420 and 1603RG. Because of low-molar-mass background interference, only *m*/*z* values higher than 480 units were considered. For the HF2000 sample, a significant background remains in the region up to approximately 1000 *m*/*z* units, under the chosen noise-threshold level (25 units of intensity on the MS). However, since there also appears to be polymer in this mass region, it was not removed. The first peak in the ELSD trace (1.6 min) was not seen by the mass spectrometer, probably because it corresponded to the fraction of the polymer with non-polar (and therefore non-ionisable) end-groups. Since this fraction can be extracted from the polymer, its MS characterisation was not required. For all of the polymers, a strong band corresponding to the main peak in the chromatogram (11–18 min) was seen. Two of the samples (BL0420 and 1603RG) showed a peak eluting at around 8 min, assumed to correspond to the mono-functional fraction of the polymer. For the purest sample, i.e. HF2000, no mono-functional fraction was present. However, all three samples had a group that eluted after the main peak that was not apparent in the ELSD trace. Each group in the 2D plot represents a separate polymeric fraction, each fraction presumably having a different end-group combination.

A second way to look at the data is to view it as a three-dimensional (3D) surface plot. In this case, the intensity of the peaks in the mass spectrum is plotted on a third axis. The surface plot highlights the relative intensity of each of the groups present in the LC–MS plot. An example of a surface plot is given in Fig. 6. Significantly, the 3D plot highlights a valley running along the low-molar-mass region of the main peak. The second (minor) peak could be due to multiply charged polymer ions, but since inspection of the isotopic





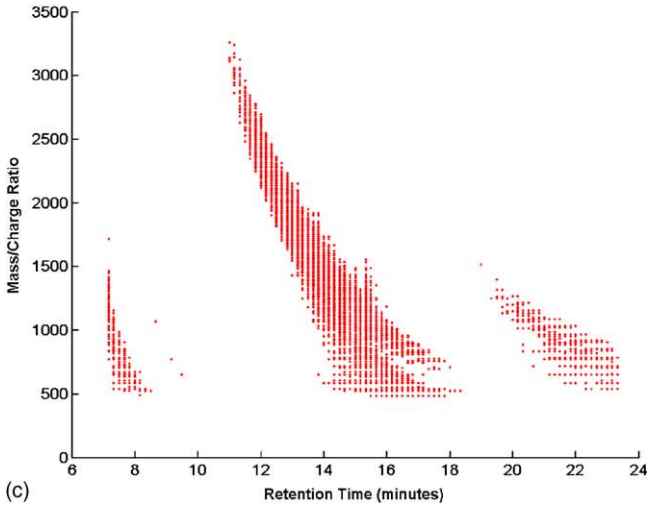


Fig. 5. 2D Plots of mass vs. retention time for (a) HF2000, (b) BL0420 and (c) 1603RG.

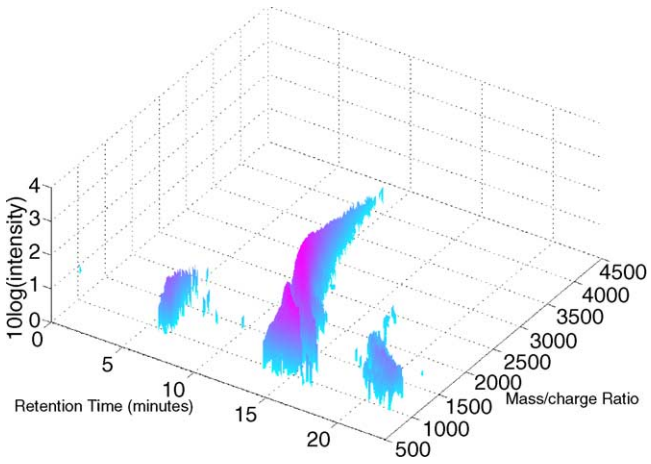


Fig. 6. Quasi 3D surface plot of sample 1603RG.

pattern showed differences between peaks of one unit, this was not the case. It is more likely that the minor group differs slightly in its chemical nature from the main group.

Once the various groups in the separation are recognised, each group must be identified, i.e. the correct end-group combination must be assigned in order to determine the functionality-type distribution of the polymeric samples.

3.5. Determination of the ion of abstraction

If the end groups of the polymer are to be determined from the mass spectrum, it is necessary to know the ion that is abstracted from the molecule in the ionisation process. For the PFPE samples, the ion of abstraction appears to come from the end groups. If an ion was abstracted from the polymeric backbone, it is likely that more than one ion would be abstracted (since the monomeric units are all similar), resulting in multiple charging. Also, since the first peak seen in the ELSD chromatogram (assumed to contain no polar end groups) is not seen by the mass spectrometer, it seems reasonable to assume that the polar end groups of the polymer play a dominant role in the ionisation process. Since the main peak in sample HF2000 is known to contain the 'standard' $–CF_2CH_2OH$ end groups, the ion of abstraction was established by assigning two $–CF_2CH_2OH$ end groups to the masses observed for that peak. The difference between the experimentally observed masses and the theoretical masses of the standard end group copolymer should then equal the mass of the abstracted ion. The highest number of matches was found for the abstraction of one entire end group, i.e. $–CF_2CH_2OH$. It is also possible that only the $–CH_2OH$ part of the end group is abstracted. However, since the difference between these two ions is a CF_2 group and that is the also the difference between the two monomeric units, it is impossible to differentiate between them using mass spectrometry. A simple H^+ ion abstraction did not result in masses that corresponded with the experimentally observed masses.

3.6. Determination of the end groups

The end groups present in the bulk polymer have been identified by NMR (Table 1). By calculating the masses that would be present for a given end-group combination, a comparison between these masses and the experimentally observed values can be made. Using an automated 'matching' program, the number of matches per extracted scan could easily be calculated. Within the program, the user can define how close the theoretical and experimental mass values must be in order to be considered a reasonable match (e.g. ±0.5 *m*/*z* units). For each end-group combination, the number of 'hits' (i.e. correct matches) are calculated and plotted against time. An example of this, for the BL0420 sample, matching two $–CF_2CH_2OH$ end groups, is given in Fig. 7. The matches are represented in three different ways. The first is a histogram outlining the number of 'hits' per scan. The second plot is a 2D plot. This is similar to the 2D plot in Fig. 5, but in this case, highlighting only those masses that could be matched to the chosen end-group combination. This format indicates the *structure* of the resulting matches. This is an important consideration when assigning the correct end-group combination, because even if there are a high number of hits (seen in the histogram), unless they are present in an ordered fashion, the assignment does not make sense. The third plot shows the average monomeric ratio of the hits in each scan. It is known (from NMR), that the average chemical composition of the copolymer monomeric units is around 1:1 (*n*:*m* value). Although this is an *average* value that will have a spread corresponding to the chemical composition distribution, it is reasonable to assume that most of the copolymer will have monomeric ratios somewhere around 1:1. The program has a function that allows the monomeric ratio to be considered when matching. By restricting the monomeric ratio to (for example) between 10:1 and 1:10, a reasonably broad range of chemical compositions will be accepted, without allowing too wide a variation in the chemical composition.

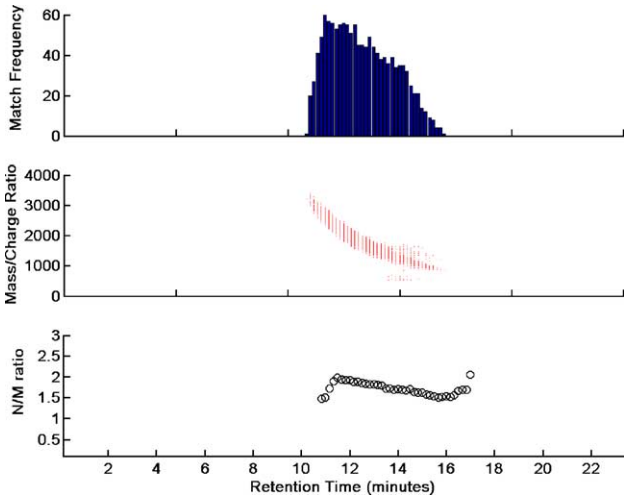


Fig. 7. Matches that were found for sample BL0420, using the standard end groups (two $–CF_2CH_2OH$).

3.7. Simplification of data analysis to avoid 'false positive' matches

The number of masses observed in each scan for the PFPE copolymers was very high and as masses increased, the difference between consecutive values decreased. For instance, a copolymer with eight CF_2O groups and one CF_2CF_2O group has a theoretical mass of 643.92 amu, while a copolymer with one CF_2O group and five CF_2CF_2O groups has a theoretical mass of 645.93 amu. When both polymers have the same end groups and the abstracted ion is the same, the mass difference between the two copolymers is 2.01 amu. In the high mass range, the difference between successive masses becomes small enough for many of the different monomer/end group combinations to fit the observed masses, leading to 'false-positive' matches. Isotopic patterns also complicated the data, because the intensity of isotopic peaks for high molar-mass molecules is quite significant.

In order to avoid misleading false-positive hits, the program can look specifically at the *n* most intense peaks in a given scan. The number of peaks that are chosen is arbitrary. However, it was necessary to ensure that the extracted peaks were representative of the *entire* polymer fraction that was eluted at that time. When a second (less-intense) group co-elutes, then information on the smaller group could be lost using this approach. In order to avoid this problem, the 2D plots obtained using both approaches (all peaks extracted versus top-*n* most-intense peaks) must be compared to ensure that *all* groups are present in both plots. For the PFPE samples, while there are points in the chromatographic run where there is some co-elution, all of the groups present in the fuller 2D representation are also present in the second, cleaner plots (in which only the ten most intense peaks were extracted). See Fig. 8a–c. Some information may be lost on the leading and tailing edges of co-eluting groups, but once the intensity of the tailing edge of the first group has decreased sufficiently, the second group will become the significant one and will then be considered in the end-group-matching process. In fact, by cleaning the plot of low intensity peaks, the *m*/*z* pattern of the main peak splits into two, similar to the surface plot in Fig. 6. Although the second group co-elutes with the main peak, it is sufficiently strong in intensity to still be seen.

Using this approach, there were much fewer 'false positive' hits for the end group matches. Since only ten peaks were extracted for each scan, the maximum number of hits in this case was ten. Matching the standard two $–CF_2CH_2OH$ end groups to the main peak gave a strong match in each case. Examples of some of the other matches are given in Fig. 9a–c. In some cases, matches are seen in more that one group. In Fig. 9a, the theoretical masses of a copolymer with one standard end group ($–CF_2CH_2OH$) and one ester end group [$C(=O)OCH_3$] were compared

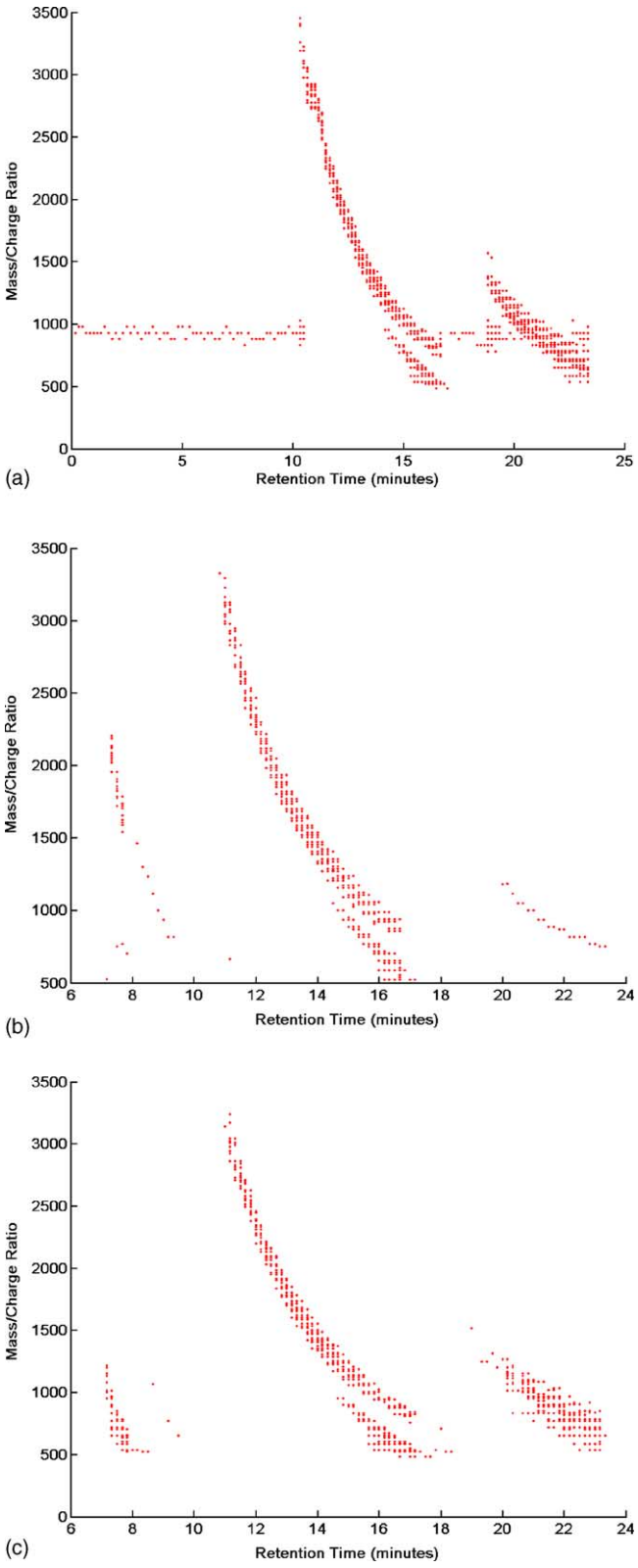


Fig. 8. 2D plots, similar to Fig. 5 but with only the 10 most-intense peaks in each scan plotted: (a) HF2000; (b) BL0420; and (c) 1603RG.

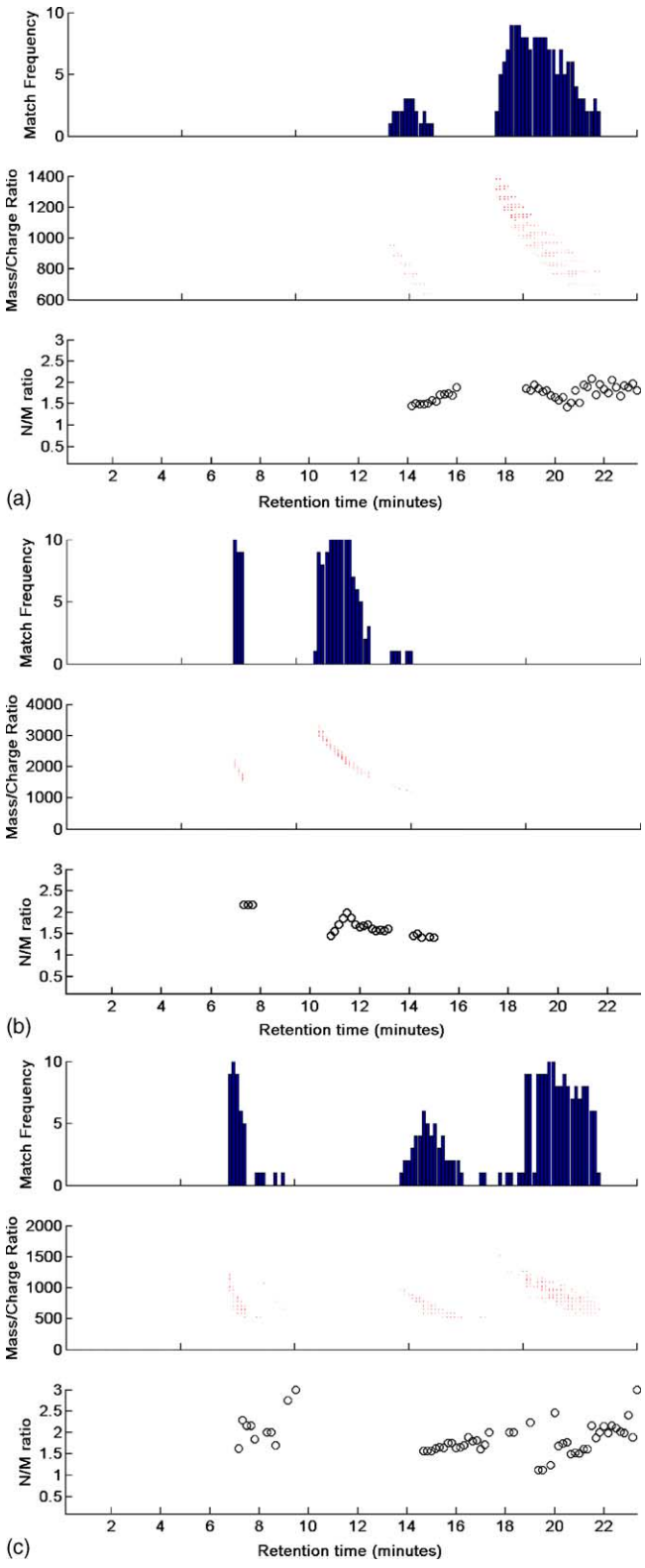


Fig. 9. Matches that were found for various end-group combinations when only the 10 most intense peaks in each combined spectrum are considered. (a) HF2000, end groups: CF_2CH_2OH and $C(=O)OCH_3$; (b) BL0420, end groups: CF_2CH_2OH and CF_3; and (c) BL0420, end groups: CF_2CH_2OH and CF_3.

with the ten most intense peaks in each of the scans of the HF2000 sample. This is the only combination that gave matches in the minor (low-molar-mass) group of the main peak (eluting between 14 and 18 min). Matches are also seen for the last peak (between 19 and 23 min). However, this is most likely due to further 'false positive' matches. Since it is known that the main group has two $–CF_2CH_2OH$ end groups, it can be argued that because an ester group is *less* polar than a hydroxyl group, it should not be eluted after the main (hydroxy capped) group. The matches in the earlier group therefore make more sense chromatographically. These end groups were also seen to match the minor group of the main peak for the other two samples. Fig. 9b shows the hits for the first peak of the BL0420 sample. This peak was assumed to be due to mono functional polymer and the best match that was found was with one hydroxy and one non-functional group (CF_3). Again, some hits were seen in the second group, however, since no real structure was present, it is not considered significant. For the last group in the chromatogram, the polarity of the end groups should be greater than the polarity of the end groups in the main group. For the 1603RG sample, the best match for the latest eluting peak (between 19 and 23 min) was with two carboxy end groups, i.e. COOH. Carboxyl groups are more polar than hydroxyl groups, so once again, this assignment makes sense chromatographically (Fig. 9c).

4. Conclusions

LC–ESI–MS of PFPE copolymers yields highly informative, but also highly complex data sets. When several different end groups are present, manual interpretation of the multi-distributed polymeric samples becomes virtually impossible and computer-aided data-handling programs are required for an automated approach to the problem. Presenting the data-rich LC–MS runs in various graphical formats simplifies data analysis and interpretation and gives a clear overview of the analysis. A significant number of possible end-group combinations can be quickly eliminated and the probability of other end-group combinations can be ranked. Nevertheless, the complexity of these PFPE samples does not allow a comprehensive characterisation of the polymer with only one technique. Further investigation of complimentary analysis methods is required to completely elucidate the structures and distributions of the PFPE copolymers. Techniques such as LC coupled to positive-mode ESI–MS, MALDI–MS and infrared spectroscopy are currently being investigated.

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