Coupling of liquid chromatography and fourier-transform infrared spectroscopy for the characterization of polymers
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5 Qualitative analysis of polymers and additives
5.1 A novel multivariate-data-analysis approach for the analysis of degraded polyvinylchloride by size-exclusion chromatography – Fourier-transform infrared spectroscopy

Summary
A new multivariate-data-analysis method DifSub, based on principal-component analysis, is introduced to reveal differences between large size-exclusion chromatography–Fourier-transform-infrared spectroscopy (SEC–FTIR) data sets and to obtain interpretable Fourier-transform-infrared spectral information. A feasibility study has been carried out, using differently aged polyvinylchloride (PVC) samples and reference PVC. The results of DifSub are compared to those obtained using conventional techniques and DifSub could be used to reveal differences between the samples. So-called Q-residual plots showed that newly formed functional-groups were present in all aged samples, but that they were not evenly distributed over the molar-mass region. Even for severely overlapping absorption bands of a reference PVC sample and an aged PVC sample, the interpretation is rather precise and minor differences between different PVC samples are easily revealed. The spectra obtained from DifSub showed subtle differences in the aromatic C–H stretch region. Although the DifSub method is very new and it has not been tested in a suitably large number of practical cases, it promises to yield much more detailed information from large data sets, such as those obtained by SEC–FTIR, than can possibly be obtained by manual interpretation. In the case of SEC–FTIR, this information can be combined with molar-mass data obtained from SEC with refractive-index (RI) detection. In comparison with manual interpretation, DifSub is very much faster and easier in any situation in which more than a few samples are being considered.

5.1.1 Introduction
The most widely used technique for the separation of polymers is size-exclusion chromatography (SEC). This technique separates according to hydrodynamic volume, which is related to the molar mass of a particular polymer, so that molar-mass information on the polymer can be obtained [1]. However, conventionally used detectors (e.g. refractive index, RI, ultra-violet, UV) do not provide detailed molar-mass-dependent chemical functional-group information. On the other hand, Fourier-transform infrared (FTIR) spectroscopy is a highly selective detection technique that can also provide structural information. Obviously, combining these two techniques (SEC–FTIR) has the advantage that molar-mass information and information on specific functionality changes in the
Polymers can be obtained simultaneously, even for analytes without chromophoric groups [2, 3].

Polymer aging has been the subject of investigations during many years, as aging will lead to a reduced service lifetime of polymeric products. A rapid method to provide information on the expected service lifetime of a polymer can be obtained by studying artificially aged specimens, subjected to accelerated aging procedures under controlled conditions (e.g. temperature, UV-light intensity, humidity). However, such conditions are different from those encountered with naturally aged samples in real applications. The chemical and physical processes occurring in these types of aging, give rise to changes in chemical composition and in structure and it can be expected that new functional-groups are formed. These processes are accompanied by a loss in mechanical integrity (e.g. impact toughness, expansion, breaking strength, brittleness) [4-6] that can be related to chain scission and cross-linking [6, 7].

In polyvinylchloride (PVC), under investigation in this work, chain scission and cross-linking may occur during the aging process, induced by thermal aging and photo-oxidation [6, 7]. The impact of these processes on the molar mass can be studied with conventional SEC-RI. Another important aspect specific for PVC involves colour changes (from yellow through brown to black [4]), which can be explained by the development of long polyenic sequences \((n \geq 6)\) arising from dehydrochlorination [5, 7]. The PVC aging process also gives rise to the formation of other functional-groups, such as peroxides, carbonyl groups, and hydroxyl groups. During processing double bonds, hydroperoxides, and ketones may be produced [6].

In principle, these functional-groups can be detected as function of the molar mass using SEC–FTIR. It is our aim to detect minor differences between aged PVC samples and a reference ("virginal") PVC sample. However, when employing SEC–FTIR very large data sets are obtained, which consist of many FTIR spectra. A comparison has to be made between a number of such very large data sets obtained for aged samples and the reference sample. Manual interpretation is tedious and time consuming, especially when the presence or absence of certain aging products is not unambiguous and when minor differences are expected between the samples. Pattern recognition techniques (e.g. multivariate data analysis, MVDA) can be used to reveal differences between reference and the aged samples. For example, principal-component analysis (PCA) has previously been used as data-reduction technique in the SEC–FTIR analysis of an artificially aged polymer-additive system [8]. However, PCA models were built for every individual sample. The loadings and concentration profiles (scores) provided insight in the degradation mechanism, but a manual comparison of the samples was still necessary.
In this work, we will introduce a new form of PCA [9] called DifSub to determine the exact molar-mass-dependent chemical differences between aged PVC samples mutually and relative to a reference PVC sample. In the initial stage of this feasibility study, FTIR-imaging was used to gain global chemical-composition information on the samples, i.e. the depth-distribution of aging products in the samples. The results of the DifSub procedure will be compared to results obtained from conventional SEC-RI and from SEC-FTIR without data treatment. Several stages can be discerned in the DifSub procedure.

1. A PCA model [9] is built from the SEC–FTIR data obtained for the reference PVC sample using the FTIR data in its original form without treatment (e.g. smoothing and/or the calculation of first or second derivatives). This allows full spectral interpretation at a later stage. Contrary to conventional PCA modelling, not just a few principal components (PCs) are used to describe the variation in the SEC–FTIR data set, but all relevant PCs are used to obtain a full descriptive model (spectral subspace).

2. SEC–FTIR data obtained from the analysis of naturally and artificially aged PVC samples are projected on the established spectral subspace. From this process the corresponding residual vectors orthogonal to the spectral subspace can be calculated for every aged sample.

3. In this way, interpretable spectra are obtained that reflect the difference between an aged sample and the reference PVC sample. The set of such spectra as a function of SEC elution time contains functional-group information as a function of hydrodynamic volume.

A feasibility study has been carried out to explore the possibilities of the DifSub procedure. It is not the objective of this study to elaborate on the mechanisms of PVC aging. For assessing the applicability of the DifSub procedure we made use of a data set acquired during the SEC–FTIR analysis of aged PVC samples. A suitable reference PVC sample was included in this set. This particular data set was chosen, because manual interpretation of the original FTIR spectra did not easily reveal the formation of new functional groups. We have investigated whether functional-group information can be obtained using the DifSub procedure.
5.1.2 Experimental

Sample description
Artificially aged, naturally aged and reference PVC drain-pipe samples (thickness, 2 mm) were all gifts from Dyka B.V. (Steenwijk, The Netherlands). Artificial aging was claimed to be equivalent to two years of outdoor exposure. This resulted in a brown discoloration in the top layer (denoted as PVCt). The colour of the underlying bulk (further referred to as PVCb) remained grey. Naturally aged PVC (PVCn) had been subjected to seven years of outdoor exposure (south-side oriented at an angle of 45°) in The Netherlands. A visual inspection showed that the sample was white throughout. The reference PVC (PVCr) sample was homogeneously grey with no discoloration. Prior to aging, all PVC samples contained 89% PVC, 1.5% stabilizer (unknown composition), 9% filler (CaCO$_3$) and 0.3% pigment (all by weight). The PVC samples were prepared by weighing and dissolution in HPLC-grade THF (ca. 10 mg/ml). Cloudy solutions, containing undissolved matter, such as CaCO$_3$ and possibly cross-linked material, were filtered by means of a single-use PTFE 0.45-µm filter (Waters, Milford, MA, USA). The PVC drain pipes and sample solutions were stored in the dark at 6°C.

Chromatography
A Waters 2695 Separations Module equipped with a vacuum degasser and a thermostatted column compartment was used for SEC separations. The sample volume injected was 50 µl. On-line UV/Vis and refractive-index (RI) detection were performed with a Waters photodiode array (PDA) model 996 and a Waters model 2410 refractive-index detector, respectively. A computer using Waters Millenium32 (version 3.2) software controlled the system and was used to monitor the detector signals. Separations were carried out on two 300 × 7.6 mm I.D. PLgel Mixed-B columns (particle diameter, 10 µm; Polymer Laboratories, Shropshire, UK) which were thermostatted at 35°C. HPLC-grade dichloromethane (J.T. Baker, Deventer, The Netherlands) was used as eluent and the flow rate was 0.5 ml/min. Narrow polystyrene standards (Polymer Laboratories) were used to construct a relative molar-mass calibration curve. All molar mass data for PVC samples are expressed as PS equivalents and all analyses were carried out in duplicate.

Interfacing SEC and FTIR was performed using an LC-Transform Model 500 (Lab Connections, Northborough, MA, USA) interface module. The start of the SEC–FTIR interface was triggered via an electronic pulse from the chromatographic system. Interface parameters were set to obtain an optimum solute deposit and included 105°C interface temperature, 11 mm nozzle height, and 103 kPa nitrogen pressure. The optimum substrate
moving speed was determined as described elsewhere [10] and was 3 mm/min. Labcon 3.9 software (Lab Connections) controlled the interface. After analyte deposition on a 60 × 2 mm (diameter × thickness) rear-surface-aluminized germanium substrate, spectra were acquired by a stepwise rotation of the substrate using an automated optical FTIR accessory, controlled by LCT 1.6.1 software (Lab Connections).

Initially, the SEC experiments were carried out utilizing THF as eluent. However, solvent elimination and subsequent deposition of the separated PVC appeared to be difficult. In contrast with the dry deposits obtained for the narrow PS standards under optimum evaporation conditions, the PVC flooded the deposition substrate, as the nebulizer temperature was set to the high temperatures (135°) necessary for evaporating the eluent at flow rates above 0.1 ml/min. Under these circumstances, the interface temperature may be above the $T_g$ of PVC ($T_g = 87 °C$) or residual THF may function as a softener. To obtain dry deposits for PS and PVC, DCM was used as the eventual eluent and mild interface conditions (see above) could be used.

**Spectroscopy**

FTIR-microscopy was utilized for depth-profiling. The spectra were acquired on a Perkin Elmer (PE, Norwalk, CT, USA) spectrometer model Spectrum One, comprising a PE Spotlight 300 microscope and a narrow-band mercury/cadmium/telluride (MCT) array detector. The aperture was 25 μm and each spectrum consisted of two scans acquired at 16 cm$^{-1}$ resolution. The system was controlled by PE Spectrum Spotlight 5.00 software.

For the acquisition of SEC–FTIR spectra, a PE spectrometer model Spectrum GX, equipped with a medium-band MCT detector was used. The scan resolution was set at 4 cm$^{-1}$ and 8 scans were accumulated for each spectrum (scan range, 400-4000 cm$^{-1}$). The FTIR sample and detector compartments were continuously purged with nitrogen, which was dried using a Zander Adsorption Dryer, Type KM5 TE (Essen, Germany) to minimize the interference from carbon dioxide and water vapour present in the atmosphere. Data acquisition was performed using PE Spectrum 3.02 software. Background spectra were obtained at unused positions on the deposition substrate. FTIR functional-group chromatograms were constructed from integrated absorption bands using PE Spectrum TimeBase 1.1.

**Multivariate data analysis**

All data analysis steps described were performed in Matlab 6.1 (The MathWorks, Natick, MA, USA). The PLS Toolbox 2.1 (Eigenvector Research, Manson, WA, USA) was used to
calculate a PCA model. For a detailed description of the MVDA procedure, the reader is referred to the Appendix.

The SEC–FTIR data sets consisted of $n$ FTIR spectra measured as function of elution time. Each FTIR spectrum ($m$ wavenumbers), reflects the functional-group composition of the SEC eluate, but can also contain some unwanted spectral variation (e.g. caused by scatter or varying amounts of water vapour in the light beam). As a first approximation each FTIR spectrum can be assumed to be a linear sum of underlying component FTIR spectra. A complete SEC–FTIR dataset ($n$ spectra) is also available for the reference sample. All spectral variation caused by chemical compounds in the reference sample is reflected in the spectral subspace, $S_{\text{ref}}$. The tool for establishing and describing $S_{\text{ref}}$ is PCA [9]. Note that the approach deviates from the conventional use of PCA in two ways: (i) no mean centering is used and PCA is performed on the raw spectral data, and (ii) instead of using only the most

![Diagram](image)

**Figure 5.1:** Explanation of interpretation of the orthogonal vector ($S^{\perp}$). Shown is the case that the reference PVC spectrum (A) contains two functional groups, represented by the plane $S_{\text{ref}}$ (spanned by the principal component vectors $PC_1$ and $PC_2$ shown in D). When an aged sample only exhibits intensity changes for these functional groups, the projection of this spectrum will fall in the plane $S_{\text{ref}}$, which is represented by $S_{\text{aged}, 1}$. However, an aged sample can contain a newly formed functional group (C), represented as $S_{\text{aged}, 2}$. The vector diagram (D) shows that $S^{\perp}_{\text{aged}, 2}$ is equal to $S_{\text{aged}, 2}$ minus some vector that is in the direction of $S_{\text{ref}}$. In the spectral domain this means that spectral bands of $S_{\text{ref}}$ multiplied by some factor is subtracted from spectrum $S_{\text{aged}, 2}$. As FTIR spectra are positive quantities, wavenumbers at which $S^{\perp}_{\text{aged}, 2}$ is negative will contain information about the reference PVC sample. The remaining positive phases left in $S^{\perp}_{\text{aged}, 2}$ carry information about new functional groups formed in the aged PVC sample.
important principal components to analyse the major variation in the data set, all significant components are used to fully describe the spectral subspace. Essentially, PCA is not used as a data-reduction technique, but rather to obtain a full mathematical description of the entire SEC–FTIR data set. For the application described in this work, this means that a high number of principal components (e.g. 10 to 20) are anticipated. Determination of the correct number of principal components \( K \) involved the construction of a series of spectral subspaces for PVCr of increasing chemical rank \( (K = 10 \text{ to } 20) \). Q-residual plots as function of hydrodynamic volume were constructed from subsequent orthogonal projection of the PVCr sample on the subspace \( S_{\text{ref}} \). The true dimensionality of the spectral subspace was assumed to be that of the PCA model where statistically 95% of the projected data set fall within the Q-limit indicated by the PCA model. In this manner, the PCA subspace fits the PVCr data set within acceptable limits.

In order to analyse chemical differences between the PVC samples, the orthogonal vectors of the FTIR spectra in each of the aged sample data sets are calculated with respect to the spectral subspace \( S_{\text{ref}} \) (see Appendix, step iii). The wavenumbers at which the orthogonal vectors have positive intensities indicate the possible formation of new functional groups in the aged PVC. For a simple case, this is illustrated in Figure 5.1. This information can be obtained for any FTIR spectrum at any point in the chromatogram and this orthogonal part of the data set, denoted as \( X_{\text{aged}}^\perp \), is used here for spectral interpretation. The entire procedure is referred to as DifSub.

5.1.3 Results and discussion

Analysis of PVC by SEC-RI and SEC–FTIR

In contrast to thermal oxidation, which can occur in subsurface regions of permeable polymers, photo-oxidation is limited to reactions at or near the polymer surface [11]. In order to investigate the distribution of degradation products across all samples, cross sections of 50-μm thickness were obtained by cryo-microtoming and analysed by transmission-FTIR microscopy imaging. Investigation of cross sections of PVCr showed a homogeneous distribution in the functional-group image of carbonyl \( (1740 \text{ cm}^{-1}) \), which was present with a moderate intensity. When PVC is exposed to artificial weathering conditions, dehydrochlorination has been suggested as the main degradation mechanism, resulting in a strong brown discoloration of the PVC top surface [5, 7]. The availability of oxygen is limited by diffusion into the sample. Therefore, cross-linking is thought to dominate oxidation. FTIR imaging of a cross-section of artificially aged PVC showed more-intense carbonyl absorption bands \( (1734 \text{ cm}^{-1}) \) in the top 100 μm of the sample,
where oxidation apparently had taken place. The underlying bulk PVC did not exhibit any characteristic functional groups that could be ascribed to aging. These results are in agreement with the work of Anton-Prinet and co-workers [12], who studied artificially aged PVC samples by microtoming followed by FTIR microscopy. Because of this inhomogeneity, it was decided to divide the artificially aged PVC sample in a top layer (PVCt, discoloured, from 0 to 100 µm) and a bulk sample (PVCb, 100-200 µm) for further analysis.

To study possible chain scission, samples were subjected to SEC-RI. The chromatograms are shown in Figure 5.2A and the statistical moments (\(M_n\), \(M_w\), \(M_p\)) are summarized in Table 5.1. A small decrease in molar mass and an unchanged polydispersity were found for PVCn compared with PVCr. Major changes in molar mass (\(M_n\), \(M_w\)) and polydispersity were found for PVCt, indicating that chain-scission may have occurred. In the low-molar-mass (low-MM) tail an increased response is found for PVCt. Extensive cross-linking would result in an insoluble gel-fraction, which cannot be measured using SEC. However, initial cross-linking between two or a few molecules should be revealed in SEC in the form of an increased response in the high-MM tail. This is not observed for the PVCt sample. The molar mass and polydispersity for PVCb were less affected by the aging process and are comparable to the PVCn samples. This implies that only the top layer of the sample is strongly affected in artificial weathering.

In order to compare SEC-RI and SEC-FTIR, functional-group chromatograms were constructed from the total absorption of the aliphatic C–H stretching vibrations (Figure 5.2B). The high-MM parts of the functional-group chromatograms are in agreement with the chromatograms obtained from SEC-RI measurements in the corresponding molar-mass region. The low-MM tail for the PVCt sample shows a similarly increased response as seen in the SEC-RI chromatogram. Additionally, the width of the polymer peak is comparable for both detectors. More detail is revealed in the low-MM region when an FTIR detector is used. In this range some remarkably differences are found. First of all, the response for the PVCt sample has increased in the SEC-FTIR analysis. Inspection of the corresponding IR spectra mainly reveal the presence of an aliphatic compound for all samples. Furthermore,

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<tr>
<th>sample</th>
<th>molar mass (g/mol)</th>
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<td>reference PVC (PVCr)</td>
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<td>(M_n)</td>
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<td>naturally aged PVC (PVCn)</td>
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<td>artificially aged PVC (0-100 µm, PVCt))</td>
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<td>artificially aged PVC (100-200 µm, PVCb))</td>
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Figure 5.2: (A) SEC-RI chromatograms and (B) SEC-FTIR functional-group chromatograms constructed from the total absorption arising from C–H stretch vibrations for: PVCr (solid line), PVCn (dashed line), PVCb (dotted line) and PV Ct (dashed-dotted line). For details, see text.

the functional-group chromatogram for the PVCn peak has shifted to a higher MM compared to the corresponding RI-chromatogram. It must be noted that the local decrease in response at the apex for PVCb in the SEC–FTIR functional-group chromatogram can be ascribed to deposition irregularities that are inherent to the solvent-elimination SEC–FTIR technique. Nevertheless, FTIR detection provides a suitable and valuable alternative for RI-detection. However, it was difficult to construct functional-group chromatograms for the relatively small amounts of newly formed functional groups from the untreated data, as characteristic PVC absorption bands were dominating the spectra. To investigate the presence of any such newly formed functional groups in aged PVC samples, the SEC–FTIR data sets were analysed by MVDA (DifSub).

Analysis of SEC–FTIR data using DifSub

In the previous section, we discussed (chemical) differences between the PVC samples that were revealed by SEC-RI and SEC–FTIR. In order to obtain detailed functional-group information further analysis via SEC–FTIR combined with MVDA will be explored. We will focus on the polymer peak, because the changes in this region are indicative for changes in the PVC itself. After the collection of SEC–FTIR data, visual inspection of the
spectra revealed baseline offsets and sloping baselines as a result of optical effects (e.g., scattering). These were corrected for as described in the Appendix (step i).

**Determination of the spectral subspace**

Following the procedure described in Section 2.4, a spectral subspace consisting of 14 principal components was established, describing 100% of the spectral variation in the PVCr sample. This is a rather high, but realistic number, especially since the chromatographic elution window taken into account is rather broad (23-33 min.) and possibly contains many different components (of different molar mass). Furthermore, it is likely that deposition effects, which influence the spectral quality, cause an increase in the number of PCs required. Capturing all this spectral variation in the PCA model of the PVCr has the advantage that small spectral changes caused by the aging of PVC are better detectable. Inspection of the PCA model loadings showed that characteristic absorption bands for PVC were represented in the first loading although higher loadings also contain PVC features. It should be mentioned that two other approaches to determine the chemical rank were also investigated. The first approach included the visual inspection of Eigenvalue plots. In this particular case, it was found that most of the variance (99.8%) was captured when three principal components were used. However, it is likely that PVCr contains more chemical components. The method described by Meloun et al. [13] was also applied. This resulted in a dimensionality of 19, which was probably too high. Both approaches appeared to be less straightforward for the initial determination of the number of PCs than the preferred method described in the Experimental section.

**Inspection of Q-residuals**

Plots of Q-residuals are obtained from the projection of the SEC–FTIR data sets of individual aged PVC samples on $S_{ref}$. The Q-residuals are a measure of the differences from $S_{ref}$ and indicate the formation of new products (Figure 5.3). Considerable differences can be observed between all aged PVC samples. For example, in the PVCn sample the formation of new products are only found in the high-MM range, indicating that natural aging affects the high-MM part only. This was partly reflected in the SEC–FTIR functional-group chromatogram (cf. Figure 5.2B), but it was not apparent from the SEC-RI chromatograms. The low-MM range remains unaffected by the aging process, although the molar-mass averages (Table 5.1) show a decrease. In PVCt and PVCb differences with respect to PVCr are found across the entire MMD and thus the entire range may be involved in the artificial aging process. The apexes found in the SEC-RI and SEC–FTIR chromatograms coincide with the apexes in the Q-residual plots for these samples. The
most intense differences were found for the PVCt sample. The low-MM tail is in agreement with the functional-group chromatogram shown in Figure 5.2B, including the increased baseline in the part below approximately $10^4$ g/mol. For all PVC samples, it can be seen that in the range of the highest MM, no substantial amounts of new compounds are formed. Finally, the high-MM part of the untreated data in Figure 5.2B already starts at a higher MM value than the region where new functional groups are detected in the DifSub procedure. A simple visual inspection of the Q-residual plots already shows that these are of great value to determine differences between samples and to provide additional information to that obtained from SEC-RI or from SEC–FTIR with manual interpretation.

**Projection of aged PVC samples and interpretation of $X_{aged}^\perp$**

The positive parts obtained after orthogonal projection ($X_{aged}^\perp$) are shown in an overall contour-plot (Figure 5.4). These plots indicate at which wavenumber and at which molar mass the spectral features are enhanced in the spectra of the aged PVC samples. The negative parts of $X_{aged}^\perp$ do not provide differential information, as these reflect the functional groups that are also present in PVCr. It can be seen that PVCn and PVCb have much in common in the 1200 to 1700 cm$^{-1}$ wavenumber range, whereas PVCt is markedly different. Hydroxyl functionality can be clearly seen around 3200 cm$^{-1}$ for PVCn and
Figure 5.4: Contour plots for $\mathbf{x}_{\text{aged}}$ (after projection on spectral subspace $\mathbf{S}_{\text{ref}}$) as function of the molar mass for aged PVC samples, indicating newly formed functional groups. (A) PVCn; (B) PVCt; (C) PVCb.

PVCb. All PVC samples show C–O functionalities between 1300 and 1000 cm$^{-1}$. Further details will be discussed below.

The chemical rank of $\mathbf{X}_{\text{aged}}$ can now be determined by the method mentioned earlier [13]. This provides an estimate of the number of newly formed functional groups in the aged samples. The chemical rank determined for PVCn was 16. For PVCt and PVCb a chemical rank of 19 was found, which indicates that more new functional groups may have formed in the artificially aged samples than in the naturally aged ones. Thus, the DifSub procedure provides information of the evolvement of functional groups and the orthogonal distance from $\mathbf{S}_{\text{ref}} (\mathbf{X}_{\text{aged}})$ is related to the intensities of newly formed absorption bands. However, the SEC–FTIR interface used in this study is only of limited use in quantitative analysis [10]. Interpretation of the orthogonal vectors ($\mathbf{x}_{\text{aged}}$) is possible, because no data pre-treatment was applied and the spectra remained in their original format during processing. More-detailed spectral information is provided in Figure 5.5. Here, overlays of ten drift-corrected spectra around the apex from the raw SEC–FTIR chromatograms and overlays of ten $\mathbf{x}_{\text{aged}}$ residual spectra (at identical elution positions) are presented. For reference purposes, spectra from PVCr are given in Figure 5.5 F.
Figure 5.5: Overlay of ten drift-corrected spectra around the apex of the polymer peak from the SEC-RI chromatogram for (A) PVCn, (B) PVCt, (E) PVCb, and the corresponding $\chi_{\text{aged}}^+$ vectors for (C) PVCn, (D) PVCt and (G) PVCb. Drift-corrected spectra around the apex of the polymer peak for PVCr (F) are given for reference purposes.
Comparison of PVCr spectra with the spectra from the aged samples mainly reveals absorption-band intensity differences in the C–O stretch region (1100-1300 cm\(^{-1}\)) and in the aliphatic C–H stretch region. From the untreated FTIR-spectra (Figure 5.5, A, B, E) there is no clear evidence for the formation of aromatic or unsaturated C–H when inspecting the wavenumber range above 3000 cm\(^{-1}\). However, in the \(x^+_{\text{aged}}\) (Figure 5.5, C, D, G) a new absorption band appeared at 3060 cm\(^{-1}\) for all PVC samples. Additionally, for the PVCt and PVCb samples a new absorption band arose at 3022 cm\(^{-1}\). Both bands indicate the formation of aromatic rings or, more likely, double bonds and they are not visible in the untreated FTIR-spectra. Furthermore, the untreated FTIR spectra show no distinct absorption bands in the region 1780 to 1680 cm\(^{-1}\). Yet, DifSub revealed the presence of a band at 1694 cm\(^{-1}\) (PVCn) that may be due to an \(\alpha\beta\)-unsaturated ketone, as the carbonyl-stretch wavenumber range (typically, 1725 to 1705 cm\(^{-1}\)) tends to decrease by 30 cm\(^{-1}\) when unsaturated groups are present [14]. When electronegative groups are attached in the \(\alpha\)-position of saturated ketones, the wavenumber of the carbonyl-stretch absorption band tends to increase [14], which may explain the absorption band present at 1777 cm\(^{-1}\) in PVCt and PVCb. Unfortunately, small and sharp absorption bands of water vapour interferences (around 1700 cm\(^{-1}\)) show up in \(x^+_{\text{aged}}\). This can be ascribed to the relatively low spectral resolution used during spectra acquisition. Consequently, a small shift in the water-vapour absorption-band maximum will be revealed by DifSub. DifSub can clearly reveal changes in the shapes and positions of absorption-bands when, for instance, the chemical environment of a functional group has changed in comparison with the reference sample. Evidence for this is found in the aliphatic C–H stretch region (cf. Figure 5.5). In addition, the characteristic absorption bands for C–O stretch (1300–1000 cm\(^{-1}\)) are present in all samples, including PVCr. Major differences in absorption-band shape and intensity can be revealed from the untreated FTIR spectra (Figure 5.5, A, B, E). However, from these spectra it is very difficult to discern newly formed absorption bands and to determine their exact position, even with the use of spectral subtraction procedures for PVCr and one of the aged samples. At first sight, the changes are probably caused by differences in intensity. On the other hand, the \(x^+_{\text{aged}}\) spectra (Figure 5.5, C, D, G) show distinct peak maxima for all the aged samples in the C–O stretch region. For example, an absorption band at 1258 cm\(^{-1}\) is present in all samples, absorption bands at 1157 and 1060 cm\(^{-1}\) appear in PVCn and PVCb and new absorption bands for PVCt are found at 1228 and 1104 cm\(^{-1}\). In all aged samples, absorption bands are found at 804 and 740 cm\(^{-1}\) (arising from C–C skeleton vibrations), but their exact band position is slightly shifted, probably due to a changing chemical environment or a change in the polymer-chain. A higher spectrometer-resolution setting could help in the determination of a more precise band position, at the expense of an
increased data-acquisition and data-processing time. It must be stressed that any functional
groups formed during synthesis or processing operations are included in the spectral
subspace and do not show up in the positive parts of the residual vectors after orthogonal
projection, provided that the reference material is truly representative of the samples before
aging. This might not be the case for the selected PVCr, since FTIR microscopy showed
that oxidation had occurred in the PVCr sample. This may explain why functional groups
associated with oxidation are not featured in the DifSub results for PVCt and stresses the
importance of the correct choice of the reference material.

For a final assignment and a complete mechanistic degradation study, complementary
structure-elucidation techniques, such as nuclear-magnetic-resonance spectroscopy (NMR)
and/or mass spectrometry (MS) are still deemed necessary. However, a comparison
between the various spectra obtained from the untreated data and from $x_{\text{aged}}$ shows a great
increase in the amount of chemical information revealed in the latter case. At best, the
manual interpretation of the SEC–FTIR data, without knowledge of the functional groups
formed, would have been a tedious and protracted procedure. More realistically, it is a
mission without any prospect of success.

To summarize, the method DifSub presented here has advantages over conventional PCA,
when spectral subspaces are determined for every individual sample. First of all, using
DifSub it is possible to find indicators for time-evolved differences in functional groups
between two or more aged samples. Furthermore, spectral interpretation of these signals is
possible. Finally, the chromatographic time-axis does not need to be included in the DifSub
model and (small) shifts of the retention times between samples are allowed. This increases
the robustness of the method. The main limitation is that intensity changes of functional
groups that are already present in the reference sample cannot be detected, as these signals
are taken into account in the full PCA model used.

5.1.4 Conclusion

DifSub is a novel multivariate-data-analysis method, which is based on PCA. It can be used
for the interpretation of large SEC–FTIR data sets. Because DifSub uses a reference
sample, all the features present in the original sample will not show up in the projection
results. Only differences between samples are revealed, as the large data sets are reduced to
the essentials. A data set comprising differently aged PVC samples and a reference PVC
sample was used in this feasibility study. It was demonstrated that DifSub is in potential a
powerful tool to reveal differences between different samples and a reference sample.
Especially, subtle differences were found in the aromatic C–H stretch region for the aged
PVC samples, which would not have been revealed using conventional interpretation of
SEC–FTIR data. The DifSub-method adds considerably to the results obtained with SEC-
RI and conventional SEC–FTIR data analysis without data treatment. This new data-
handling approach is potentially suitable to reveal aging mechanisms. Moreover, it can be
used in areas where a detailed analysis is needed for the comparison of large spectroscopic
data sets, such as (spectroscopic) imaging or multi-dimensional chromatography.

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Appendix

The procedure to analyse the spectra that are collected during the SEC–FTIR runs consists
of three steps: (i) wavenumber selection and pre-processing of FTIR spectra, (ii)
determination of the spectral subspace, $S_{ref}$, for the reference PVC sample, and (iii) analysis
of the spectra of the aged PVC samples with respect to the established spectral subspace
$S_{ref}$.
i. **Wavenumber selection and pre-processing of the FTIR spectra**

The part of the FTIR spectrum between 1900 cm\(^{-1}\) and 2500 cm\(^{-1}\) does not contain much spectral information. Therefore, this wavenumber range is discarded. The spectral ranges 550-1900 cm\(^{-1}\) and 2500-3300 cm\(^{-1}\) are baseline-drift corrected separately, to prevent the effect of baseline curvature from jeopardizing the construction of a valid model. The baseline drift is assumed to be mainly an instrumental and sample effect (*i.e.* scattering). Table A lists the wavenumber ranges that were used to estimate the drift. The baseline-fitting regions were used for linear interpolation over the specified wavenumber range. This interpolated line was subtracted from the entire FTIR spectrum.

<table>
<thead>
<tr>
<th>Wavenumber regions for PCA modelling (cm(^{-1}))(^a)</th>
<th>Range used for baseline-fitting (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>550-1900</td>
<td>400-590</td>
</tr>
<tr>
<td>2500-3300</td>
<td>2500-2650</td>
</tr>
</tbody>
</table>

\(^a\) Since IR activity is absent in the 1900-2500 region, this region is omitted.

All measured spectra, *viz.* the spectra of the reference sample and of the aged samples, are treated in the same manner. The resulting spectral data of each SEC-FTIR analysis are collected in a \((I \times J)\) data matrix \(X\), in which \(I\) is the number of spectra and \(J\) is the number of wavenumbers remaining after pre-treatment. The matrix that contains the data for the reference sample is called \(X_{\text{ref}}\) and the matrix that contains the data of an aged sample is called \(X_{\text{aged}}\).

ii. **Determination of the spectral subspace for the reference PVC sample**

A full spectral subspace of the matrix \(X_{\text{ref}}\) is determined by means of PCA [I, II]. The number of principal components (\(K\)) is chosen in such a way that all spectral variation of chemical origin is included in this subspace. The model for the data matrix is:

\[
X_{\text{ref}} = TP^T + E
\]  

(A.1)

in which \(T\) is a \((I \times K)\) matrix with scores and \(P\) is a \((J \times K)\) matrix with loadings. \(E\) is an \((I \times J)\) error matrix. When the number of principal components (\(K\)) is selected correctly (see Experimental), this latter matrix will contain only instrumental spectral variation (mainly noise). The loading vectors (represented by the columns of the \(P\) matrix) are of interest here. These vectors span the spectral subspace and form an orthonormal basis for spectral subspace \(S_{\text{ref}}\).
iii. Analysis of the spectra of the aged samples.

Each row in the matrix $X_{aged}$ is a spectrum of the aged PVC sample corresponding to a specific SEC elution time (hydrodynamic volume). It is rearranged as a $(J \times 1)$ column vector $x_{aged}$. This spectrum is projected orthogonally [III] on the spectral subspace $S_{ref}$:

$$x^\perp_{aged} = (I - PP^T)x_{aged} \quad \quad (A.2)$$

in which $I$ is the $(J \times J)$ unity matrix. Inspecting these orthogonal vectors as a function of the hydrodynamic volume for each sample supplies information about the evolution of new functional groups (compounds) as a function of the sample molar mass. As discussed in the main text only the positive intensities are indicative of these newly formed groups (compounds). The root-mean-square values ($e_{new}$) of these positive intensities are used as measures of the new phenomena. The (pseudo) rank of matrix $X^\perp_{aged}$, i.e. the matrix that contains all vectors $x^\perp_{aged}$ as its rows, is indicative of the number of new chemical components that are present in the SEC chromatogram of an aged sample.

References


Chapter 5
5.2 Polymer-additive analysis by coupling high-performance liquid chromatography and Fourier-transform infrared spectroscopy

Summary
A commercially available solvent-elimination LC–IR interface was used for the analysis of polymer additives. The additives used in this study included a selection from various classes, viz. hindered amines, hindered phenols and phosphorous antioxidants, covering a wide range of polarity and molar mass. An explorative flow-injection-analysis study was carried out into the possibilities to obtain good-quality deposits for all additives. Using the deposition interface, the presence of additive classes was successfully verified by functional-group screening of an additive mixture separated by gradient RPLC. Finally, the application of gradient-elution LC–IR to the analysis of a real polymer sample, viz. polystyrene containing Irganox 1076, was investigated. It is shown that gradient-elution LC–IR can be used for the functional-group screening of additives present in a polymer sample, without any extraction procedure. Spectral interpretation revealed the presence of a hindered phenol in the polymer sample.

5.2.1 Introduction
Polymer additives are often incorporated in synthetic polymer systems to improve physical, mechanical or optical properties and to extend the functional lifetime. They include antioxidants, plasticizers, stabilizers, processing aids, etc.. Additive analysis is important to (i) obtain insight in the production process, (ii) obtain insight on degradation processes during either polymer processing or usage, (iii) identify migration from food-contact materials into foods, (iv) identify unknown additive systems in plastics, and (v) obtain insight in the stability of additives during the incorporation into plastics or during the service life of the final product. [1–4].

The wide variety of additives in polymeric materials complicates their analysis and identification [5]. Most additives are pure monomeric compounds, but other ones consist of complex oligomeric or isomeric mixtures. Additives cover a wide range of molecular weight, volatility and polarity. Furthermore, the so-called primary anti-oxidants are easily degraded during polymer processing, while the so-called secondary anti-oxidants are degraded slowly during the lifetime of polymers. Additives may react with each other or with the polymeric material, making the mixture of additives even more complex. Finally, the additive concentrations are usually small (from less than 0.1 to about 5%), which makes their detection and identification more difficult.
As a consequence spectroscopic analysis, \textit{i.e.} infrared (IR), nuclear-magnetic-resonance or UV/Vis spectroscopy, of the complete polymer is only of limited value. The majority of additives require (chromatographic) separation techniques for characterization. Widely used methods include additive isolation by extraction or precipitation and subsequent analysis by liquid chromatography \cite{1, 6-8} or, less frequently, by gas chromatography \cite{9}. Very promising techniques are the coupling of liquid chromatography with mass spectrometry (LC-MS) \cite{4, 10} or Fourier-transform infrared spectroscopy (LC–IR) \cite{11-16}. Although MS provides molar-mass information for identification, its sensitivity primarily depends on the ionization efficiency of the additive to be analyzed. On the other hand, IR can be used as a universal detection technique. It can easily identify classes of additives present in the sample, due to its selectivity for functional groups. IR, therefore, offers many advantages in the analysis of additives.

Coupling of LC and FTIR has been realized basically by \textit{(i)} on-line flow-cell and \textit{(ii)} solvent-elimination techniques. In the on-line approach, the effluent of the liquid chromatograph is passed through a flow cell and IR spectra are acquired continuously. The second approach utilizes a heated nebulizer, often aided with nebulizer gas, to evaporate the effluent and to deposit the analytes on a suitable substrate. IR spectra are acquired (shortly) after deposition. Major advantages of solvent-elimination interfaces are \textit{(i)} the absence of interfering effluent absorption bands, \textit{(ii)} increased off-line scanning time resulting in an improved signal-to-noise ratio, and \textit{(iii)} generation of transmission-like spectra that can be searched against commercially available KBr-disc libraries. This makes such interfaces more attractive than flow cells. Therefore, research on the analysis and identification of polymer additives by LC–IR has focused on solvent-elimination interfaces.

In the early 1990's, Jansen \cite{11} has shown the use of a thermospray/moving-belt interface combined with diffuse-reflectance optics for the sensitive qualitative detection of polymers and additives. Several polymers, containing di-ethylhexylphthalate as plasticizer and pentaerythritol tetra-stearate as release-agent, were subjected to size-exclusion chromatography (SEC) with dichloromethane as eluent at 1 ml/min. Isocratic RPLC–IR with water/methanol (30/70\% \textit{v/v}) at a flow rate of 0.5 ml/min was used for the analysis of Irganox 1010 and Irganox PS 800. Detection limits in the 100-ng range were reported, depending on the IR-sensitivity and volatility of the solutes. Later, Robertson \textit{et al.} \cite{12} used Irganox 565 as a test compound for the assessment of a similar interface. The interface was used with various compositions of methanol–acetonitrile–water at high flow rates, viz. 0.5–1 ml/min. Identification down to 50 \textmu g/ml (approx. 1 \textmu g on-column) was achieved at high thermospray temperatures (165–186°C) without degradation. The detection limit is ten times worse than the results obtained by Jansen \cite{11} with a similar interface. A linear
calibration curve was obtained for Irganox 565 from 50 to 1000 µg/ml (injected volume 20-50 µl). However, there was no quantitative advantage over UV and the system was recommended only for qualitative purposes. Furthermore, six phenolic antioxidants at the 1 mg/ml-level were successfully separated and deposited from a 100% aqueous mobile phase.

Somsen et al. [13] presented a highly sensitive method for the analysis of additives using a micro-HPLC system, a heated pneumatic nebulizer, and ZnSe as substrate. Spectra were acquired by FTIR microscopy. The flow rate was 20 µl/min of methanol/water (95/5% v/v). Spectra obtained from the ZnSe substrate indicated a minimum identifiable quantity of about 10 ng.

Jordan and Taylor [14] used a commercially available interface from Lab Connections (model not stated), Ge as substrate, and methanol-water gradient HPLC separations (from 94 to 100% methanol; flow rate, 1 ml/min) in combination with nebulizer-temperature programming. A study was carried out on twenty additives to demonstrate the feasibility of depositing these additives in well-defined spots. Eventually, this interface was employed to analyze nine selected additives at the 2-µg (on-column) level. The study only featured a limited selection of highly polar compounds, due to the restricted polarity range of the gradient used. It was found that the low molecular weight of butylated hydroxytoluene (BHT; molar mass, 220.4 g/mol) prevented successful deposition. Limits of identification were estimated at 0.5–1.0 µg for accurate spectroscopic identification of the other components.

In 1999, the interface originally developed by Robertson et al. [12] was modified by adding heated nitrogen to the thermospray outlet capillary as nebulizer gas. It was then used by Mottaleb [15]. His research resulted in the separation of additives with 100% water as eluent and a flow rate of 0.5 ml/min and in their deposition on a moving belt. By comparing acquired spectra with library spectra, it was demonstrated that no oxidation took place. No limits of identification were given.

More recently, Bruheim and co-workers [16] used a commercial pneumatic nebulizer, modified to handle low flow rates, in combination with temperature-programmed packed-capillary LC. The flow rate was 5 µl/min. Separation of a mixture of three additives was demonstrated, as well as the separation and identification of Irgafos P-EPQ in its various oxidation states. The mass detection limit for Irganox 1010 was about 40 ng.

In summarizing the work done in this field, it can be concluded that sensitive analysis of polymer additives using LC–IR is best accomplished with the use of micro-HPLC and FTIR microscopy. This can be ascribed to the fact that small amounts of eluent need to be evaporated at low flow rates, resulting in small deposit spots. FTIR microscopy is able to
focus on such small spots. For a full discussion on this topic, the reader is referred to [17]. However, such instruments are complex, expensive and difficult to automate. Therefore, they are not commonly found in laboratories. When conventional flow rates (viz. 0.5 ml/min) are used, absolute detection limits around 0.1-1 µg can be anticipated. The majority of the published separations were carried out in the isocratic mode or covered a restricted eluent-polarity range when gradient LC was used [11–14, 16]. This may imply that the authors of these papers recognized the need of programming the interface temperature. Specifically, large changes in heat capacity are inherent to changing the eluent composition in gradient HPLC. Optimizing the deposition conditions can be a rather troublesome and time-consuming process. On the other hand, the applications have been limited to (almost) water-soluble, highly polar additives, requiring high interface temperatures at the flow rates used. Nevertheless, thermal degradation was not observed at these temperatures and LC–IR has proven to be a valuable tool in additive-degradation studies.

In this paper, a commercial solvent-elimination LC–IR interface is applied for the analysis of polymer additives using reversed-phase gradient LC (RPLC). A number of additives from different classes were selected, covering a broad range of molar masses, volatility and polarity. Three commercially important additive types, i.e. antioxidants, UV absorbers and light stabilizers were represented. These included hindered-amine light stabilizers (HALS), hindered-phenol antioxidants, and phosphorous antioxidants. Important deposition characteristics for each additive have been investigated, including the spreading of the deposit across the substrate, the formation of rings upon deposition, incidental obstruction of the nebulizer, and changes in spectral features, that can occur due to oxidative or thermal degradation during or after deposition. Next, an RPLC-UV method was developed and coupled with FTIR for functional-group screening and identification of an additive mixture. Finally, it will be demonstrated that RPLC–IR can be used as a functional-group screening method for polymer samples containing 'unknown' additives. To this end, a polymer sample containing polystyrene and Irganox 1076 (1.2% by weight) is analyzed in a single analysis by RPLC–IR, without prior extraction.

5.2.2 Experimental

Chemicals

Dichloromethane (DCM, HPLC grade) and ammonium acetate were obtained from J.T. Baker (Deventer, The Netherlands) and methanol was supplied by Rathburn Chemicals (Walkeburn, Scotland). Acetonitrile (MeCN, HPLC grade) and tetrahydrofuran (THF,
HPLC grade) were purchased from Biosolve (Valkenswaard, The Netherlands). A 25% ammonia solution was obtained from Merck (Darmstadt, Germany). HPLC-grade water was prepared in house using a Nanopure Infinity water purifier (Barnstead, Dubuque, Iowa, USA)

**Table 5.2:** Details of additives used in this study in order of retention time. The corresponding functional group chromatograms from Figure 5.6 are indicated in the table.

<table>
<thead>
<tr>
<th>trade name</th>
<th>chemical name</th>
<th>M (g/mol)</th>
<th>t_r, IR (min)</th>
<th>functional-group chromatogram (Figure 5.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinuvin 770</td>
<td>bis(2,2,6,6-tetramethyl-4-piperidinyl)sebacate</td>
<td>480.7</td>
<td>16.6</td>
<td>E, G</td>
</tr>
<tr>
<td>Tinuvin P</td>
<td>2-(2-hydroxy-5-methylphenyl)-2H-benzotriazole</td>
<td>225.2</td>
<td>20.2</td>
<td>H</td>
</tr>
<tr>
<td>Tinuvin 213</td>
<td>A, 52%; B, 50%; C, 13%</td>
<td>A, 637; B, 975; C, 300</td>
<td>21.0</td>
<td>E</td>
</tr>
<tr>
<td>Irganox 245</td>
<td>triethylene glycol bis-3-(3-di-tert-butyl-4-hydroxy-5-methylphenyl)propionate</td>
<td>586.8</td>
<td>22.4</td>
<td>E, F, H</td>
</tr>
<tr>
<td>Irganox 1098</td>
<td>N,N'-hexane-1,6-diybis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionamide)</td>
<td>637</td>
<td>24.8</td>
<td>F, H, I</td>
</tr>
<tr>
<td>Irganox 1035</td>
<td>2,2-thiodiethylene bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate</td>
<td>642.9</td>
<td>31.7</td>
<td>E, F, I</td>
</tr>
<tr>
<td>Irganox 259</td>
<td>hexamethylene bis (3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate)</td>
<td>638.9</td>
<td>33.5</td>
<td>E, F, I</td>
</tr>
<tr>
<td>Tinuvin 326</td>
<td>2(2-hydroxy-3'-tert.-butyl-5'-methylphenyl)-5-chloro-benzotriazole</td>
<td>315.8</td>
<td>33.9</td>
<td>F</td>
</tr>
<tr>
<td>Uvite 120</td>
<td>2,5-Bis(5'-tert-butyl-2'-benzoxazolyl)thiophene</td>
<td>431</td>
<td>35.6</td>
<td>.b</td>
</tr>
<tr>
<td>Tinuvin 327</td>
<td>2-(2'-hydroxy-3',5'-di-tert.-butylphenyl)-5-chlorobenzotriazol</td>
<td>357.9</td>
<td>37.0</td>
<td>E, F</td>
</tr>
<tr>
<td>Ultranox 626</td>
<td>bis (2,4-di-t-butylphenyl) pentaerythritol diphosphate</td>
<td>604.7</td>
<td>40.0</td>
<td>B, C</td>
</tr>
<tr>
<td>Irganox 1010</td>
<td>pentaerythritol tetrakis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate]</td>
<td>1177.7</td>
<td>40.5</td>
<td>E, F, I</td>
</tr>
<tr>
<td>Irganox 1330</td>
<td>1,3,5-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-2,4,6-trimethylbenzene</td>
<td>763.2</td>
<td>43.2</td>
<td>F, I</td>
</tr>
<tr>
<td>Irganox 1076c</td>
<td>octadecyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate</td>
<td>530.9</td>
<td>40.5</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

* This additive consists of three ingredients A to C. A, a-[3-[3-(2H-Benzotriazol-2-yl)-5-(1,1-dimethylethyl)-4-hydroxyphenyl]-1-oxopropyl]-w-hydroxypoly(oxy-1,2-ethanediyl); B, a-[3-[3-(2H-Benzotriazol-2-yl)-5-(1,1-dimethylethyl)-4-hydroxyphenyl]-1-oxopropyl]-w-[3-[3-(2H-benzotriazol-2-yl)-5-(1,1-dimethylethyl)-4-hydroxyphenyl]-1-oxopropoxy]poly(oxy-1,2-ethanediyl); C, polyethylene glycol.

* All absorption bands of Uvite 120 interfere with other additives present in the mixture and no characteristic bands could be selected.

* Irganox 1076 was present in the PS sample analyzed by RPLC–FTIR. It was not included in the deposition optimization and RPLC–FTIR of the additive mixture.
Additives were obtained from several manufacturers. Trade names, chemical names and molar masses can be found in Table 5.2. Stock standard solutions of additives were prepared by weighing and dissolution in DCM at the 10-mg/ml level. Mixtures were prepared by combining the stock solutions to a concentration of 2 mg/ml for each standard. Polystyrene containing 1.2% (w/w) Irganox 1076 was prepared at TNO Industry (Eindhoven, The Netherlands) and a solution was prepared in THF (ca. 120 mg/ml). All standard solutions were stored in the dark at 6°C.

**Chromatography and spectroscopy**

A Waters 2695 Separations Module (Milford, MA, USA), equipped with a vacuum degasser and a thermostatted column compartment, was used for gradient HPLC separations. The sample volume injected was 1 μl, unless otherwise stated. UV-detection was performed with a Waters PDA model 996. A computer using Waters Millenium32 (version 3.2) software controlled the system and was used to record the detector signals.

Separations were carried out on a 150 × 3.0 mm I.D. Waters Xterra MS C18 column (particle diameter 5 μm), which was thermostatted at 35°C. The flow rate was 0.2 ml/min. The eluent consisted of aqueous 0.01-M ammonium-acetate (eluent A) and MeCN (eluent B). The initial mobile-phase composition was 50-50% (v/v) A-B and the gradient was started 2 minutes after injection (gradient steepness, 2%/min). The final eluent composition of 100% B was maintained for 13 minutes, before returning to the initial conditions.

Interfacing LC and IR was performed using an LC-Transform Model 500 interface (Lab Connections, Northborough, MA, USA). The system was controlled by Labcon 3.9 software (Lab Connections). The interface temperature was programmed to facilitate eluent evaporation with changing composition. The nozzle height was set to 10 mm and nitrogen at 103 kPa pressure was used for nebulization. After analyte deposition on a 60 × 2 mm (diameter × thickness) rear-surface-aluminized germanium substrate, spectra were acquired using a three-times focusing optical IR accessory, controlled by LCT 1.6.1 software (Lab Connections). During scanning, the substrate was stepwise rotated.

The FTIR system consisted of a Perkin Elmer (Norwalk, CT, USA) spectrometer model Spectrum GX, equipped with a medium-band mercury/cadmium/telluride (MCT) detector. The scan resolution was set at 4 cm⁻¹ and for each spectrum 8 scans were accumulated. The FTIR sample and detector compartments were continuously purged with nitrogen, which was dried using a Zander Adsorption Dryer, Type KM5 TE (Essen, Germany) to minimize the interference from carbon dioxide and water vapor present in the atmosphere. In all cases, data acquisition was performed using Perkin Elmer Spectrum 3.0 software.
Background spectra were obtained at unused positions on the germanium substrate. FTIR functional-group chromatograms were constructed using Perkin Elmer Spectrum TimeBase 1.1. For the construction of these chromatograms, the maximum absorption within a selected IR window was used. An increase in retention time (by ca. 0.7 min at a flow rate of 0.2 ml/min) was observed for the IR chromatograms, due to the extra system volume between the PDA detector and the LC–IR interface. Library searching was performed using the Polymer-Additives library, which was obtained from Bio-Rad Laboratories (Hemel Hempstead, United Kingdom) and which contained 1740 spectra of additives.

For the additive-deposition studies, 5 µl of each additive standard (concentrations approximately 10 mg/ml) where spray-deposited on a Ge-Al substrate, using the chromatographic system without any separation column (flow-injection analysis mode, FIA). The mobile phase used was 100% MeCN and the flow rate was set to 50 µl/min. The LC–IR interface parameters were set to obtain an optimum solute deposit and included: 75°C interface temperature, 7-mm nozzle height, and 172-kPa nitrogen pressure.

The effects of morphology on the IR spectra of some deposited additives were verified by recording IR spectra of the pure additive by using a diamond attenuated-total-reflectance (ATR) accessory (Specac, Orpington, Kent, England).

5.2.3 Results and discussion

Deposition characteristics of additives
To explore the deposition characteristics of a wide range of additives, all additives listed in Table 5.2 were deposited in the FIA mode. The LC–IR interface parameters were optimized to obtain a stable, narrow spray, completely evaporating the effluent when impinging on the substrate. However, for certain additives it was difficult to obtain good quality (i.e. dry and homogeneous) deposits. For example, Irganox 1330 produced a wet spot. Spreading of the deposit and “ringing” effects [12] were observed, where a high concentration of additive was found at the outer edges of the deposit. Deposition of Irganox 1098 resulted in an unctuous trace. The remainder of the additives yielded dry deposits. We obtained no evidence for an important role of the additive molar mass in the deposition process. An elevated evaporation temperature could lead to dryer spots. However, at the current interface settings some additives were already building up in the nozzle. They were pushed out of the capillary as solute particles, bouncing off the substrate without being deposited. Gaining good-quality deposits for a wide range of additives proved difficult. The optimum deposition conditions for the entire range of additives necessarily constituted a compromise.
Qualitative analysis of polymers and additives

In order to examine the possible degradation of deposits, IR spectra were acquired (i) directly after deposition, (ii) after keeping the substrate during two days in an inert (nitrogen gas) atmosphere, and (iii) after storing the substrate during two days in the laboratory environment at room temperature. For most additives there were no apparent changes in spectral features after storage in an inert atmosphere. However, at both storage conditions Ultranox 626 exhibited a fusion of three absorption bands (ν region, 970-1070 cm⁻¹) into one large and broad band, in conjunction with the appearance of a broad O-H absorption band at 3345 cm⁻¹. Furthermore, the hydroxyl-stretching vibration arising from Irganox 1330 appeared as one single absorption band at 3647 cm⁻¹ when the deposit was measured directly after deposition. After two days of storage, either under laboratory conditions or under nitrogen, this single band turned into three sharp absorption bands at 3648, 3629, and 3614 cm⁻¹. In order to confirm that no oxidative degradation occurred during deposition, reference spectra were recorded from the powder of both additives using an ATR IR accessory. The ATR IR spectra obtained showed similar spectral features as the LC-IR spectra of Ultranox 626 and Irganox 1330 deposits after two days of storage in either nitrogen or air. Apparently, no oxidative degradation had yet occurred immediately after deposition and another possible explanation of this phenomenon is discussed below.

Due to the steric hindrance by the t-butyl groups present in the ortho positions in Irganox 1330, intramolecular and intermolecular hydrogen-bonding interactions are absent. Therefore, hydrogen-bonding effects are not likely to explain the spectral differences. Possibly, residual eluent influences the morphology of the analyte directly after deposition, although no characteristic eluent-absorption bands arising from MeCN were present in the spectra obtained in the FIA mode. To investigate this possibility, a solution of Irganox 1330 in acetone was applied to the diamond ATR. The strong carbonyl absorption of acetone could easily reveal traces of solvent. After partial evaporation, traces of acetone were still present, which was obvious from the carbonyl stretching vibrations in the ATR spectrum. Only one hydroxyl-stretching band was observed, similar to the absorption band previously seen in FIA-IR experiments. This could indicate that in FIA-IR the eluent is still present in small amounts shortly after deposition, possibly affecting the morphology. When eluent interactions can play a role, library identification must be performed with care. A similar splitting of sharp hydroxyl-absorption bands was observed by Somsen et al. for Irganox 1076 deposited on ZnSe as substrate and examined on different days [13].

Gradient HPLC of additive test mixture

Flow-injection experiments have shown that additives covering a wide molar-mass range can be successfully deposited and analyzed by LC-IR. The selected classes of additives are
well suited for functional-group screening. For example, hindered amines, hindered phenols, and phosphorous antioxidants can be selectively detected by constructing their corresponding functional-group chromatograms. This will reveal the presence or absence of various types of additives in a mixture or in a sample containing unknown additives. Therefore, a test mixture containing 13 additives (for details, see Table 5.2) was separated by gradient RPLC and detected by IR spectroscopy.

After the separation was completed, the following absorption bands were selected for detection [18]. For the detection of phosphorous antioxidants, the P–O–C stretching vibration at 1016 cm\(^{-1}\) was selected. Because this vibration occurs in the fingerprint region, where many other vibrations are found, the P–O–C stretching band at 1190 cm\(^{-1}\) was monitored simultaneously to prevent a false positive detection. The strong carbonyl vibration at 1644 cm\(^{-1}\) was chosen for amide-containing additives. The strong C=O stretching vibration (1732 cm\(^{-1}\)) was used for the detection of carbonyl-containing additives. For additives containing aromatic groups, the aromatic C–H stretching vibration occurring at 3002 cm\(^{-1}\) was chosen for detection. The N–H stretching vibration at 3323 cm\(^{-1}\) was selected for amine-containing additives. Finally, for additives containing a phenol group two absorption bands were selected. These were the broad (b) hydroxyl vibration at 3200-3500 cm\(^{-1}\), for additives in which hydrogen bonding is present, and the sharp (s) hydroxyl band at 3619 cm\(^{-1}\) in case strong intramolecular hydrogen bonding is inhibited due to steric hindrance (i.e. hindered phenols).

The selection of functional-group chromatograms specific to the classes of additives is illustrated in Figure 5.6. All of the additives could be selectively detected, except Uvitex 120. Given the structure of Uvitex 120, the spectrum is rather featureless in the range from 1640 cm\(^{-1}\) to higher wavenumbers, which hampered the selection of a single specific wavenumber for constructing a functional-group chromatogram. All spectra acquired were of good quality (Figure 5.7).

Spurious positive or negative peaks can occur in the functional-group chromatograms, intrinsically because of the way in which baseline points are defined in the IR spectra used for their construction. For example, the chromatographic baselines in the phosphite functional-group chromatograms (Figures 5.6 B and C) drop below zero in regions where other additives elute. When baseline points coincide with an absorption band for a different additive this will give rise to a negative value in the functional-group chromatogram. Other functional-group chromatograms show such baseline artifacts to a lesser extent. Spurious peaks in functional-group chromatograms can also arise from neighboring absorption bands that protrude into the selected regions. For example, a functional-group chromatogram optimized for the N-H stretch vibration covering a limited wavenumber region (range 3340-
Figure 5.6: RPLC–IR Gram–Schmidt (A) and functional-group chromatograms (B-I) for the analysis of a mixture containing the following additives: 1, Tinuvin 770; 2, Tinuvin P; 3, Tinuvin 213; 4, Irganoxx 245; 5, Irganox 1098; 6, Irganox 1035; 7, Irganox 259; 8, Tinuvin 326; 9, Uvitex 120; 10, Tinuvin 327; 11, Ultranoxx 626; 12, Irganox 1010; 13, Irganox 1330. Functional-group chromatograms B-I were constructed from the maximum absorption band height over a narrow wavenumber region or at a discrete wavenumber as follows: B, 1016 cm⁻¹; C, 1188-1193 cm⁻¹; D, 1644 cm⁻¹; E, 1720-1745 cm⁻¹; F, 3002 cm⁻¹; G, 3323 cm⁻¹; H, 3200-3500 cm⁻¹ (broad (b) OH absorption band); I, 3687-3552 cm⁻¹ (sharp (s) OH absorption band). Peaks marked with an asterisk were identified as spurious peaks (see text for details).
Figure 5.7: FTIR spectra obtained from the RPLC separation of an additive test mixture. Spectra assignment: 1, Tinuvin 770; 2, Tinuvin P; 3, Tinuvin 213; 4, Irganox 245; 5, Irganox 1098; 6, Irganox 1035; 7, Irganox 259; 8, Tinuvin 326; 9, Uvitec 120; 10, Tinuvin 327; 11, Ultranoxx 626; 12, Irganox 1010; 13, Irganox 1330. The spectra numbers correspond with the peak labelling from Figure 5.6.
3300 cm\(^{-1}\)) falls within the broad hydroxyl-stretch vibration (arising, for example, from Irganox 1098; absorption range 3518-3140 cm\(^{-1}\)). This gives rise to a large (off-scale) spurious peak. It is, therefore, important to visually inspect the entire set of functional-group chromatograms and IR spectra in order to determine which chromatographic peaks truly correspond to the functional group chosen and to discern the presence of components left undetected.

Visual inspection of the substrate after deposition showed that the first-eluting additive (Tinuvin 770) was spread across the substrate, resulting in a broad and tailing peak in the chromatogram. Spreading of this additive was not observed in the FIA–FTIR experiments described in section 3.1, where depositions were carried out at constant conditions (isocratic chromatography and isothermal nebulization). During the chromatographic gradient the temperature of the nebulizer was linearly decreased. A possible explanation for the spreading of Tinuvin 770 could therefore be that the nebulizer temperature was not optimal for the momentary eluent composition at the retention time of Tinuvin 770. This illustrates the compromise that has to be struck to establish optimum deposition conditions for a broad range of additives. In the case of overlapping peaks, such as Irganox 259 (t\(_r\), 33.5 min) and Tinuvin 326 (t\(_r\), 33.9 min), fully resolved functional-group chromatograms could be obtained (for example for the carbonyl trace of Irganox 259; Figure 5.6, trace C).

**Gradient HPLC of polystyrene containing additives**

The isolation and identification of additives present in polymer samples often requires several steps [1, 6–8]. The sample is typically subjected to an extraction procedure (step 1) after which the extract is concentrated (step 2). Thereafter, the additives are separated and identified, for example by HPLC with UV detection (step 3). Such a multi-step procedure is time-consuming and generates large amounts of (organic) waste. Alternatively, in case of soluble polymers the entire sample can be dissolved and the solution containing polymer and additives can be directly analyzed by gradient-elution chromatography [19–21]. Here, a complete dissolved (polymer) sample is injected in a weak eluent, so that the polymer is retained at the top of the LC column. Upon starting a gradient and increasing the concentration of a strong eluent, the polymer will redissolve and can be separated from other components present in the original sample. When IR spectroscopy is utilized as a detection technique, functional-group screening can reveal the presence of certain classes of additives in the polymer sample.

A feasibility study into gradient RPLC–IR was carried out by analyzing a PS sample containing 1.2% (w/w) Irganox 1076. From the analysis of the standard additive mixture it could be derived that a good-quality spectrum for Irganox 1076 required an injected mass
of at least 1 to 2 µg. Therefore, a 4-µl sample aliquot, corresponding to 5.8 µg of additive, was analyzed by RPLC–IR. The RPLC gradient used was based on the gradient used for separating the additive mixture. Test injections of an Irganox-1076 standard showed elution of the additive towards the end of the gradient at 100% MeCN. Therefore, the gradient was adapted and the mobile-phase composition was rapidly changed from 50-50% (v/v) A-B to 100% B in 2 minutes and held constant during 40 min (which is equivalent to ten column volumes). Thereafter, a separate gradient segment was run from 100% MeCN (solvent B) to 100% THF in 10 minutes. The final composition was maintained for 10 minutes. This latter segment was added in order to redissolve and elute the PS from the HPLC column.

In the optimization process, the peak profiles for PS and Irganox 1076 were observed from the UV signal at 276 nm and baseline separation was obtained \( t_{Irganox\,1076} = 39.8\,\text{min}; t_{PS} = 58.8\,\text{min} \). The nebulizer temperature of the LC–IR interface was optimized for the additive deposition.

The Gram–Schmidt chromatogram (Figure 5.8), which is the overall reconstructed chromatogram based on the IR information at all wavelengths, did not reveal a distinct peak corresponding to Irganox 1076. Both the selectivity and sensitivity for Irganox 1076 were enhanced by the construction of several functional group chromatograms in a similar way as described for the analysis of the previous additive mixture (cf. Figure 5.6). Because of the large differences in sample amounts for the polymer and the additive, only the Irganox-1076 elution region is shown in Figure 5.8. It was not our goal to detect and identify the bulk of the sample, as this is accomplished much more quickly and easily with direct FTIR spectroscopy. It is shown that carbonyl, aromatic C–H and sharp (s) hydroxyl vibrations are present at 40.5 min. These are typical absorption bands for additives containing a hindered-phenol group. The corresponding IR spectrum taken at the apex could be identified as that of Irganox 1076 by library searching using the Euclidean-distance algorithm (match qualifier of 0.858 on a scale of 1). The apex IR spectrum extracted from the chromatogram is shown in Figure 5.9, together with a reference spectrum of Irganox 1076, which was recorded from a 2-µg FIA deposition. The screening of functional groups as described above can reveal information on the classes of additives present in a sample, even when a Gram–Schmidt chromatogram does not indicate the presence of any components.
Figure 5.8: Functional-group-screening chromatogram of a polystyrene sample containing 1.2% (w/w) Irganox 1076. For details on the construction of functional-group chromatograms, see Figure 5.6.
Figure 5.9: A. IR spectrum from the peak eluting at 40.5 min in Figure 5.8. Library searching showed a match qualifier of 0.858 (on a scale of 1) for Irganox 1076. B. Reference spectrum recorded from a FIA deposition of Irganox 1076. For further details, see text.

5.2.4 Conclusions

FTIR spectroscopy coupled to HPLC using a solvent-elimination interface has proven to be a valuable tool for the screening of various types of additives (i.e. hindered amines, hindered phenols, phosphorous antioxidants). The molar mass did not seem to influence the deposition characteristics, such as obtaining a dry or a wet deposit. The vast majority of the selected additives could be deposited very well. Good-quality IR spectra were obtained from an additive test mixture (2 μg of each additive) separated by RPLC–IR. A few additives exhibited changes in morphology when deposited. This was revealed from their corresponding IR spectra. Therefore, care must be taken when automated library searches are performed. Other types of molecules may also exhibit polymorphism when subjected to solvent-elimination LC–IR.

A real polymer sample containing polystyrene and Irganox 1076 was used to demonstrate that polymer additives could be characterized by gradient elution LC–IR. In this case no extraction procedure proved to be required. Even at the low concentration levels of the
additive present in the sample, a characteristic spectrum was obtained and identified by library searching. Functional-group screening may be used to provide a reliable prediction on the presence of specific classes of additives in polymer samples.

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References