Summary

Ultra-high pressure liquid chromatography (UHPLC) is a contemporary type of liquid chromatography (LC) that uses instrumentation capable of generating pressures above the conventional 40 MPa (400 bar) limit of high-performance liquid chromatography (HPLC). In addition to enhanced pressure capabilities, UHPLC systems feature reduced extra-column volumes. They are used in combination with very fast detectors equipped with low-volume cells. These characteristics make it possible to employ columns packed with sub-2-μm particles at optimum linear velocities without significant loss of efficiency caused by undesired band-broadening effects. From chromatographic theory it is known that small particles at high pressures can offer improvements in analysis speed and/or efficiency compared to larger particles used at typical HPLC conditions. These improvements are fundamentally feasible for both low-molecular-weight compounds and polymers. Whereas the number of UHPLC applications for analysis of complex low-molecular-weight samples has grown dramatically in the recent years, almost no UHPLC polymer separations have been reported in literature. The goal of the present thesis is to study possibilities and challenges associated with analysis of polymers using UHPLC. We aim to demonstrate the usefulness of this technique for separations of synthetic polymers based on molecular weight and chemical composition.

In Chapter One the history of LC and the place of UHPLC in contemporary chromatographic analysis are briefly discussed. This chapter outlines the quest for smaller particles and higher pressures which arose as soon as the fundamentals of liquid chromatography were established.

Chapter Two describes challenges that a chromatographer can face when analyzing polymer samples. Many synthetic polymers are not soluble in conventional chromatographic solvents. Several approaches to LC analysis of poorly soluble polymers are described (sandwich injection, using high temperatures, derivatization and chemical degradation). Different LC separation mechanisms (size-exclusion chromatography, hydrodynamic chromatography, reversed-phase or normal-phase gradient-elution LC, isocratic separation at critical conditions, etc.) that can be applied for polymer analysis are also discussed in this chapter. The separation principles of these techniques are described, as well as practical challenges associated with each of them. Different
combinations of two separation mechanisms in a two-dimensional (2D) set-up for polymer separations are discussed. Aspects of the optimization of polymer separations in terms of speed and resolution are addressed. The role of the contemporary trends in LC – viz. core-shell stationary phases, monolithic columns and UHPLC – in improving the trade-off between speed and efficiency is discussed. Finally, the most common detection techniques applied for polymers are described and their advantages and disadvantages are summarized.

Chapter three addresses difficulties associated with performing (size-based) polymer separations using UHPLC. A number of problems are identified, including a lack of designated SEC columns, significant extra-column contributions to the peak width, deformation and degradation of polymers at UHPLC conditions, and limited resistance of UPLC equipment to some chromatographic solvents. Using wide-bore (4.6 mm I.D.) UPLC columns, the extra-column contribution to the peak width can be sufficiently minimized and fast, yet efficient size-based separations of polystyrene (PS) polymers can be obtained. PS molecules with molecular weights up to ca. 50 kDa penetrate the pores of the UPLC packing material and elute in the SEC mode. Higher-molecular-weight PS macromolecules are too large to enter the pores and they elute via the HDC mechanism. The influence of flow rate, column length and temperature on the separations has been investigated.

Chapter Four studies in detail the fundamental problem of deformation (transition from coil to stretch shape) and degradation of polymers at the high-stress conditions generated in UHPLC. It is confirmed that the simultaneous action of slalom and hydrodynamic chromatography can cause the appearance of two chromatographic peaks for a polymer with a uniform molecular-weight distribution. The nature of the two peaks is investigated by collection and re-injection of the polymer fractions as well as by off-line two dimensional UHPLC. The conditions corresponding to the onset of PS deformation and degradation in UHPC are established. No degradation of PS with molecular weights up to 3 MDa has been found at common UHPLC conditions. For larger PS macromolecules degradation has been observed. Column frits have been identified as the main source of PS degradation.

Chapter Five describes an application of UHPLC for the separation of polyurethane (PU) samples used in the coating industry. Gradient-elution UHPLC separations are
compared with HPLC separations for this type of polymers. HPLC coupled with electrospray ionization mass spectrometry has been used to identify the low-molecular-weight products present in the samples. These products are formed from the excess diisocyanate present during PU synthesis. LC fractions containing the polymeric part of the sample have been studied with matrix-assisted laser desorption/ionization mass spectrometry. The influence of synthesis parameters (the order in which monomers are added to the reactor and the ratio between isocyanate and polyol) on the obtained products is investigated. Also UHPLC separation of PU polymers containing carboxylic groups is studied at different pH-values.

The possibility to perform comprehensive two-dimensional UHPLC analysis of polymers is demonstrated in Chapter Six. Gradient-elution UHPLC in the first dimension provides efficient separations of polymers based on their chemical composition. Ultra-high pressure SEC in the second dimension offers very fast size-based separations of macromolecules. The resulting two-dimensional UHPLC×UHPLC plots can be obtained in (less than) 1 hour, which is significantly faster than conventional HPLC×SEC analysis. The 2D analysis gives information on chemical-composition and molecular-weight distributions in a polymer sample and on their mutual dependence. The usefulness of this UHPLC×UHPLC approach is demonstrated for the analysis of a mixture of poly(methyl methacrylate) and poly (n-butyl methacrylate) homo- and copolymers. Two-dimensional UHPLC is also applied for the analysis of industrial PU prepolymers. When the first-dimension separation is performed at alkaline conditions, the 2D plot allows estimating a ratio between PU macromolecules that contain carboxylic groups and PU macromolecules that do not contain such groups. This information cannot be provided by one-dimensional separations, neither by gradient-elution UHPLC, nor by SEC.