Coupling of liquid chromatography and Fourier-transform infrared spectroscopy for the characterization of polymers
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4 Assessment of quantitative aspects in solvent-elimination-based liquid chromatography–Fourier-transform infrared spectroscopy for the analysis of polymers
4.1 Fourier-transform infrared spectroscopy with a sample-deposition interface as a quantitative detector in size-exclusion chromatography

Summary

The use of a state-of-the-art commercial solvent-elimination interface for liquid chromatography–infrared spectroscopy is discussed from the perspective of quantitative analysis. The effect of eluent flow rate is investigated with respect to the homogeneity of the deposit and the trace width along the deposition trace. Low flow rates (50 µL/min or less) turn out to be favorable for obtaining a good trace and a high sensitivity. The trace width decreased from 2.20 mm to 1.13 mm when the flow rate was reduced from 500 to 25 µL/min. Preservation of chromatographic detail during deposition was evaluated at different substrate moving speeds. The additional (extra-column) band broadening that is inherent to nebulizer-deposition interfaces, causes a loss in resolution in SEC–FTIR in comparison with SEC-UV. The repeatability of the deposition is evaluated by SEC–FTIR analyses of polystyrene standards (peak molar masses: 1,250 to 2,950,000 g/mol) and the RSD is found to range from 3.2 to 5.8% in response and from 0.21 to 0.47% in retention time.

4.1.1 Introduction

Size-exclusion chromatography (SEC) is often used in the characterization of polymers and copolymers. Because SEC only separates according to hydrodynamic volume, the effects of molar mass and chemical composition cannot be distinguished. Thus, for non-homogeneous polymers, neither the molar-mass distribution (MMD), nor the chemical-composition distribution (CCD) or the functionality-type (FTD) distribution can be determined from SEC alone. The detectors most commonly used in SEC, such as refractive index (RI) and evaporative light scattering (ELS) detectors, are non-specific. Though UV/Vis detectors are more selective, many polymers do not show any UV activity at all, so detectors of this type cannot be generally used for polymer-composition analysis.

Fourier-Transform Infrared (FTIR) spectroscopy is a very powerful tool for analyte identification and for obtaining detailed chemical (functional-group) information. In many cases it is therefore preferred to the previously discussed detectors. Thus, the most widely accepted method to obtain better insight in the chemical (micro-)structure of synthetic polymers is the coupling of SEC with FTIR. Combining liquid chromatography (LC) with FTIR spectroscopy will increase the amount of information obtained from chromatographic
data. Such information is essential in the development of new polymers or in product-failure analysis.

A large number of interfaces between LC and FTIR have been described and two approaches can be distinguished, viz. techniques that use flow cells and techniques involving solvent elimination. In on-line flow-cell-based LC–FTIR interfaces [1–3] spectra are acquired on-the-fly. However, applications are limited to IR transparent solvents and this approach shows rather poor detection limits. Because of these limitations, solvent-elimination based LC–FTIR interfaces are more successful. These make use of thermospray [4–6], particle beam [7, 8], electrospray [9], ultrasonic [10–13], or pneumatic [14–20] nebulizers. The resulting interfaces yield mid-IR spectra free of solvent interferences and show lower detection limits than the flow-cell-based LC–FTIR interfaces. Nowadays, the most widely used pneumatic nebulizer is commercially available and such an instrument has been used in this study.

While the identification power of solvent-elimination interfaces has been amply demonstrated [12, 19, 21], quantitative aspects have hardly been investigated in polymer analysis. Proverd et al. [20] studied the effects of specific instrument settings (viz. substrate scanning speed and number of spectra collected) on the reconstructed (Gram–Schmidt) SEC–FTIR chromatograms, which were only visually compared to the obtained SEC-RI chromatogram. The effect of varying the substrate moving speed during deposition was not investigated. Schunk et al. [22] investigated the location and distribution of deposited polymer films by optical microscopy imaging of deposited polymers. Liu et al. [13] noticed an inhomogeneous deposition, but accepted the inherent variance without optimizing the LC–FTIR conditions, because IR-absorption-band ratios within FTIR spectra remained constant. Recently, quantitative analysis of polymers was investigated by Schunk and co-workers [23, 24]. Their research focussed on the development of FTIR calibration models and they discussed various aspects, including quality of the polymer film, molar-mass effect, and data pre-processing.

To obtain quantitative data from solvent-elimination-based LC–FTIR interfaces, it is necessary that the FTIR spectra represent all (or a given constant fraction) of the material deposited on the substrate. However, spreading of the deposit outside the area covered by the FTIR beam is a phenomenon inherent to the pneumatic spray nebulizers commonly used for interfacing LC and FTIR. Deposit spots spread out due to the convection of evaporated eluent and nebulizer gas across the substrate when both impinge on the deposition substrate. In addition, the sample trace may not be completely homogeneous (i.e. it may not have a constant thickness and composition throughout the spot). Finally, the performance parameters of the FTIR system (e.g. beam diameter and beam positioning) are
of great importance. To study these effects, an explorative study into quantitative aspects and settings of a commercially available LC–FTIR interface has been carried out with the aim to improve the quantitative interpretation of data obtained from LC–FTIR systems. To study fundamental aspects we focused only on LC separations in which the solvent composition was not changed during the analysis. New findings are presented which allow a better understanding of the performance of pneumatic nebulizers. In particular, deposition effects are documented that occur with a commercially available solvent-elimination interface, used in conjunction with an FTIR sampling accessory and using an FTIR microscope. The trace widths proved to depend on the eluent flow rate, affecting the quantitative figures of merit. After optimization of the eluent flow rate, the chromatographic resolution obtained for the SEC–FTIR analysis of a mixture of polystyrene standards was compared to that obtained with conventional SEC-UV. From triplicate collections and FTIR analysis of the polystyrene mixture, the repeatability of deposition was determined across a broad mass range.

4.1.2 Experimental

Chemicals
Unstabilized tetrahydrofuran (THF; HPLC-grade) from Labscan (Dublin, Ireland) was used for all experiments. Polystyrene standards were supplied by Polymer Laboratories (Church Stretton, Shropshire, UK) and polycarbonate standards were obtained from Scientific Polymer Products (Ontario, NY, USA). Stock standard solutions of polystyrene and polycarbonate were prepared by weighing and dissolution in HPLC-grade THF. A mixture of polystyrene standards with peak molar masses (Mₚ) of 1.25, 34.5, 465 and 2,950 kg/mol, was prepared by combining the stock solutions and dilution with HPLC-grade THF to final concentrations of ca. 0.4 mg/ml for each standard. All standard solutions were stored in the dark at 6°C.

Size-exclusion chromatography
The chromatographic system consisted of a Waters 2690 Separations Module (Milford, MA, USA), equipped with a vacuum degasser and a thermostatted column compartment. This system was used for flow-injection experiments, in which a 50-μl sample plug was introduced into the eluent stream without using any separation column, and for SEC experiments. On-line UV detection was performed with a Waters PDA model 996 at 260 nm. A computer using Waters Millenium32 (version 3.2) software controlled the system and was used to monitor the detector signals.
SEC separations were carried out on two 300 mm × 7.5 mm I.D. PLGel linear MixedB columns (particle diameter 10 µm; Polymer Laboratories) using THF at a flow rate of 0.5 ml/min. The injected sample volume was 50 µl. During all analysis the column compartment was maintained at 35°C and the sample compartment was set at 20°C. An Acurate model ICP-04-20-CR (LC Packings, Amsterdam, The Netherlands) flow splitter was used for measuring the chromatographic resolution and the repeatability. One-twentieth of the total flow through the column (i.e. 25 out of 500 µl/min) was diverted to the LC–FTIR interface.

**Interfacing liquid chromatography with FTIR spectroscopy**

An LC-Transform Model 500 (Lab Connections, Northborough, MA, USA) interface, based on the principle described by Gagel and Biemann [14–16], was used to evaporate the eluent and to collect the chromatogram on a 60 × 2 mm (diameter × thickness) rear-surface-aluminized germanium substrate. The interface was controlled by Labcon 1.0 software (Lab Connections). For flow-injection and continuous-flow-injection experiments (i.e. where a continuous flow of polystyrene containing THF was delivered to the LC–FTIR interface) by means of a syringe pump and SEC analyses, the conditions are summarized in Table 4.1.

**Table 4.1:** Experimental LC–FTIR-interface conditions used to obtain a dry deposit at various THF flow rates.

<table>
<thead>
<tr>
<th>flow rate (µl/min)</th>
<th>$h_{\text{nebulizer}}$ (mm)</th>
<th>$T_{\text{nebulizer}}$ (°C)</th>
<th>$p_{\text{nitrogen}}$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>8</td>
<td>40</td>
<td>138</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>50</td>
<td>172</td>
</tr>
<tr>
<td>75</td>
<td>6</td>
<td>55</td>
<td>138</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
<td>55</td>
<td>172</td>
</tr>
<tr>
<td>250</td>
<td>7</td>
<td>95</td>
<td>172</td>
</tr>
<tr>
<td>500</td>
<td>8</td>
<td>110</td>
<td>172</td>
</tr>
</tbody>
</table>

Nitrogen was used as nebulizer gas. The substrate moving speed was 1.7 mm/min, unless otherwise stated. Collection of the analytes on the substrate was delayed until the actual elution of the samples. The LC–FTIR interface was triggered via an electronic pulse from the chromatographic system. The substrate movement during sample deposition was controlled by an x,y-stepper motor. In deposition-homogeneity and response-optimization studies, a Harvard Apparatus 22 (Holliston, MA, USA) syringe pump was used to deliver a constant flow of 25 µl/min of THF containing 0.2 mg/ml of polystyrene to the LC–FTIR interface. The syringe pump and the chromatographic system were connected to the LC–FTIR interface via a T-piece and a make-up flow of pure eluent was added by the chromatographic system to obtain a total flow rate of 500, 250, 100, 75, and 50 µl/min. In
flow-injection studies, the LC–FTIR interface was coupled directly to the injector of the chromatographic system. In other experiments the interface was operated in parallel with the UV detector via a flow splitter.

After sample deposition, the collection substrate was manually transferred to a three-times-focusing optical FTIR accessory, controlled by LCT 1.6.1 software (Lab Connections), and spectra were acquired by stepwise rotation of the substrate.

**Spectroscopy**

FTIR spectra were acquired using 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 sample and detector compartments were continuously purged using nitrogen gas, which was dried using a Zander Adsorption Dryer, Type KM5 TE (Essen, Germany). 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 total area of the absorption or the maximum peak height from the selected IR absorption band in each spectrum was used.

FTIR-microscopy experiments were performed in the reflectance mode on a Perkin Elmer System 2000 spectrometer, equipped with an Perkin Elmer Autoimage microscope with a beam spot size of 100 × 100 μm and 100-μm steps. A narrow-band MCT detector was used and the microscope was controlled by Autoimage 4.1 software. The acquisition parameters were identical to the settings used for the Perkin Elmer Spectrum GX.

### 4.1.3 Results and discussion

**Homogeneity of deposits**

To evaluate the regularity and homogeneity of deposition along the trace, a constant flow of a solution of polystyrene in THF was directed at 0.5 ml/min to the LC–FTIR interface without chromatography. The polystyrene was deposited as an arc-wise, continuous trace of constant mass and composition from 25 to 340°. After obtaining the FTIR spectra of the deposit, the maximum IR absorption band heights at 698 cm\(^{-1}\) and 3026 cm\(^{-1}\) were calculated against the disk angle. As shown in Figure 4.1 (only profile at 698 cm\(^{-1}\) shown), the functional-group profile turned to be wave-shaped and the intensity of the response varied from 0.0350 to 0.0904 AU. The stability of the response, expressed as the relative
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Figure 4.1: Functional-group profiles obtained by continuous, arc-wise depositions of polystyrene from 25 to 340° at a flow rate of 500 µl/min (solid line) and 25 µl/min (dashed line). The response was constructed from maximum absorption-band heights at 698 cm⁻¹. The total mass deposited was kept constant.

standard deviation (RSD) of the response along the deposit, was 26.6% (see Table 4.2). Such a variation will not impart the use of this LC–FTIR interface for qualitative purposes (spectrum identification), but it will jeopardize quantitative interpretation of the absorption intensities.

A combination of two interface-related factors provides a plausible explanation for the observed deposition pattern. Firstly, the actual contour of the spray leaving the nozzle was oval rather than circular. This is often observed in LC–FTIR solvent-elimination interfaces [25]. Secondly, the movement of the deposition substrate is determined by an x,y-stepper motor. In other designs of deposition interfaces, the substrate is rotated around its center, while the position and orientation of the nozzle (and thus of the spray) relative to the substrate surface is constant. When, in case of moving the complete substrate in the x,y-plane, at a disk angle of 0° a part of the spray is pointing to the center of the substrate, it will be pointing to the outside of the substrate after a rotation by 180°. A combination of these two factors results alternately in narrow traces, where the deposited mass is concentrated on a small area of the substrate, and broad traces, where the deposited mass is spread over a larger area.

Similar experiments were carried out by Liu and Dwyer [13]. However, they used an ultrasonic nebulizer interface, in which the substrate movement was different from that used in our system, in that it was rotated under the nozzle. The FTIR optics accessory was
Table 4.2: Response, stability of the response (expressed as RSD), and response-ratio for arc-wise depositions of polystyrene from 25 to 340°C, calculated from maximum absorption band height at 698 cm⁻¹ and 3026 cm⁻¹.

<table>
<thead>
<tr>
<th>flow (μl/min)</th>
<th>response (698 cm⁻¹)</th>
<th>response (3026 cm⁻¹)</th>
<th>response ratio of 696 cm⁻¹ and 3026 cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>average (AU)</td>
<td>RSD (%)</td>
<td>average (AU)</td>
</tr>
<tr>
<td>500</td>
<td>0.0601</td>
<td>26.6</td>
<td>0.0253</td>
</tr>
<tr>
<td>250</td>
<td>0.0955</td>
<td>27.2</td>
<td>0.0389</td>
</tr>
<tr>
<td>100</td>
<td>0.0978</td>
<td>27.2</td>
<td>0.0427</td>
</tr>
<tr>
<td>75</td>
<td>0.118</td>
<td>25.2</td>
<td>0.0497</td>
</tr>
<tr>
<td>50</td>
<td>0.128</td>
<td>23.0</td>
<td>0.0504</td>
</tr>
<tr>
<td>25</td>
<td>0.206</td>
<td>13.5</td>
<td>0.0768</td>
</tr>
</tbody>
</table>

Similar. At a flow rate of 1 ml/min, Liu and Dwyer obtained an average spectral peak area of 9.4 AU/cm and a response stability, expressed as RSD, of 4.6% for the 700 cm⁻¹ absorption band, calculated from a 62° arc-wise deposition (start position unknown) on the substrate. In our experiments, the response stability was 14.9% (RSD) over a similar angle (rotation angle, 40–102°; flow rate, 500 μl/min). This is more evidence that the substrate-to-nozzle position and its orientation are important factors for obtaining a homogeneous deposition along, circular traces. If not, it is essential that the scanning conditions are adapted to the deposition conditions so as to obtain the best possible results, i.e., both deposition and scanning should either take place linearly or by a rotation around the center of the substrate.

One needs to be aware of this phenomenon in case the commercial LC–FTIR interface used in this paper is applied as a detector for quantitative purposes. Scanning the entire deposit rather than small spots, by surface mapping with FTIR-microscopy, might overcome the present problem, at the expense of much-increased complexity.

FTIR-microscopy experiments were performed to confirm our hypothesis of oval deposition by acquiring FTIR spectra across polystyrene depositions which were deposited by moving the substrate in the x or y direction only. This will also unravel any inhomogeneity across the deposition trace. To this end, a polystyrene (Mₚ, 195,900 g/mol; ca. 0.1 mg/ml) solution in THF was linearly deposited at a flow rate of 500 μl/min. FTIR absorption band heights at 2924 cm⁻¹ showed significant differences in the deposited mass perpendicularly across the trace, as can be seen in Figure 4.2. The deposition pattern observed when depositing in the x-direction is strongly asymmetrical and depositing in the y-direction results in a more symmetrical pattern. However, in both cases, little material was deposited in the center of the trace. Previously published results [22], where FTIR-microscopy scans were taken across a polymethyl methacrylate deposit, showed the left and the right part of the trace to be of almost equal intensity.
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Figure 4.2: Response of functional-group profile perpendicularly to the trace obtained by linearly deposited polystyrene and subsequently scanning by FTIR-microscopy. The response was constructed from absorption band heights at 2924 cm⁻¹. Deposition flow rate, 0.5 ml/min; x-direction (dashed line) and y-direction (solid line).

To eliminate a faulty nozzle as the cause of our observations, the gap where the capillary protrudes into the nozzle and where the spray is surrounded by sheath gas was verified to be circular. Furthermore, the stainless-steel nozzle capillary was replaced by a perfectly circular and perpendicularly cut fused-silica capillary of 200 µm I.D. to eliminate distortion at the edges of the original mounted capillary as a possible cause. This still resulted in the previously described deposition pattern. Until now, many parameters may influence the spray shape, and an exact correlation between these parameters and the spray shape are not known. Data produced by other workers (cited above) indicated that the way in which the substrate is moved under the nozzle is of major importance for producing a continuous trace. Because the principle of deposition movement of the LC–FTIR interface used in this study can not be changed, it was investigated whether the trace inhomogeneity can be overcome by changing the operating parameters as described below.

**Determination of trace width and optimization of the response**

In addition to a high response, a stable and constant response (*i.e.*, low RSD) is desired to obtain quantitative results from LC–FTIR data, independent of the deposit position on the substrate. In principle, a maximum response with a small variation around arc-wise depositions can be obtained by producing a deposition trace sufficiently narrow for it to be fully contained within the IR beam. In that case, small variations in the deposition pattern...
will be obscured. The main factor responsible for the trace width is the eluent flow rate, while other parameters, such as the nitrogen pressure and the interface temperature, must be simultaneously optimized to obtain a dry deposit.

The trace width was measured at several flow rates, viz. 500, 250, 100, 75, 50, and 25 µl/min with the interface temperature and the nitrogen pressure being adjusted to obtain a dry deposit. Polycarbonate (ca. 1.0 mg/ml) was used as test compound and was deposited in linear traces in the flow-injection mode. Subsequently, FTIR-microscopy scans were obtained perpendicularly across the polycarbonate trace and concentration profiles were constructed of the carbonyl absorption band height at 1776 cm⁻¹. Upon decreasing the flow rate from 500 to 100 µl/min the trace width was significantly reduced from 2.20 mm to 1.24 mm. No carbonyl absorption was observed in the center of the trace at flow rates of 500 and 250 µl/min. At 75, 50, and 25 µl/min flow rates, the trace width decreased to 1.17, 1.15, and 1.13 mm, respectively (see Figure 4.3), and carbonyl absorption appeared in the center of the trace.

In order to investigate the response and the stability of the response expressed as RSD along the trace, arc-wise depositions of polystyrene (Mₚ, 465 kg/mol) were prepared in the constant-flow mode (no column) at the eluent flow rates listed earlier. During all depositions, the mass flow (5 µg polystyrene/min) and the total mass deposited were held constant. The polystyrene deposit was scanned from 25 to 340°, resulting in 316 spectra.

**Figure 4.3:** Trace width (in mm) obtained after linear deposition of polycarbonate at different eluent deposition flow rates. The trace width was calculated from the carbonyl absorption band height at 1776 cm⁻¹ obtained from FTIR-microscopy scans perpendicularly across the trace. For details, see text.
The average response and stability of the response (expressed as RSD) were calculated by averaging the response from functional-group profiles constructed from maximum IR absorption band heights at 698 cm\(^{-1}\) and 3026 cm\(^{-1}\) (see Table 4.2 and Figure 4.4). An example of the response along the trace at 698 cm\(^{-1}\) is given in Figure 4.1. As expected, at low flow rates (25 μl/min) the sensitivity for the IR absorption band at 698 cm\(^{-1}\) has increased significantly (by a factor of 3.4) compared with the response at a flow rate of 500 μl/min. At the same time, the RSD decreased from 26.6 to 13.5%. Apparently a more compact deposition was produced and the trace width was reduced to a value more in line with the FTIR beam diameter, obscuring some of the fluctuations in the deposition. In addition, in spectral-band-ratio analysis, where concentration effects are cancelled out, the standard error was of the order of 2 to 6% at all flow rates smaller than 250 μl/min (see Table 4.2).

**Chromatographic resolution**

Working with off-line detectors or spectrometers, after immobilizing or fractionating chromatograms, can have adverse consequences for the chromatographic resolution. While in fractionation the chromatographic resolution is limited by the number of fractions collected, in solvent-elimination LC–FTIR the chromatographic resolution is affected by the substrate moving speed [22]. In order to find deposition conditions at which FTIR
Table 4.3: Chromatographic resolution determined from SEC-UV and SEC-FTIR at different substrate moving speeds. Peak molar masses (in kg/mol): A, 2.95 × 10^3; B, 465; C, 34.5; D, 1.25. For chromatograms, see Figure 4.5.

<table>
<thead>
<tr>
<th>peak</th>
<th>UV detection (260 nm)</th>
<th>FTIR detection at various substrate moving speeds (mm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>A-B</td>
<td>2.2</td>
<td>1.0</td>
</tr>
<tr>
<td>B-C</td>
<td>3.3</td>
<td>1.5</td>
</tr>
<tr>
<td>C-D</td>
<td>3.3</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Chromatograms show comparable resolution as chromatograms obtained with in-line detectors, substrate moving speeds of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mm/min were selected to collect and subsequently analyze a polystyrene-standard mixture. Resolution from the UV chromatogram was compared with that from the total areas of the absorption band at 698 cm⁻¹. From Table 4.3 it can be seen that the most significant increase of SEC-FTIR resolution is observed when increasing the substrate moving speed from 1.0 to 2.0 mm/min. Furthermore, it can be seen that even at a high substrate speed, it is hard to preserve the chromatographic resolution for polystyrenes of relatively low molar mass due to molar-mass effects [24].

An undesirable consequence of a high substrate speed is a loss in sensitivity, because less mass is deposited on the same area of the substrate. This can be seen from Figure 4.5, where the response of low-molar-mass polystyrene (Mₚ, 1,250 g/mol) decreases more strongly with increasing moving speed compared to polystyrenes of higher molar-mass.

For polystyrene standards with peak molar masses of 2,950,000 and 465,000 g/mol, SEC-FTIR resolution is nearly as good as SEC-UV resolution at a rather high substrate moving speed of 3.5 mm/min. Thus, chromatographic resolution is not only determined by the substrate moving speed, but also by the molar mass of the deposited polymer [24]. This is true for polystyrene, but similar results are anticipated for other types of polymers, probably depending on the physical properties, such as crystallinity and viscosity. This needs to be explored through future experiments. A substrate moving speed of 2 mm/min was used in further experiments and offers a good compromise between chromatographic resolution, sensitivity, and the time needed to collect FTIR spectra.
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Figure 4.5: SEC-UV chromatogram and SEC-FTIR functional-group chromatograms obtained after deposition of a polystyrene-standard mixture at various substrate moving speeds. Functional-group chromatograms were constructed from the total area of the absorption at 698 cm$^{-1}$. Peak molar masses (in kg/mol): A, 2.95 x 10$^3$; B, 465; C, 34.5; D, 1.25.

Repeatability

Validation of the repeatability of deposition was performed by analyzing a polystyrene-standard mixture by SEC–FTIR in triplicate and by comparing the data with those obtained from UV detection. Peak areas and retention times of all four polystyrene standards were calculated from FTIR functional-group chromatograms (absorption band area at 698 cm$^{-1}$) and from UV chromatograms at 260 nm. The latter data were used to estimate the conventional chromatographic repeatability.

The repeatability of chromatographic peak areas over a broad molar-mass range varied between 3.2 and 5.8% and the repeatability of retention times varied between 0.21 and 0.47% (see Table 4.4). This is in the same range as data obtained using the in-line UV detector. Interfacing LC to FTIR with the present type of interface will produce reliable
Table 4.4: Repeatability of peak area and retention time for SEC–FTIR analyses of a mixture of polystyrene (PS) standards (n=3), calculated from UV detection at 260 nm and FTIR absorption band areas at 698 cm⁻¹ (Mₚ, peak molar mass).

<table>
<thead>
<tr>
<th>PS, Mₚ (kg/mol)</th>
<th>Peak area</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV FTIR</td>
<td>UV FTIR</td>
</tr>
<tr>
<td>Average (AU/min)</td>
<td>RSD (%)</td>
<td>Average (min)</td>
</tr>
<tr>
<td>2.95 × 10³</td>
<td>4.74 1.02</td>
<td>2.44 4.0</td>
</tr>
<tr>
<td>465</td>
<td>4.59 1.13</td>
<td>2.35 3.5</td>
</tr>
<tr>
<td>34.5</td>
<td>4.53 0.91</td>
<td>1.26 3.2</td>
</tr>
<tr>
<td>1.25</td>
<td>4.46 0.64</td>
<td>0.86 5.8</td>
</tr>
</tbody>
</table>

Data that can be compared with those from in-line detectors. In addition, SEC–FTIR provides structural information throughout the chromatogram.

4.1.4 Conclusions

SEC–FTIR is a mature technique for obtaining qualitative (spectral) information. A few quantitative studies have been performed in research environments using home-built interfaces, but the application of commercial LC–FTIR interfaces for the quantitative analysis of polymeric samples has hardly been explored. It has been demonstrated that the eluent flow rate is a critical factor in obtaining a homogeneous deposition in commercially available solvent-elimination interfaces, in which the deposition substrate is moved by an x,y-stepper motor. The trace width was reduced from 2.20 mm to 1.13 mm by reducing the eluent flow rate through the LC–FTIR interface from 500 to 25 μl/min. Much of this effect is reached by relatively small reductions in the flow rate (e.g. down to 100 μl/min). Upon a reduction in the flow rate, the depositions of polystyrene showed an improvement in response by a factor of 3.4, while the stability of the response, expressed as RSD was improved from 26.6 to 13.5%. The chromatographic resolution in SEC–FTIR is not only affected by the substrate moving speed, but also by the molar mass of the polymer to be analyzed. The repeatability of three consecutive depositions with a polystyrene standard mixture was found to be 4.0% in response and 0.47% in retention time for high-molar-mass polystyrene and 5.8% in response and 0.21% in retention time for low-molar-mass polystyrene.

Acknowledgements

The authors wish to thank dr. Tom Visser from the Vibrational-Spectroscopy Department of the Utrecht University (The Netherlands) for his assistance in performing the FTIR-microscopy experiments.
References

25. Dr. T. Visser, Utrecht University, The Netherlands, personal communication.
4.2 Potential of a solvent-elimination interface for liquid-chromatography–Fourier-transform-infrared spectroscopy in quantitative analysis of polymers

Summary

It is demonstrated that various parameters may complicate copolymer quantification in a commercial interface for liquid chromatography–infrared spectroscopy (LC–IR) using absorption-band ratios. The parameters include spectroscopic effects, the optical configuration and deposit properties. Various methods have been investigated to obtain valid and consistent absorption-band ratios for polymer films on Ge, Ge-Al and ZnSe substrates for use in univariate calibration (absorption-band ratio vs. co-monomer ratio). These methods include the reduction of IR scattering and the deposit inhomogeneity. Furthermore, the influence of the refractive index of the substrate and the optical configuration (i.e. transmission and transflection) have been investigated. Finally, the molecular orientation and the effects of thin films have been examined. It was found that an incorrect representation of absorption bands was mainly due to a limited film thickness of the deposits on the various substrates. Major changes in absorption-band ratios were found at deposited quantities smaller than 10 µg. Consequently, in the quantification of copolymers univariate calibration curves depend on the injected quantity. Still inconsistent absorption-band intensities were found for deposits of higher quantities, which might be ascribed to the varying film thickness inherent to solvent-elimination LC–IR.

4.2.1 Introduction

Knowledge of the chemical composition of (co-)polymers as a function of molar mass is important to enlarge insight in the synthesis as well as the aging of polymers. A widely applied method to obtain such information is the combination of liquid-chromatography and infrared spectroscopy (LC–IR), in particular using size-exclusion chromatography (SEC) and solvent-elimination IR interfaces [1–11]. In such an interface the analyte is deposited on a moving flat substrate, while the solvent is removed by evaporation. Spectral data are subsequently acquired by passing the substrate through the IR-beam. Commonly used substrates are made of IR-transparent materials (ZnSe, Ge, or, less often, CaF₂) and IR spectra are acquired in the transmission mode. When the substrate is coated on the back (non-deposition) side with an IR-reflective coating (e.g. aluminum) transflection measurements are facilitated.
For copolymer quantification using SEC–IR two univariate-calibration methods are commonly used, i.e. the intensities of specific absorption bands are plotted against the deposited quantity (method I) [9, 10] or, the absorption-band ratios are plotted against the relative copolymer composition (method II) [2, 5, 12, 13]. In method I the total deposited quantity can be derived from the injected amount. For example, the mass fraction eluted during a finite elution time can be obtained from area slices in the refractive-index (RI) chromatogram. In the latter case the specific RI response (dn/dc) must be known. The method is difficult to apply under all circumstances for a number of reasons. Firstly, for non-homogeneous copolymers the dn/dc values vary with the elution volume. Secondly, the use of an RI detector is not a viable option if solvents are used with refractive indices close to that of the polymer. Finally, RI detection cannot be used in combination with gradient LC. In LC–IR, deposition trace widths have proven difficult to control [5, 14] and depend, for example, on the injected amount, and on the physical properties of the sample, the eluent, and the substrate. A varying deposit trace width in combination with a fixed IR-beam diameter will hamper quantification in cases where the deposition trace width exceeds the IR-beam diameter [14].

Method II requires copolymer standards in the chromatographic analysis but such standards are often not available. Alternatively, a series of polymer “blends” (i.e. mixtures) with known compositions may be used. The advantage of using absorption-band ratios is that concentration effects are cancelled out. However, the method requires isolated absorption-bands for each of the components (or monomeric units). Because the absolute absorption-band intensities along a deposited trace proved to be variable and because of the other limitations of method I [5, 14], quantification of copolymers should preferably be performed using absorption-band ratios.

Several authors report the use of absorption-band ratios to obtain functional-group information on copolymers and polymer blends as a function of molar mass in SEC effluents using solvent-elimination SEC–IR. These reports are based on transmission spectroscopy on KBr [1, 2] and Ge [9, 15] substrates and on transfection spectroscopy on rear-surface-aluminized germanium (Ge-Al) substrates [3–7, 11, 12]. The composition of a copolymer was determined using copolymer standards or from a series of blends with known composition, from which linear calibration curves were obtained. In most cases, the copolymer compositions were expressed in concentration units (i.e. percentage or quantity per volume) [1–5, 9, 10] but in some reports only the absorption-band ratios were given [6, 12].

Absorption-band ratios on KBr discs acquired in the transmission mode were successfully used for the determination of the composition drift in ethylene-propylene rubber [1]. A
linear relationship was obtained for deposited amounts ranging from 1 μg to 1 mg (absorption-band ratios of \( \nu_{as} \text{CH}_3 \) and \( \nu_{as} \text{CH}_2 \)). This relationship was later applied by Wheeler and Willis [3] on similar copolymers. However, a different substrate (Ge-Al) was used than used during the calibration (KBr-discs) and the IR spectra from the samples were acquired in the transflection mode (range of deposited amounts 450-750 μg). Dekmejian and coworkers determined the polyester content in a hydrocarbon-polyester (HC-PES) copolymer using absorption-band ratios, and calibration based on HC/PES blends [2]. Relative absorption-band intensities were used for the compositional analysis of styrene-butadiene-rubber (SBR) copolymers, using SBR copolymers of known composition for calibration [5]. A linear relationship between the styrene/butadiene concentration ratio and the ratio of the respective absorption-band intensities was obtained for copolymers with 10% to 44.5% styrene on Ge-Al substrates measured in transflection. All calibration standards were presumably deposited in equal concentrations. It was stated that the absorption-band ratios for a homopolymer should remain constant across the SEC-eluate, even if the actual deposited quantities vary. Recently, polystyrene (PS) and poly(methyl methacrylate) (PMMA) polymers were used for film-casting and to create deposits on Ge. These were both measured in the transmission mode. The absolute-band intensity (films) and the area of the absorption-band second derivative (deposits) were plotted vs. the polymer mass, which resulted in linear relationships [10, 15]. The home-built interface used in that work featured a series of small Ge discs (13 mm diameter) configured in a circle. On each disc a given fraction of the chromatogram was deposited, after which IR spectra were measured in the transmission mode. Consequently, this configuration limits the chromatographic resolution, as the number of fractions collected is limited by the number of Ge-discs. Presumably, the entire deposit was covered by the IR beam, producing spectral intensities in proportion with the deposited quantities. The interface used in our study is technically different [13, 16]. It does allow most of the chromatographic resolution to be maintained [14], but quantification via absorption-band ratios is thought to be the best option [14].

The use of absorption-band ratios for applications other than polymers was not reported to our knowledge. Plots of the absorption-band intensity vs. the deposited quantity are invariably used and linear relationships have been reported, e.g. for the deposition of dyes on ZnSe (deposition range, ~500 ng to 15 μg) [17] or for the deposition of phenanthrenequinone on Al-mirrors (deposition range, 16 ng to 250 ng) [16]. Spectra were obtained in the transmission and transflection modes, respectively. A linear relationship was also obtained for sucrose standards from 53 to 5300 pg, obtained using a recently
introduced LC-microdispenser-IR interface, that produces trace widths as narrow as 30-60 μm [18].

From Figure 4.6 it appears that (at least in some cases) erroneous composition data can be obtained in quantitative SEC–IR analysis of copolymers. We obtained a linear calibration curve for the absorption-band ratios vs. the monomer ratio for a series of PS–PMMA blends of known composition (Method II). These blends were deposited in the flow-injection analysis (FIA)-mode on a Ge-Al substrate. The calibration curve was validated by using a homogeneous styrene-(methyl methacrylate) (SMMA) copolymer of known composition which was subjected to SEC and deposited on an identical substrate. Surprisingly, quantification based on the absorption-band ratios from the copolymer spectrum (i.e., 3026 cm⁻¹/1154 cm⁻¹) resulted in a large difference between the estimated styrene-content (55%)

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**Figure 4.6**: The effect of anomalous absorption-band intensities on copolymer quantification. Shown on the left is the calibration curve obtained from a series of PS–PMMA blends with a total deposited quantity of 5 μg per composition. The functional-group chromatograms from the integrated absorption at 1154 cm⁻¹ (—) and 3026 cm⁻¹ (-----) were obtained from subjecting a PS-co-PMMA to SEC–IR (top-right). The copolymer composition was 40% styrene (determined via elemental analysis). The absorption-band ratios for the copolymer have been calculated (bottom-right) and subsequently projected on the calibration curve. Following this procedure, a copolymer composition of 55% PS was obtained. (SEC conditions: column, two 300 mm x 7.5 mm I.D. PLGel MixedB (d_p=10μm); eluent, THF; flow rate, F=0.5 ml/min; injection volume, 50 μl; sample concentration, 1.0 mg/ml.)
and the actual (known) composition (40%) as can be seen from Figure 4.6 (dashed line). Besides, the absorption-band ratios were found to vary across the chromatographic peak. Clearly, copolymer quantification by SEC–IR is complicated even in case absorption-band ratios are used.

The aim of this study was to determine the different parameters that affect the absorption-band ratio for quantitative analysis for polymer films across a wide concentration range obtained from solvent-elimination LC–IR using Ge, Ge-Al and ZnSe substrates. These parameters include light-scattering effects, molecular orientation, the measurement mode (i.e., transmission, transflection), the refractive index of the substrate, the homogeneity of the deposit, and the film thickness. Alternatively, if anomalous spectra are obtained from various experimental set-ups we aim to provide clear insights into the limitations of the solvent-elimination technique and recommendations on how and when it can be used successfully.

4.2.2 Theory

Different effects can be discerned that may lead to errors in the quantification process. These include spectroscopic effects (e.g. scattering, refraction, reflection) and the optical configuration (e.g. transmission, reflection, or transflection and the substrate refractive index), in conjunction with sample properties, such as molecular orientation, a varying film thickness, and the deposit morphology. These factors will be considered below.

Scattering and discontinuous films

The process of solvent-evaporation and analyte-deposition in pneumatically assisted solvent-elimination LC–IR interfaces may result in a dispersed deposition phase consisting of domains of sufficient size to create different morphologies of deposits [19] from which anomalous IR spectra may be obtained [20]. For example, when the polymer deposit is inhomogeneous, scattering of the incoming IR radiation may occur in case domain sizes are in the order of the mid-IR wavelength and this will result in drifting baselines in the spectra. Furthermore, film inhomogeneity can – in extreme cases – result in asymmetrical absorption bands, due to large changes in the refractive index in the vicinity of an absorption band, also known as the Christiansen effect (see Appendix) [19, 21]. Finally, a combination of specular reflection, diffuse reflection and transmittance can arise from an imperfect polymer film and these factors may affect the absorption-band frequency and intensity [16, 22–24].

In addition, agglomerated polymer domains ("droplets") separated by empty regions may be formed during solvent-elimination and analyte deposition [4, 19]. The concentration
Figure 4.7: Calculated effect of an inhomogeneous film and bare spots on the observed absorption compared to the theoretical absorption. Shown are the scenarios for different fractions of bare surface interrupting the film indicated in the graph (solid lines) [25]. The broken lines show the absorption deviation calculated for deposits of varying film thickness as shown in Figure 4.11 (50 ng PS, dashed line; 200 ng PS, dotted line). For details, see text.

profile across a polymer deposit has proven to be variable and an increased concentration has been found at the edges compared to the center of the deposit [14]. The effect of a varying concentration on the absorption was first discussed by Jones [25], and it has recently been reviewed by Cheung et al. [26]. Open regions or domains with a decreased concentration will result in a reduced absorption and thus in an underestimation of the amount of polymer present. It has been shown that the higher the absorption of the band, the stronger the effect of open regions (cf. Figure 4.7). Variations in the film thickness can therefore result in inconsistent absorption-band ratios in practice.

Optical configuration and substrate refractive index

The optical configuration, e.g. transfection or transmission, can affect the nature and appearance of the IR spectra. In addition, the refractive index of a particular substrate and the angle of incidence determine the amount of reflected and transmitted IR radiation in these configurations.

Moreover, it was shown that for a number of analytes deposited on flat substrates the absorption-band ratios in transfection spectra can be different from those in the transmission spectra of the same analytes prepared as KBr discs [27]. Furthermore, an
increased band asymmetry has been observed in transflection measurements on front-surface-aluminized mirrors as the deposited quantity was increased, indicating a contribution from specular reflection from the analyte rather than from the surface of the mirror [24].

The influence of the substrate’s refractive index and the contribution of the transmission and transflection components for a given polymer deposit can be determined from Snell’s law and the Fresnel equations (cf. Appendix). This is illustrated in Figure 4.8 for a polymer deposit on Ge and ZnSe. The results are calculated for the transflection accessory used in this study with a nominal incident angle of 18.5°. Only a fraction of the IR radiation (4.9%) is specularly reflected at the top-surface of the deposit, which will not be measured in transmission measurements. The remainder of the IR radiation (95.1%) experiences absorption through the deposit. A significant increase in the transflection component is calculated for Ge (18.1%) when compared to ZnSe (2.7%), because of the high refractive index of Ge. Most of the IR radiation is lost at the rear of the substrate when a ZnSe substrate is used. However, when a Ge-Al substrate is used this part is reflected towards the

![Figure 4.8: Schematic showing the specular reflected, transflected and transmitted intensities from IR radiation on a PS film deposited on a Ge and ZnSe substrate. Conditions: $n_{\text{air}} = 1.00$, $n_{\text{PS}} = 1.57$, $n_{\text{Ge}} = 4.00$, $n_{\text{ZnSe}} = 2.20$, $\theta_1 = 18.5^\circ$, $\theta_2 = 11.7^\circ$, $\theta_3(\text{PS-Ge}) = 4.6^\circ$, $\theta_3(\text{PS-ZnSe}) = 8.3^\circ$.](image-url)
detector and contributing to the IR spectrum. Consequently, the pure specular-reflection component is relatively small. From rough surfaces, \textit{i.e.} from real deposits, specular reflection and, in addition, diffuse reflection can occur. Diffusely reflected light can only be effectively collected when specific parabolic mirrors are included in the optical accessory. Thus in either mode (transmission or transflection) diffuse reflection appears as background absorption. Finally, a part of the IR radiation may experience total-internal-reflection (TIR) inside the substrate, which can lead to a loss in energy. However, this effect does not occur in transflection measurements with common substrates (\textit{viz.} Ge, ZnSe) and with the current incidence angle.

\textbf{Molecular orientation}

In solvent-elimination-based LC–IR, the analytes are usually still partly in solution when they are deposited on the substrate [24, 28]. The slower the evaporation rate of the remaining solvent, the more likely is the analyte to form an oriented crystal on a smooth substrate. This can affect the analyte spectrum [24] and it can complicate quantification for two reasons. Firstly, the beam emerging from most interferometers featured in contemporary Fourier-transform (FT) IR spectrometers has some residual polarization leading to selective interaction with certain molecular vibrations. In addition, the principle of operation of the current LC–IR interface may possibly enhance molecular orientation as scanning of the deposit is not executed in a manner that corresponds to the deposition [14]. If orientation effects, however small, arise from the deposition, than they may be enhanced by the (angle-dependent) positioning of the substrate during scanning. It can further be expected that molecular orientation occurs more easily on the highly crystalline Ge rather than on non-crystalline substrates, such as ZnSe, CaF$_2$, or NaCl. To prevent the eventual occurrence of molecular orientation the use of substrates with a rough surface has been suggested, for example a thin layer of an infrared-transparent powder on a suitable substrate [24]. However, the absorption is not strongly dependent on the nature of the polarization of the incident beam at a small incidence angle, even when molecular orientation and polarization are present. Only if measurements are performed under grazing angle conditions, \textit{i.e.} at an incident angle preferable greater than 80° [29], these effects may play a role.

\textbf{Effect of thin films on the absorption}

The film thickness can further influence the absorption-band intensity [24, 30]. When absorption occurs in the deposit, the incident IR light is modulated by the substrate, depending on the absorption coefficient ($\kappa$) of the imaginary refractive index. A standing
wave will originate at the substrate (for $\kappa > 0$) and it will be dampened as function of the distance in the deposit from the substrate surface [30].

In the special case where the film thickness is less than a quarter of the wavelength, the intensity of the s-polarized component of the incident radiation rapidly approaches zero and it will not appear as absorption. This can be ascribed to the sum of the resultant of the incident and reflected radiation, taking into account the phase shift of the light reflected from the surface. As a result, a complex interference pattern will be created for specularly reflected IR radiation. Specifically, since the phase shift for the s-polarized component at the reflecting surface is close to $180^\circ$, this part of the transflected radiation will experience destructive interference, leading to anomalous absorption-band intensities [31].

The effect of a limited film thickness on transfection spectra was modeled for a number of polymers, substrates, and film thicknesses by Pacansky et al. [30]. It was revealed that the transfection oscillates with an increasing film thickness (investigated range, 0 to 5 $\mu$m) and the transreflectivity decreases with increasing film thickness. A higher oscillating frequency of the reflection was found for short wavelengths when compared to long wavelengths. This effect occurs at both investigated incidence angles ($i.e.$, $25^\circ$ and $50^\circ$). Fuoco et al. [24] found a non-linearity in the absorption-band intensity vs. injected quantity for transfection measurements from supercritical-fluid chromatography–IR deposits on Al. For low sample quantities, the absorption-band intensities were found to be lower than the corresponding intensities obtained by transmission spectroscopy, but larger when the injected sample quantities exceeded a certain value. This suggests a transition point related to the deposited quantity and thus to the film thickness ($v.i.z.$ a critical film thickness). This explanation may also be applicable for deposits on Ge and ZnSe substrates when anomalous absorption-band ratios are found.

4.2.3 Experimental

Chemicals

Unstabilized HPLC-grade tetrahydrofuran (THF) was supplied by Labscan (Dublin, Ireland), chloroform was purchased from Biosolve (Valkenswaard, The Netherlands) and toluene was obtained from Baker (Deventer, The Netherlands). Polystyrene (PS, peak molar mass $M_p=465$ kg/mol) and poly(methyl methacrylate) (PMMA, $M_p=185$ kg/mol) standards were supplied by Polymer Laboratories (Church Stretton, Shropshire, UK). Syndiotactic PMMA (s-PMMA), isotactic PMMA (i-PMMA), and isotactic PS (i-PS) were obtained from Scientific Polymer Products (Ontario, NY, USA). Stock solutions of PS, PMMA, s-PMMA and i-PMMA were prepared by weighing and dissolution in THF. Stock
solutions of i-PS were weighed and dissolved in chloroform. For spin-coating experiments on Ge-Al substrates, PS ($M_w=330 \text{ kg/mol}$) was obtained from Dow Chemical and a 32% standard solution was prepared in toluene. In these experiments the film thickness was determined using a Dektak$^3$ST surface profiler (Veeco Instruments, Santa Barbara, CA, USA). All standard solutions were stored in the dark at 6°C. Spectroscopy-grade KBr was obtained from Merck (Darmstadt, Germany).

**Flow-injection analysis (FIA)**

A Waters 2695 Separations Module (Milford, MA, USA), equipped with a vacuum degasser and a thermostatted column compartment was used in the flow-injection experiments. The eluent (THF) flow rate was 25 μl/min. Sample plugs of 5 μl were introduced into the eluent stream and directly transferred to the LC–IR interface without using a separation column, while holding the substrate stationary. A computer running Waters Millenium32 (version 3.2) software controlled the system.

**Interfacing liquid chromatography with IR spectroscopy**

An LC-Transform Model 500 (Lab Connections, Northborough, MA, USA) interface, based on the principle described by Gagel and Biemann [13], was used for effluent evaporation and analyte deposition. Rear-surface-aluminized germanium (Ge-Al), bare Ge (Lab Connections), and bare ZnSe (International Crystal Laboratories) were used as substrates (all 60 mm in diameter). The substrate moving speed was set to 2 mm/min, the nebulizer gas was nitrogen, and the nebulizer temperature was set to 40°C. The interface was controlled by Labcon 1.0 software (Lab Connections). After analyte deposition, the collection substrate was manually transferred to an IR accessory. For transfection measurements an accessory was used that was supplied with the LC–IR interface. It comprised of two three-times-focussing mirrors and a stepper motor for substrate rotation. A transmission accessory was constructed in-house. It was used in combination with the same stepper motor for substrate rotation. An aperture was placed in front of the latter accessory, to adjust the beam diameter to the deposit size. Both accessories were controlled by LCT 1.6.1 software (Lab Connections) and spectra were acquired by stepwise rotation of the substrate.

**Spectroscopy**

A Perkin Elmer (Norwalk, CT, USA) spectrometer model Spectrum GX, equipped with a medium-band mercury/cadmium/telluride (MCT) detector was used for IR-spectra acquisition. Each spectrum consisted of 8 accumulated scans and the optical resolution was...
set at 4 cm\(^{-1}\). Absorption-band intensities were kept below 1 AU. The sample and detector compartments were continuously purged with nitrogen gas, which was dried using a Zander Adsorption Dryer, Type KM5 TE (Essen, Germany). Data acquisition was performed using Perkin Elmer Spectrum 3.0 software. Background spectra were obtained at unused positions on the substrate. Selected IR absorption bands for detection of PS were the aromatic out-of-plane C–H rocking deformation (695 cm\(^{-1}\)), the C=C stretching vibration (1486 cm\(^{-1}\)), and the aromatic-ring C–H stretching vibration (3026 cm\(^{-1}\)). These absorption bands covered a wide wavenumber range, in order to include the wavenumber-dependent variation in the refractive index. All absorption-band ratios mentioned are calculated relative to the 1486 cm\(^{-1}\) absorption-band. Measurements of the peak areas of baseline-corrected absorption bands were performed by the IR software package. IR spectra from deposits were acquired directly after deposition.

For orientation studies, various polymers were deposited in duplicate in the FIA mode and a ZnSe polarizer (Spectra Tech, Stamford, CT, USA) was placed in the IR beam in front of the substrate. Background spectra were recorded with the corresponding polarization.

Reference transmission spectra were acquired from PS films prepared on KBr discs by applying a PS solution and drying under a heat source. Background spectra were obtained from each disc before the polymer solution was applied. IR-microscopy spectra were acquired on a Perkin Elmer spectrometer model System 2000, equipped with an Perkin Elmer Autoimage microscope and a narrow-band MCT detector. The aperture was set at 100 x 100 \(\mu\text{m}\) and the \(x,y\)-sample stage was moved at 100-\(\mu\text{m}\) steps. Each spectrum consisted of 8 scans, acquired at 8 cm\(^{-1}\) optical resolution. The system was controlled by Perkin Elmer Autoimage 4.1 software.

### 4.2.4 Results and discussion

#### Spectral integrity

In principle, the relative band intensities in an IR spectrum for a pure compound should remain constant, irrespective of the concentration. Because IR spectra are essentially independent of molecular weight, this should also be the case for a polydisperse homopolymer. A narrow PS standard was deposited in the FIA-mode on a Ge-Al substrate to verify that constant absorption-band ratios can be obtained across a broad concentration range (range 0.25 to 250 \(\mu\text{g}\)). IR spectra were acquired in transfection. As a reference, transmission spectra of PS on KBr discs were acquired. Absorption-band intensities at 3026 and 695 cm\(^{-1}\) were calculated relative to the absorption-band at 1486 cm\(^{-1}\) and were plotted against the total quantity deposited (Figure 4.9, Table 4.5).
Figure 4.9: Plot of the absorption-band ratios versus the deposited quantity (in µg) (a) before and (b) after a brief exposure to dichloromethane vapour (solvent annealing). Circles, A(3026 cm\(^{-1}\))/A(1486 cm\(^{-1}\)); triangles, A(695 cm\(^{-1}\))/A(1486 cm\(^{-1}\)). Indicated are the relative absorption band areas for a PS reference film (measured in transmission): A(3026 cm\(^{-1}\))/A(1486 cm\(^{-1}\)), dashed line; A(695 cm\(^{-1}\))/A(1486 cm\(^{-1}\)), dotted line.

At high amounts (region 25 to 250 µg) the absorption-band ratios are comparable. However, a large variation in the absorption-band ratios is found when amounts less than 10 µg are deposited, jeopardizing any attempts at quantitative analysis. Inspection of the IR spectra obtained from the PS deposits revealed that scattering effects were present, as was evident from sloping baselines or even baseline shifts. This is an indication for diffuse and specular reflection from an irregular surface, where the IR radiation is not directed towards the detector. Christiansen distortion was observed for the 695 cm\(^{-1}\) absorption-band. It was shown by Cheung et al. that solvent annealing may improve the film quality and reduces scattering by reducing the number of discrete domains and removing film irregularities [19, 20]. Therefore, the deposits were subjected to solvent annealing prior to the acquisition of transflective IR spectra.
Table 4.5: Absorption-band ratios determined from the integrated area under the absorption bands at 3026 cm\(^{-1}\), 1486 cm\(^{-1}\) and 695 cm\(^{-1}\) of (i) depositions of various amounts of PS on various substrates, before and after solvent annealing, and measured with different optical configurations and of (ii) spin coating of PS on Ge-Al. Relative absorption band areas for a PS reference film (measured in transmission): \(A(3026 \text{ cm}^{-1})/A(1486 \text{ cm}^{-1}) = 1.18; A(695 \text{ cm}^{-1})/A(1486 \text{ cm}^{-1}) = 2.23\).

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<td>Ge-Al(^c)</td>
<td>0.20</td>
<td>1.63</td>
<td>4.01</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.72</td>
<td>1.34</td>
<td>1.77</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3</td>
<td>0.81</td>
<td>1.82</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0</td>
<td>1.36</td>
<td>2.36</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>1.11</td>
<td>1.68</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

\(^a\)\(n=4\), \(^b\)\(n=2\), \(^c\)\(n=1\)
After annealing, the scattering effects disappeared and horizontal baselines close to zero absorption were obtained (Figure 4.10). Furthermore, all absorption-bands appeared much narrower, band asymmetry was reduced, Christiansen distortion disappeared and the number of well-resolved absorption bands increased. At large deposited amounts (i.e. 250 µg), the film thickness is too large for the annealing process to remove all of the film irregularities, which becomes clear from a visual inspection of the deposit. Solvent-annealing greatly enhances spectral features across the spectrum. However, annealing of the deposit resulted in greater variations between duplicate deposits and it did nothing to help overcome the observed variations in absorption-band ratios (see Figure 4.9B).

**Figure 4.10:** IR spectra of (a) 25 µg and (b) 250 µg PS deposits on Ge-Al before (dotted line) and after (solid line) a brief exposure to dichloromethane vapour (solvent annealing).
Effect of the film-thickness variations on the absorption-band ratios

An irregular surface (or varying layer thickness) of the deposit can result in anomalous absorption-band intensities (Figure 4.7). In order to get a detailed insight in the effect of a varying layer thickness on the absorption-band ratios, IR spectra from small portions across PS deposits were acquired by IR-microscopy. In addition, it was expected that solvent-annealing would result in a (more) homogeneous film and this was also studied. For this purpose, various amounts of PS were deposited on Ge-Al in the FIA-mode (10–200 ng) and transflection-microscopy spectra were acquired across the deposits from 100 × 100 μm regions before and after annealing.

Figure 4.11 shows the absolute absorption-band intensity (3026 cm⁻¹) and absorption-band ratios (3026 cm⁻¹/1486 cm⁻¹) of a cross-section for a PS deposit of 50 and 200 ng. A large increase in concentration can be observed at the outer edges of the deposit, illustrating the variation in film thickness. Although all the collected spectra were of good quality and no Christiansen distortion was present, the absorption-band ratios across the deposit varied inconsistently when the integrated absorption was smaller than 0.05 AU. However, at the two apexes of the concentration profile (i.e., maximum film thickness), the absorption-band ratios were comparable for all deposits (AU > 0.05). In the center of the deposit, where the deposited quantity was minimal, the absorption-band ratio varied, unless samples with an

![Figure 4.11: Concentration profiles across PS deposits (A, 50 ng; B, 200 ng) as characterized by IR microscopy from the integrated absorption at 3026 cm⁻¹ (solid line). The absorption-band ratios (3026 cm⁻¹/1486 cm⁻¹) are represented as dotted lines. The concentration profile obtained from the integrated absorption at 1486 cm⁻¹ shows concentration maxima and minima at similar substrate positions and is therefore not shown.](image-url)
increased film thickness, \textit{i.e.} 200 ng, were deposited, resulting in an integrated absorption larger than 0.05 (Figure 4.11B). To summarize, even when spectra are acquired from small homogeneous areas of relatively constant film thickness, similar anomalies in absorption-band ratios were observed as with spectra acquired from the entire deposit.

After solvent annealing, the characteristic concentration profile as shown in Figure 4.11 was still present. Therefore, we have assessed the effect of a varying film thickness for real deposits on the total absorption as discussed in Section 2.1. For the deposition profiles given in Figure 4.11 the observed overall absorption is plotted as function of the theoretical absorption in Figure 4.7 (broken lines). The absorption deviation follows the absorption corresponding to a fraction of bare surface interrupting the film of 0.4. This demonstrated that a varying film thickness can result in a significant underestimation of the absorption-band intensities. This effect will be enhanced when the ratio is calculated from absorption bands exhibiting different absorptivities.

**Molecular orientation**

To study molecular orientation, highly symmetrical polymers, \textit{viz.} \textit{i}-PMMA, \textit{s}-PMMA and \textit{i}-PS, which are more likely to form oriented crystals, and polymers without symmetry, \textit{viz.} \textit{a}-PS, \textit{a}-PMMA, were deposited in duplicate at 0.5 and 1.0 \(\mu\)g on a Ge and a Ge-Al substrate. Spectra were acquired in transmission and transflection at polarization angles of 0° and 90°. Spectra acquired from the symmetrical and asymmetrical polymers did not exhibit characteristic differences that could be related to orientation. It is therefore proven that molecular orientation is not implicated for PS and PMMA in the observed anomalous absorption-band ratios arising from the deposition on highly crystalline Ge-substrates measured under transmission and transflection conditions at near-normal incidence angle.

**Effect of substrate on transmission spectra**

The impact of different substrates and transmission measurements on the absorption-band ratios were determined from PS deposits on Ge and ZnSe substrates. In addition, the deposits were also exposed to a solvent-annealing procedure as discussed previously. The results are summarized in Table 4.5.

In general, the absorption-band ratios tend to decrease with increasing amount of PS deposit. After solvent annealing, a larger variation was found in the absorption-band ratios for the duplicate measurements. This will therefore not be further discussed. For \(A(695\ \text{cm}^{-1})/A(1486\ \text{cm}^{-1})\) on Ge-Al the intensity ratios increased compared to the transflection results obtained on Ge, but are comparable to the transflection intensities found for deposits on ZnSe. When the same absorption bands were measured in the transmission mode, the
relative intensity was approximately doubled due to an increased absorption at 695 cm$^{-1}$. This effect is demonstrated in Figure 4.12, where baseline corrected IR spectra are shown that were obtained from a 50-μg PS deposit (to facilitate the comparison, the absorption of the 1486 cm$^{-1}$ absorption bands was normalized to 1.0 AU). It can be seen that intensities relative to 1486 cm$^{-1}$ decreased for many absorption-bands when measurements were carried out in transmission. The absorption-bands affected can be ascribed to the following vibrations: aromatic ring and aliphatic C–H stretch (3100-2800 cm$^{-1}$), the C–C aromatic ring stretch (1601 cm$^{-1}$), the aliphatic C–H deformation (1454 cm$^{-1}$) and one of the aromatic out-of-plane C–H rocking deformations (695 cm$^{-1}$). On the other hand, an increased absorption-band ratio is observed for the other aromatic out-of-plane C–H deformation (750 cm$^{-1}$). Obviously, the changes in absorption-band ratios are affected by molecular vibrations arising from similar functional groups in a different way.

Comparing the absolute absorption-band intensities can reveal information on the reflected and transmitted amounts of IR radiation. The transflection intensity for the absorption-band at 1486 cm$^{-1}$ from the untreated IR spectra acquired from Ge-Al is three times higher than that of a similar deposit on Ge measured in transmission, while the double-pass-transmission mechanism is expected to result in a two-fold intensity increase. The

![Figure 4.12: IR spectra acquired from 50-μg PS deposits on various substrates with (a) transflection and (b) transmission spectroscopy. All spectra were baseline corrected and normalized to 1.0 AU for the absorption-band at 1486 cm$^{-1}$. Substrate types are indicated in the Figure.](image-url)
absorption (1486 cm\(^{-1}\)) for transfection measurements on a Ge substrate was intermediate between the absorption values found on Ge-Al and transmission on Ge, suggesting a high reflectivity at the deposit-Ge interface. When transmission measurements were carried out, the absolute absorption-band intensities were remarkably smaller. The absorption intensity (1486 cm\(^{-1}\)) decreased in going from Ge to ZnSe to KBr, possibly due to differences in substrate surface-roughness.

**The effect of thin films on the absorption**

For the calibration curve shown in Figure 4.6, a different slope was obtained when the calibration was performed with a total quantity of 25 \(\mu\)g for all PS-PMMMA blends (linear least-squares fit: \(Y = 0.0258 X + 0.006\), as opposed to \(Y = 0.0653 X - 0.009\)). Apparently, the film thickness causes anomalous absorption-band ratios. In order to investigate whether the film thickness could lead to erroneous absorption-band ratios in the absence of bare regions, deposits with a constant film thickness were prepared by means of spin-coating on a Ge-Al substrate. The film thickness and the relative absorption-band ratios are given in Table 4.5. A large deviation in the absorption-band ratios was observed for all film thicknesses, despite the fact that these have been determined from a homogeneous deposit. Probably, a film thickness in the order of the mid-IR wavelengths affects the absorption-band intensities. A weak absorption is expected when the film thickness (\(d_f\)) is smaller than \(\lambda/4\) [24]. This affects the absorption-bands at high wavenumbers differently than the absorption bands at low wavenumbers due to a varying absorptivity of the \(s\)- and \(p\)-components. For example, at \(v = 4000\) cm\(^{-1}\) weak absorption is expected at \(d_f < 0.625\) \(\mu\)m, while at \(v = 500\) cm\(^{-1}\) the absorption will be affected at \(d_f < 5\) \(\mu\)m. When \(d_f\) exceeds \(\lambda/4\), the absorptivity of different bands will not increase equally and will result in inconsistent absorption-band intensities, as both polarizations will be differently absorbed. When \(\lambda/d_f\) is much larger than 0.5 both polarizations are absorbed equally and consistent absorption-band intensities can be obtained. However, for all absorption bands in the IR-spectrum this condition can only be fulfilled when \(d_f\) exceeds 10 \(\mu\)m (i.e., an absorption band at 500 cm\(^{-1}\)). In practice, this equals a deposited quantity of 37 \(\mu\)g (deposit diameter, 2 mm; \(\rho_{PS} = 1190\) kg/m\(^3\)).

For PS deposits on Ge it was found that all absolute absorption-band intensities were smaller for transfection than the corresponding intensities obtained by transmission spectroscopy for low sample quantities (< 10 \(\mu\)g, \(d_f=1.3\) \(\mu\)m) and larger for quantities higher than 10 \(\mu\)g (see Table 4.5). This turning point is a lower quantity than expected for the current LC-FTIR interface. The largest variation in absorption-band ratios was observed at low amounts deposited (i.e. < 5-10 \(\mu\)g). For low quantities, the film thickness
and the observed anomalous absorption-band intensities are of the same order as modeled by Pacansky et al. [30].

Previously, we have shown linear calibration curves (absolute absorption-band intensities) [32] for a copolymer and a blend where the injected amount varied from 10 to 200 µg. In that case, correct absorption-bands intensities were obtained as the film thickness exceeded the critical value and all IR-radiation was absorbed without discrimination of polarized components.

**Concluding remarks**

After assessing the various attempts to obtain consistent absorption-band ratios, we conclude that two effects affect the absorption-band ratios. These are the underestimation of the absorption as a result of changes in concentrations across the deposit (i.e. inhomogeneous deposits) and the small film thickness, causing anomalous absorption for the different polarizations. However, because of this combined effect, it is very well possible that other sources of error are present and the origin of the anomalies is still not fully understood. Previous research [24, 26] has focused on other aspects influencing the absorption-band ratios. However, in the present study alternative explanations, such as molecular orientation and the refractive index of the substrate, were excluded as significant factors. It is likely that for chromatographic peaks, where spectra are acquired from quantities of 1 to 25 µg, anomalous absorption-band intensities impede quantification.

### 4.2.5 Conclusions

With the present (commercial) solvent-elimination LC–IR interface, the deposited amounts (of polymer) greatly influence absorption-band ratios in the spectra. Two main potential effects have been identified. Firstly, an inhomogeneous deposit (i.e. a varying film thickness) can result in an underestimation of the absorption, depending on the absolute intensity of an absorption band. This was modeled using IR-microscopy spectra obtained across a real deposit. A solvent-annealing procedure could not fully homogenize the deposit. Secondly, when the film thickness is in the order of, or smaller than, the mid-IR wavelengths, the $s$- and $p$-polarized components are absorbed differently and interference effects can occur. A relatively thick film, i.e. larger than 10 µm, is therefore preferred. Of minor importance is the occurrence of specular reflection. This contribution depends on the films thickness and on the wavelength. It is most likely to occur for thin films (i.e. less than 10 µm) arising from the typical injected quantities encountered in LC–FTIR. It was also revealed that the Christiansen effect, molecular orientation, the substrate refractive-index, and the optical configuration did not significantly influence the absorption-band ratios. On
the other hand, scattering effects and shifting baselines occurred when the deposited amount exceeded 10 μg for PS, further complicating quantification. With the use of micro-LC the quantification problems can be (partly) overcome and quantification will be less complicated. At the typical low flow rates used, a narrow deposit is anticipated and, although injected quantities are smaller, an increased film thickness can be obtained.

References

Appendix

Reflection and transmission according to Fresnel

The reflection and transmission of electromagnetic waves is described by the Fresnel equations. These equations give the ratio of the reflected intensity to the incident intensity for the parallel polarized (p-component) and perpendicularly polarized (s-component, ‘senkrecht’) vector to the plane of incidence.

Assume an electromagnetic wave at an incidence angle of 18.5° (θ) passing the air-polymer film, where \( n_{air}=1.00 \) and \( n_{polymer}=1.57 \). The refracted angle (θr) can be calculated according to Snell’s law:

\[
\sin \theta_r = \frac{n_{air} \sin \theta_i}{n_{polymer}} = \frac{1.00 \times \sin 18.5}{1.57}
\]

\[
\theta_r = 11.66°
\]

The reflection coefficients can now be calculated for the p- and s-components:

\[
r_{||} = \frac{\tan(\theta_i - \theta_r)}{\tan(\theta_i + \theta_r)} = 0.206 \quad \text{(A.2)}
\]

\[
r_\perp = -\frac{\sin(\theta_i - \theta_r)}{\sin(\theta_i + \theta_r)} = -0.237 \quad \text{(A.3)}
\]

and the reflectivity, representing the reflected power, is given by:

\[
R = \frac{r_{||}^2 + r_\perp^2}{2} = 0.049 \quad \text{(A.4)}
\]
Assessment of quantitative aspects...

Due to the preservation of energy it follows that the transmittance will be:

\[ T = 1 - R = 0.951 \]  

(A.5)

A special situation occurs when the electric field parallel to the plane of incidence \( (E_{||}) \) equals zero \( (\tau_{||}=0) \) and occurs when \( \theta_{i} + \theta_{r} = 90^\circ \). The use of Snell’s law gives and expression for this so-called Brewster angle (or polarizing angle).

\[ n_{\text{air}} \sin \theta_{i} = n_{\text{polymer}} \sin(90 - \theta_{i}) \]  

(A.6)

\[ \tan \theta_{i} = \frac{n_{\text{polymer}}}{n_{\text{air}}} = \frac{1.57}{1.00} \]  

(A.7)

\[ \theta_{i} = 57.5^\circ \]

At the Brewster angle, the reflected light is linearly polarized with its electrical field perpendicular to the plane of incidence \( (E_{\perp}) \).

The Christiansen effect [I, II, III]

Strong absorption bands from coarsely ground samples can be distorted due to the change in refractive index across the absorption band. This is often called the Christiansen effect. For IR-transparent materials, such as KBr, the refractive index decreases slowly with increasing wavelength. However, for a sample with vibrational frequencies in this region, large changes in the refractive index of the sample are observed in the vicinity of the absorption band (anomalous dispersion). When the absorption band is approached from the low wavenumber side, the refractive index increases until it reaches a maximum. Since the amount of scattered radiation is proportional to the square of the difference in refractive indices between the sample and the surrounding medium, the sample becomes highly scattering and tends to make the sample opaque on the low wavenumber side of the absorption band (see equation (1)).

\[ R = \left( \frac{n_{\text{medium}} - n_{\text{sample}}}{n_{\text{medium}} + n_{\text{sample}}} \right)^2 \]  

(A.8)

Passing through the absorption band, the refractive index suddenly falls so that losses by scattering are reduced. The transmission increases markedly at wavenumbers at which the refractive indexes of the particles match that of the surrounding medium. This transmission
is reduced again as the wavelength moves out of the range in which the absorption band has a large affect on the refractive index. The resulting asymmetrical absorption band may resemble a derivative curve rather than an absorption spectrum. Reducing the sample particle size below the wavelength of incident radiation minimizes the effect.

![Graphs showing the ChristianSEN effect](image)

**Figure 4.A:** Artificial example of the Christiansen effect. (A) Variation of the refractive index across the absorption band, (B) the unaffected absorption band and (C) the resulting absorption spectrum. (All in arbitrary units (AU)).

**References**