Interactive liquid chromatography for the characterisation of polymers

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SUMMARY

The primary goal of this research was to improve our understanding of interactive liquid chromatography for the characterisation of polydisperse macromolecules. This goal was achieved and some significant progress was made in highlighting the practical advantages of the technique for the characterisation of polymers.

In Chapter 1, a brief outline on the need for polymer characterisations is presented and the state of the art for chromatographic and hyphenated chromatographic analyses of polymers is discussed. The basis of LC models, for the prediction of the retention behaviour of analyte molecules, is also introduced.

In Chapter 2, a previously described retention model for reversed-phase chromatography is applied to the gradient LC separation of polystyrene. It is shown that the model can be expanded to cover a polydisperse sample using correlations between the model parameters and between the model parameters and molar mass. The expanded model is used to predict retention behaviour as a function of molar mass. The model is incorporated into a spreadsheet application so that once a model is validated for a given separation, it can be used to predict the retention time of a polymer of any molar mass and polydispersity (within the defined limits of the model). The spreadsheet automatically constructs chromatograms and calibration curves (retention time versus molar mass) to help show the chromatographer how changes in the chromatographic conditions influence the resulting separation. Most significantly for polymeric samples, the spreadsheet can also predict how the peak shapes of polydisperse samples vary with the experimental conditions (i.e. mobile phase composition/gradient), the average molar mass of the sample and the molar-mass distribution of the sample. This allows a specific chromatographic separation to be optimised on a computer rather than in the laboratory.

The correlation between the model parameters is also used to highlight the existence of a so-called critical composition (i.e. a mobile-phase composition where retention is independent of molar mass). The critical composition can be predicted using the correlations and it is proven experimentally that the prediction was accurate for a specific polymer. It is also shown that for high-molar-mass copolymers, the retention behaviour can be predicted as a function of chemical composition. Crucially, the prediction is only based on the retention behaviour of its component homopolymers.
In Chapter 3, the use of retention models for the prediction and optimisation of separations of polydisperse samples is considered in some more detail. Using the same model as in chapter 2, a more thorough investigation of the relationship between the model parameters and molar mass is undertaken. It is found that a straight-line correlation does not provide the best relationship between the model parameters and molar mass. A more accurate relationship is established by examining narrower segments of the molar-mass range and finding the best (straight) line in each specific range. The overall relationship is then determined by plotting all the lines in one figure. Using the improved relationship between molar mass and the model parameters, excellent predictions for the retention of a wide range of molar masses (for the specific polymer) can be made. Within the spreadsheet, calibration curves showing the separation according to molar mass are constructed, allowing easy optimisation of separations. The predicted calibration curves (and chromatograms) clearly indicate that (under suitable conditions) interactive LC can exhibit extremely high selectivity in a targeted molar-mass range. The curves also show that the chromatographic peaks of polydisperse samples should not necessarily be symmetrical and that asymmetry (such as tailing, fronting or even split peaks) should be expected and can be understood when the selectivity changes across the molar-mass range of its distribution. The selectivity predicted using the model is verified for a particular separation by taking narrow fractions across the eluting peaks and measuring the MALDI-MS spectrum of these fractions. In some cases, essentially monodisperse fractions are collected. The experimental calibration curve is described mathematically using a series of third-order polynomial curves, which are then used to calculate the molar-mass distribution of a number of polystyrene standards. Interactive LC is found to give lower polydispersities than size-exclusion chromatography, for the determination of molar-mass distributions of low-to-medium-molar-mass, narrowly distributed samples, although MALDI-MS analysis suggest that the actual polydispersity may be even lower.

In Chapter 4, a second approach to establishing the validity of retention models for polydisperse samples is introduced. This approach uses non-linear regression and is compared to methods used in previous chapters. Similar results were found for both approaches. A rigorous validation of the model (as a function of molar mass, gradient slope and retention time) is presented and confidence intervals for the model parameters are calculated and found to broaden significantly with molar mass. The correlation between the model parameters and molar mass is again considered and a further improvement in the description of the relationship is made. A bilinear relationship between molar mass and \( \ln k_0 \) is found to be the most accurate. This relationship is used to predict the retention times of a broad range of polymers, measured under various experimental conditions. An
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expanded (or global) model is developed to describe the relationship between experimental conditions, molar mass and retention times. An important consequence of the global model is that a drastic reduction in the number of experiments required to characterise a polymer can be made without compromising the predictive accuracy of the model.

Chapter 5 is an investigation of an interactive-LC separation coupled to a mass spectrometer for the characterisation of a functional copolymer. The samples are multidimensional, with three primary distributions, i.e. a molar-mass distribution, a functionality-type distribution and a chemical-composition distribution. All three distributions affect the (required) properties of the polymer. For a comprehensive characterisation of such a complex polymer, a multidimensional separation system is required. Interactive-LC coupled to MS allows the polymer to be separated according to two of its distributions, i.e. functionality (by the chromatographic separation) and molar mass (by the mass spectrometer). Due to the low-polarity of the polymer and the LC mobile phase, ionisation of the polymer via an electrospray ionisation source was difficult, but could be achieved using a post-column additive. Data analysis of the resulting two-dimensional separation was a highly challenging prospect, due to the large amount of information obtained from the separation and to the complexity of the polymer sample. In order to obtain as much relevant information, in as convenient a manner as possible, an automated data-analysis system is developed. Graphical representations of the analyses that give a clear overview of the two-dimensional separation are constructed from the data. The probability of a given end-group combination is also indicated graphically through an automated ‘mass-matching’ program. These techniques allow for an expedient and thorough analysis of an otherwise data-overloaded technique.

In Chapter 6, the potential of LC coupled to a nuclear magnetic resonance spectrometer (NMR) is explored for various copolymeric samples. Block and (bimodal) randomly distributed copolymers of polystyrene and polymethyl methacrylate are separated chromatographically according to their chemical composition. The separation is coupled on-line to a nuclear-magnetic-resonance spectrometer, to quantify the ratio of styrene to methyl methacrylate in the copolymer and to establish whether randomly distributed copolymers can be distinguished from a block copolymer with the same chemical composition. The techniques are coupled in the continuous-flow mode. This is a challenging approach to LC-NMR, because the low sensitivity of NMR means that very high concentrations of sample are required for detection. The results were promising, although some further optimisation of the experimental set-up (in particular increased sample loading and residence time in the NMR cell) would be very beneficial. A clear distinction between block and randomly distributed copolymers can be made because the more homogeneous environment around
the monomers of a block copolymer results in sharper NMR peaks. Quantitation of the chemical composition using NMR proved difficult because the signal-to-noise ratio was not high enough under the experimental conditions chosen. This is particularly true for the randomly distributed copolymers which have low, broad signals for the methyl ester peaks of the polymethyl methacrylate.