Capillary electrophoresis for the characterization of synthetic polymers

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Chapter 1

General introduction

1.1 Synthetic polymers

Polymers are large molecules (macromolecules) formed from many repeated monomer units. A more extensive description of a polymer is given below [1]. The variation in the length of the polymer chains, type and distribution of the monomer units and the structure of chains result in numerous materials with various physical and chemical properties.

The degree of polymerisation of a macromolecule represents the total number of repeat monomers in a polymer chain [2 - 4]. Polymerisation reactions hardly ever yield individual chains of all the same chain length; usually there is spread around a mean chain length and molar mass (MM) value. Consequently, one of the main properties of a polymeric compound is its molar-mass distribution (MMD) that can be characterized by an average MM and polydispersity. Simple macromolecules that are composed with one type of monomer (homopolymers) differ in MMD.

Macromolecules derived from more than one type of monomer units, copolymers, have an MMD as well as a chemical-composition distribution (CCD). Synthesis processes can influence the order of the repeat pattern in the polymer chains. Patterns found in copolymers are alternating, random, block and grafted.

When homo- and copolymers differ in type or number of functional groups, either as end-groups or distributed along the polymer backbone, a functionality-type distribution (FTD) will also be present. Schematic representations of the distributions that can be present in synthetic polymers are shown in Figure 1.1.
Figure 1.1 Polymers can have many distributions.

The simplest architecture possible for a macromolecule is a linear chain with two specific end-groups. There are a number of different reaction mechanisms, such as addition- and condensation polymerisation, that yield linear polymers. Addition polymerisation is based on a step-wise growth of the polymer chains via free radicals or ionic groups. Poly(styrene) and poly(ethylene oxide) are types of polymers produced by this type of synthesis. Condensation-type polymers are formed by reactions between functional groups of monomer units. Examples of polymers belonging to this category are linear polyesters and polyamides.

When some molecules utilized in the polymerisation have more than two reactive groups, polymers with a branched structure can be produced. Branching can lead to very complicated three dimensional polymer architectures, and above a certain level of branching a cross-linked polymer network can be formed.
1.2 Characterization of synthetic polymers

As already mentioned, the distribution and average values of the polymer chain length have a significant influence on the physical and chemical properties of technical products. Therefore, analytical methods for characterizing polymer distributions are essential for industrial process monitoring and quality control. In most cases separation of the compounds is necessary in these methods. Depending on the separation mechanism of a method it provides information on a specific polymer distribution. In general, analytical systems are best suited for macromolecules with sizes in specific ranges. In Figure 1.2 separation methods commonly used for the characterization of (synthetic) macromolecules according to size, type or number of end-groups, composition and charge density are compared.

Size-exclusion chromatography (SEC), often in combination with viscosity, light scattering and/or refractive index detection, is the most commonly applied technique for characterizing the size of synthetic polymers [5, 6]. In SEC a column packed with porous particles and a ‘strong’ solvent as eluent are used for separating compounds based on their hydrodynamic molecular volume. Large polymers are excluded from the pores of the particles to a greater extent and hence elute earlier from the column than smaller compounds. After separation by SEC, the data obtained on the
polymeric compounds are commonly translated into a MMD using mass-calibration curves obtained with standard polymeric material of known average molar mass and low polydispersity.

Field-flow fractionation [7, 8] and hydrodynamic chromatography [9] are separation techniques particularly useful for the characterization of ultra-high molar mass polymers and solid particles. Both techniques may yield the average molar masses and size distributions of polymer samples. Detection is often carried out on-line with a multi-angle light scattering (MALS) detector.

While mass spectrometry (MS) is mainly used for the analysis low-MM compounds, it can also be applied in biopolymer analysis and, to a more limited extent, for synthetic polymers [10]. In MS, analytes are first ionised, then separated based on their mass-to-charge ratio and finally detected. When a polymer molecule is easily ionisable, MS analysis can provide accurate information on its chemical structure, specifically on its molar mass, and implicitly on its chemical composition and functionality. However, even with a simple homopolymer sample very complicated MS spectra can be obtained, because of the presence of isotopes, salt adducts and fragments of the main polymer chain formed in the MS source. With the development of soft ionisation sources, such as electrospray and matrix-assisted laser-desorption ionisation (MALDI) in combination with time-of-flight (TOF) MS the suitability of MS for synthetic polymers has improved.

For the determination of the chemical composition and functionality of polymers interactive liquid chromatography (iLC) can be used. The separations in iLC are based on the partitioning of the polymer chains between the stationary and mobile phases in the column. Gradient iLC can provide information on the CCD of copolymers. Unfortunately, the development of a gradient method can be time-consuming, because several parameters such as stationary phase, mobile phase composition, gradient profile and temperature need to be optimised. With theoretical LC models the retention behaviour of (co)polymers in a gradient system can be predicted [11]. Depending on the composition of the eluent, retention of polymers can be independent of the molar mass and a separation according to the type and number of functional groups can be realised. This specific LC mode is called critical chromatography.

In the past decade capillary electrophoresis (CE) has emerged as a fast and efficient technique for separating synthetic polymers [12, 13]. The basic principle of CE is the migration of ionic particles through a capillary in a background-electrolyte (BGE) solution under the influence of an electric
field. In the simplest mode, which is termed capillary zone electrophoresis (CZE), the sample ions migrate with velocities according to their charge-to-size ratio. This mode can easily be used for, e.g., the determination of the MMD of polymers with a fixed charge or to obtain information on the dispersity of the charge density of polyelectrolytes.

Micellar electrokinetic chromatography (MEKC) is often applied for the separation of neutral compounds. Similar systems, based on the interaction between surfactant ions or aggregates in the BGE and the analytes have also been used for the separation of polymers with a neutral backbone. Because the principles and instrumental aspects of CE are well documented by several authors [see e.g. ref. 14-17] this introduction is focused on the application of CE and related electrokinetic techniques to synthetic polymers.

1.3 Capillary electrophoresis of synthetic polymers: a literature overview

**Polyelectrolytes: determination of size**

Polyelectrolytes are macromolecules with ionisable groups that can dissociate in a suitable solvent into charged polymer chains (polyions) and small counterions. Linear poly(styrenesulfonates) (PSSs) belong to these polymeric compounds. Small oligomers with a chain length < 8 can be separated by free solution CZE [18, 19], while PSSs with longer chain lengths all migrate with the same mobility in free solution. Analysis of the latter type of PSS can be performed using a CE buffer containing an entangled-polymer sieving medium. Hydroxyethyl cellulose (HEC) was found to be a better polymer additive than poly(acrylamide) with regard to the resolution and migration time of the PSSs [20].

The presence of HEC additives does not significantly modify the electro-osmotic flow (EOF), which indicates that the polymeric additives do not interact with the capillary inner surface and that viscosity effects are negligible. However, the authors mentioned that a suppressed EOF resulted in better separations. To achieve this, capillaries with chemically coated inner walls were applied. With a reversed polarity of the voltage the polymers migrated towards the detector end of the capillary with the smaller polyelectrolytes eluting first, due to the effect of the sieving medium. The influence of the HEC concentration in the BGE on the separation of PSSs with different molar masses is shown in Figure 1.3. Under optimised conditions separations according to size of PSSs with an average molar mass up to 1200 kDa were achieved within 10 min.
Chapter 1

Suppression of the EOF in bare fused-silica capillaries can also be obtained by using a low pH BGE. Since PSSs are dissociated even at low pH a phosphate buffer at pH 2.5 with dextran could be used as the polymer additive [21]. Dextran was chosen because its viscosity in solution is lower than that of HEC. With this method separations of PSS standards with molar masses in the range of 1.6 – 354 kDa were achieved.

Generally, in CE a better resolution can be obtained when ionic analytes migrate against the EOF. At pH 8 the velocity of the EOF, and with that the velocity of the sieving agent, is higher than the electrophoretic mobility of PSS polyelectrolytes. With a positive electric field, the PSS anions with smaller chain lengths elute first after the migration time of the polymeric network (EOF time) [22 - 24]. A practical advantage of this mode is that variations in operation conditions that affect the velocity of the EOF can be monitored from the electropherograms obtained.

Several research groups studied the sieving behaviour of PSSs in HEC [20, 22] and PEO solutions [23, 24]. The concentrations of polymer additives in the BGE solutions applied were above the entanglement threshold, to create polymer networks in the solution. Important characteristics of such networks are the blob size (mesh size) and the reptation time of the mesh obstacles. Viscosity measurements allow the determination of these characteristics. PSS separations carried out with well-characterized entangled polymer solutions demonstrated that the optimum operation conditions
with respect to selectivity and analysis time are obtained when the reptation time of the mesh obstacles is close to the time it takes the solute to migrate over the distance of a blob size [24]. Therefore, sieving networks from a specific (technical) polymer are most suitable for the separation of a specific, and a relatively narrow molar-mass range of polyelectrolytes. It has been demonstrated that the molar-mass range could be extended by using bimodal mixtures of polymers as additives [25].

The blob size of the polymer network has a significant influence on the sieving mechanism of polyelectrolytes. With mesh sizes much larger than the radius of gyration of the polyions, the polymeric chains take on a rod-like confirmation. The Ogston model describes this sieving mechanism theoretically. Migration according to this model was observed for PSS with a molar mass < 88 kDa in HEC solutions with mesh sizes > 90 nm [24]. When the mesh size is smaller than the size of the polyelectrolytes, the polyions move through the network by a reptile-like (head first) motion. This regime is called pure reptation. The electrophoretic mobilities of the polyelectrolytes are then inversely proportional to their molar mass. In this regime the highest mass selectivity can be obtained. However, when a strong electric field is applied the polymers become more stretched in the direction of the field and loss of selectivity occurs. This phenomenon is called reptation with orientation or biased reptation. It was demonstrated experimentally that the sieving mechanism of PPSs in entangled polymer solutions was based on a combination of the Ogston and the biased reptation regimes [23]. The pure reptation regime has not been observed in any study. A possible explanation might be that the PPS chains are stiffer than the DNA backbone.

An interesting approach to the study of the separation mechanism of PSSs was by pulsed-field CE using an entangled HEC matrix [26]. The confirmation of the polyelectrolytes was dependent on the frequency of the input signal. The most efficient separations were obtained when the polymers migrated in a coiled confirmation. The mobility of the polymers was inversely proportional to their degree of polymerisation. Although it was demonstrated that the pulsed-field technique could be useful for the characterization of large polyelectrolytes, no other applications on synthetic polymers have been reported.

In principle, other linear flexible polyelectrolytes behave similar in entangled polymer solutions as PSSs. Therefore, charged (water soluble) polymeric compounds with a constant charge density can be separated according to size using entangled-polymer solutions. Other types of polyelectrolytes with anionic functional groups, for which CE methods have been developed, are poly(acrylic acids) [27, 28] and poly(phosphoric acids) [29].
CE is a useful alternative for SEC especially for the size-analysis of cationic polyelectrolytes, since such compounds often interact strongly with the stationary phase in SEC systems. In CE interactions between the polycations and negatively charged silanol groups at the capillary wall must also be avoided. With coated capillaries and dextran solutions at pH 2.5 it was possible to characterize poly(2-vinylpyridine) (P2VP) and poly(4-vinylpyridine) (P4VP) [30]. No significant adsorption of the polymers was observed. Although the chemical architecture of the two types of PVPs is very similar, significantly different sieving mechanisms of the polycations were found. Therefore, it was not possible to use a single mass-calibration curve to characterize both types of polymers.

CE with polymer matrices not only offers information on the size of polyelectrolytes, it can also be used to monitor modification processes of polymers, as has been demonstrated for the quaternization of P2VPs with dimethylsulfate [31]. After a reaction time of 24h higher electrophoretic mobilities and broadened peaks were found for the (derivatized) polymer samples.

**Polyelectrolytes: determination of the charge density**

As was shown above, polyelectrolytes above a certain degree of polymerisation with constant charge-to-size ratio have a constant electrophoretic mobility in free-solution CZE. This opens up the possibility to determine the charge density of polyelectrolytes by CZE. Gao et al. [32] developed a CZE system to determine the chemical composition distribution (CCD) of random copolymers consisting of the charged monomer 2-acrylamido-2-methylpropanesulfonate (AMPS) and the neutral monomer acrylamide (AAm). Figure 1.4A shows the relationship between mobilities and the charge density of the copolymers. It was found that the mobility of polymeric compounds with a low AMPS content (< 35%) increased linearly with increasing charge density. For polyelectrolytes with higher charge densities, a significant effect of counter ion condensation on the electrophoretic mobility was observed. As a result, the mobility of the polymers increased only slightly with increasing charge density. A similar behaviour was found for the electrophoretic mobilities of acrylicacid/AAm copolymers, as was reported by other authors [28]. Both research groups mentioned that the results obtained experimentally were close to the curve theoretically predicted by Manning's theory on counter-ion condensation.

Figure 1.4B shows the separation of AMPS-AAm copolymers with different percentages of (charged) AMPS in the backbone. Since the charge densities of the compounds were low, the different polymer products could be separated. The widths of the peaks will be a result of the
chemical composition dispersity of the compounds. Determination of the polydispersity can be performed using the (linear part of the) plot as a calibration curve.

We have studied the possibility to determine the charge density of carboxymethylcellulose (CMC) by CE (see Chapter 4). CMC is a water-soluble cellulose derivative with multiple carboxylic acid groups along the cellulose backbone. The degree of substitution (DS) represents the average number of substituted carboxylic groups per glucose unit. Stefansson [33] already demonstrated a separation of (derivatized) CMCs with different DS values by CZE with laser induced fluorescence (LIF) detection. We have developed a CZE system with UV detection for the analysis of CMCs. [34]. Separations were performed using borax buffer in fused-silica capillaries of 75 μm ID and for detection UV absorption at 196 nm was measured. For low-DS samples an increase of the mobility with DS was found, while for CMCs with high DS values the effect of counter-ion condensation on the mobility was observed. Results of experiments with indirect UV detection with two monitoring ions showed that the peak widths reflect the variation in mobility and therefore the DS of CMCs. With a DS-calibration curve electropherograms of a technical samples could be translated into DS-distributions, and the DS-polydispersity could be established.
Chapter 1

Polymers with charged end-groups

Synthetic polymers with a fixed number of charged end-groups can be characterized by free solution CZE. In CZE the electrophoretic mobility of these polymers is directly related to the size of the compounds. Several approaches have been used to apply CZE for the analysis of poly(ethylene oxide-co-propylene oxide) copolymers with amine end-groups, which are termed Jeffamine polymers. In a first study the polymers were partly derivatized with a fluorescence reagent to provide detectability [35]. Non-derivatized compounds were not detected; doubly derivatized polymers were neutral and were hence detected as a single peak at the migration time of the EOF. Singly labelled derivatives were positively charged at pH < 10, and hence migrated in order of their charge-to-size ratios (Figure 1.5). Best resolution for Jeffamines up to an average molar mass of 1200 Da was found using a BGE solution of pH 4 with 20% methanol. The main reason for this was the low EOF velocity under these conditions.

Since fluorescence detection is not available in most commercial CE systems, and the method relies on an incomplete derivatisation, an alternative method with indirect UV detection was developed for the characterization of Jeffamine samples [36]. To improve the separation of the individual oligomers a reduced EOF, obtained by dynamic coating of the capillary wall, was applied. Under optimized conditions, baseline separation of Jeffamine oligomers with average molar masses of up to 900 Da was possible.

The system used for the analysis of the Jeffamine samples was also applied for the size-separation of poly(ethylene oxide) diamine oligomers. In this application monoamine by-products could be separated from the diamine main polymers (Figure 1.6). The electropherogram demonstrates that, in principle, both the MMD and the purity of technical amine polymer samples can be determined with the optimised CZE method.

After derivatization with phthalic anhydride (PhAH) Jeffamines could be detected by UV absorption [37]. The derivatization yields UV active compounds with a charge of -2. With a buffer of pH 9.3 and bare fused-silica capillaries the derivatives migrated against the EOF with the smallest polymers eluting last. Overlapping sets of peaks were observed in the electropherograms, due to the occurrence of variable ratios of the monomers in the polymer chains, which caused some difficulties in the determination of the MMD of the copolymer samples.

CZE has been applied to characterize dendrimers of poly(amidoamine) with a core of ammonia [38] or ethylenediamine [39]. In phosphate buffers at low pH the amino end-groups of these branched polymers were protonated and the positively charged polymers migrated towards the UV detector at
General introduction

Figure 1.5  First published oligomer separation of the Jeffamine ED 600 series after derivatization with a fluorescence reagent [35].

Figure 1.6  Electropherogram of diamine oligomers of poly(ethylene oxide) separated from monoamine by-products [36].
the cationic end. Low generation dendrimers (< 6) could be separated from each other, whereas high-MM compounds had similar effective charges and therefore migrated with the same mobility. It seems that for the separation of these compounds a sieving media is required. However, both CE systems were originally developed to provide information on the homogeneity of synthesized products and the presence of by-products formed by side reactions. It was found that all technical products contained the compounds of the previous generation and side products as impurities.

Polycarboxybetaines are composed of zwitterionic monomeric units. In CZE in an acidic medium they migrate as cationic compounds. A number of studies have been devoted to the CZE separation of polycarboxybetaines [30, 40, 41]. Information on the chemical structure and pH behaviour of technical products could be obtained by performing separations at different pH values [41]. Poly(lactic acid) (PLA) is a polymer with a neutral backbone bearing negatively charged end-groups. PLA oligomers with chain lengths up to 7 monomeric units were baseline separated using CZE in the conventional mode [42]. Separations could be performed with shorter run times in the reversed EOF mode using capillaries modified with polycations [43, 44]. The systems were primarily used to monitor the kinetics of hydrolysis of the oligomers in biological samples, but also to investigate the coupling reaction between PLA and mono-amino Jeffamine oligomers [45].

Water-soluble neutral polymers

Fatty alcohol ethoxylates (FAEs) are neutral low-MM copolymers, which are often used in laundry detergents, cleaning agents, cosmetics and herbicides. After conversion of the hydroxyl end-groups with PhAH to provide for charge and UV detectability, FAEs can be determined in commercial products and wastewater disposals by CZE, as has been described by Heinig et al. [46]. The singly charged polymer derivatives migrated against the EOF. Both the length of the alkyl chains and the number of ethylene oxide (EO) monomers vary in FAE chains, hence samples of this type of surfactants are complex. The authors compared CZE and high-performance liquid chromatography (HPLC) for the analysis of technical FAEs (Figure 1.7). The CZE electropherograms showed a strong overlap of two sets of peaks of polymers with equal alkyl groups but different number of EO monomers. The CZE method was in particular valuable for fast and efficient fingerprinting of the FAEs. With LC both the alkyl and EO homologues could be separated. The alkyl homologues were eluted according to chain lengths, while the elution order of the EO homologues in LC corresponded to the migration order in CE.
Polyethylene glycols (PEGs) are an important class of water-soluble synthetic polymers. For CE analysis of PEGs derivatization of the hydroxyl end-groups is required. Bullock [36] showed CZE separations of PEGs after derivatization with PhAH. With a reduced EOF separations of the (doubly charged) PEG derivatives into individual oligomer peaks were possible up to a molar mass of 3500 Da (Figure 1.8). Similar separations were obtained using a buffer to which a high content of methanol was added to reduce the EOF [47]. With neutral coated capillaries (suppressed EOF) and a reversed polarity of the electric field it was expected to improve the resolution of PEG derivatives [37]. However, the separations were not significantly better than those obtained using fused-silica capillaries and BGE solutions containing organic solvents for EOF suppression.

Excellent resolution of high-MM PEGs (> 3000 Da) as phthalate derivatives was demonstrated using CGE [48]. The migration time of the derivatives depended on the EOF, the charge-to-size ratio of the compounds and sieving by the network. Therefore, the polymers with the shortest chains eluted first after the EOF. A linear relationship between the molar mass of the oligomers and the migration time was found, which can be used as mass-calibration curve to determine the MMD of the compounds. The major disadvantage of this method was long the run time. The oligomeric separation of a PEG 4600 sample took more than 1.5h (see Figure 1.9). More efficient separations with shorter analysis time of a PEG sample with a similar MMD were obtained using
Figure 1.8 Free solution CZE separation of PEGs derivatized with PhAH (n represents the degree of polymerisation) [36].

Figure 1.9 Separation of a blend of PEG 1000, 3350 and 4600 as phthalate derivatives by CGE [48].
1,2,4- benzene tricarboxylic anhydride (BTA) as derivatization reagent [49]. The derivatization reaction with BTA yields polymer derivatives with a charge of \(-4\). This CE system was used to determine PEG by-products in samples of ethoxylated surfactants.

An interesting approach of CZE separations of PEGs was described by Vreeland et al. [50]. To one end group of the PEGs a monodisperse, fluorescently labelled DNA molecule was attached. Since the DNA strands have an identical effective charges they tend to migrate with the same electrophoretic mobility, and a separation can be obtained due to differences in molecular friction of the DNA-PEG conjugates of different PEG oligomers. This new mode of CE has been called free-solution conjugate electrophoresis (FSCE). Results obtained on the separation of a PEG 5000 sample demonstrate that the friction coefficient of the conjugates increased linearly with increasing degree of polymerisation of the PEGs. The method allows determination of the average molar mass and polydispersity of the polymers. Values for \(M_w\) and \(M_n\) were in good agreement with results obtained by MALDI-TOF-MS experiments. Recently, a paper was published on the analysis of polypeptoids using FSCE [51].

Triton-X series polymers (alkylphenol polyethoxylates) are non-ionic water-soluble polymer surfactants. The presence of the alkylphenol group makes the macromolecules more hydrophobic than for instance FAEs. Separation of this kind of polymers can be performed by micellar electrokinetic chromatography (MEKC) [36, 52 - 55]. Separations of neutral analytes by MEKC are based on the differential distribution between the aqueous buffer and the migrating micellar pseudostationary phase. The hydrophobic alkylphenol part of the compounds strongly associated with sodium dodecylsulfate (SDS) micelles in aqueous solutions. Therefore, the polymeric compounds will elute as a single peak at the migration time of the micelles. It was necessary to add a high concentration of organic solvent to the buffer solution to brake down the micelle structure. It was demonstrated that smaller aggregates of the SDS surfactant still associated with the hydrophobic analytes. This separation mechanism has been described as solvophobic association [56]. The migration time of the Triton CF compounds increased with decreasing length of the ethoxylate chain (Figure 1.10). This indicates that the homologues with less ethylene oxide units interact more strongly with SDS than the (more polar) highly ethoxylated polymers [52].

Triton-X polymers can also be separated by MEKC in pure aqueous buffer solutions using bile salts such as sodium cholate (SC) and sodium deoxycholate (SDC) as surfactants [55]. Small amounts of organic solvents in the BGE were required to improve the stability of the UV-baseline. Good resolution between Triton-X homologues was obtained.
We have used the MEKC principle for the characterization of water-soluble PEGs and the less polar poly(propylene glycols) (PPGs) [47]. Prior to the separation both types of polymer compounds were converted into hydrophobic UV-active derivatives with phenyl isocyanate. Background-electrolyte solutions contained borax, SDS as surfactant, and high contents of organic solvent, such as methanol, acetonitrile or tetrahydrofuran (THF). It was found that only the hydrophobic phenyl isocyanate group of PEG derivatives interacted with the SDS aggregates, while the length of the PEG chain determined the frictional force. As a result the separation order of PEG-derivatives in a MEKC system was similar to that observed with free zone CZE.

Linear PPG-derivatives behaved completely different in an SDS buffer solution. It appeared that both the phenyl isocyanate end-group and the PPG chain contribute to the distribution between the micellar- and aqueous phase. Because of the different behaviour of PEG- and PPG derivatives in a SDS solution, it was possible to separate a mixture of the two types of polymers with similar MMDs.

Copolymers with different CCDs are expected to show different migration times in an MEKC system. This was shown by the group of Cifuentes [57, 58] who applied MEKC to study the synthesis of random copolymers of 2-hydroxyethyl methacrylate (HEMA) and vinylpyrrolidone (VP). As is shown in Figure 1.11, electropherograms obtained of copolymers of HEMA and VP
showed two polymer products, one rich in HEMA and others rich in VP, depending on the reaction time [57]. The size dependence of the interaction of the copolymers with SDS ions was negligible when using a background electrolyte consisting of 50% (v/v) methanol in 50 mM borate buffer at pH 9.5 with 35 mM SDS.

MEKC separations of copolymers with ionic and neutral monomers give complex patterns because the interactions between the copolymers and the SDS ions will depend on both the hydrophobicity and charge distribution of the polymer chains. A MEKC system has been developed to monitor the copolymerisation reaction of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and 2-hydroxyethyl methacrylate (HEMA) or N,N-dimethylacrylamide (DMAA). The method allowed fingerprinting of intermediate and final copolymer products synthesized under different conditions.

We have developed a CE system with an SDS containing buffer to separate tri-functional glycerin-based polyols from mono- and di-functional by-products [59]. Prior to the injection the hydroxy end-groups of the polyols and impurities were converted with PhAH. It was found that the interaction of the charged polymers and SDS ions increased with the MM and the amount of propylene oxide in the backbone of the molecules. Under optimum separation conditions the quantities of impurities in technical polyols could be determined accurately.
Chapter 1

Non-aqueous CE for hydrophobic polymers

Since most synthetic polymers are hydrophobic and soluble only in organic solvents, it may be concluded that aqueous CE systems are not often suited for their characterization. In many cases synthetic polymers can be easily analysed by SEC with organic solvents. Using non-aqueous CE for synthetic polymers is an option when analyses with conventional SEC systems are complicated or when highly efficient separations are required.

So far, a limited number of CE methods to analyse water-insoluble polymers have been described with organic solvents for the BGE solution. Since LC and GC methods for long chain surfactants, such as alkanesulfonates and fatty acids, are usually laborious and require derivatization procedures, non-aqueous CE methods were developed for the characterization of these types of low-MM polymers [60, 61]. To increase the surfactant solubility and separation selectivity specific solvent mixtures were used in these applications. The authors demonstrated certain advantages of non-aqueous CE over aqueous systems, such as the possibility to adjust the mobilities by changing the organic solvent mixtures and by exploiting specific interactions between analytes and additives that did not occur in aqueous solutions.

In a paper of Mengerink et al. [62] it was shown that a CZE system is an efficient alternative for the determination of oligomers of nylon-6 (polyamide-6). As polyamides are not soluble in water or in common organic solvents, the use of a high content of 1,1,1,3,3,3-hexafluoropropanol (HFIP) in the BGE solution was necessary. At low pH (with phosphoric acid in the BGE) the linear polymers bear a positive charge on the amino end-group and migrate according to size.

Because SEC and MALDI-TOF-MS failed to characterize metallo bist(terpyridine) diblock polymers, we have developed a non-aqueous CE system to determinate the MMD of this type of hydrophobic polymers [63]. With deactivated capillaries and barium perchlorate in N-methylformamide (NMF) as BGE it was possible to separate the macromolecules according to size. The analytical system could also be used to determine the presence of by-products with higher mobilities (charge-to-size ratios). With several technical samples mono complexes were observed as degradation products.

Research on the application of non-aqueous CZE to separate hydrophobic oligomers has been performed by Cottet et al. [64]. In his work cationic oligomers of N-phenylaniline with a chain length of 2, 4, 6 and 8 were used as model compounds. As in the applications described above, the
General introduction

Solubility of the oligomers was the main limiting factor in the choice of the solvents. Mixtures of methanol with non-polar organic solvents such as THF, chloroform, dichloromethane and 1,4-dioxane were tested as potential candidates for the electrokinetic system. Moreover, the effect of the ionic strength, injection time, electric field, and temperature on the resolution of the polymeric compounds was investigated. Under optimised conditions baseline resolution between the low-MM oligomers could be obtained.

The use of specific interactions between neutral polymers and charged surfactants in organic solvents to generate electrophoretic migration of the macromolecules through a sieving medium has been studied recently [65]. It was found that neutral polystyrene and poly(methylmethacrylate) (PMMA) show electromigration in N,N-dimethylformamide (DMF) containing the surfactant stearyltributylphosphonium bromide (STBPB). Gels of high-MM polyethylene oxide were used as sieving matrix. The authors pointed out that the high-MM polymeric compounds eluted first followed by lower molar masses. However, the results experimentally obtained to determine the elution order of polystyrene standards of 50 and 2.3 kDa showed an opposite migration behaviour. This agreed with sieving behaviour under aqueous conditions. Run times were about 60 min. Nevertheless, this research is an attractive development towards applying electrokinetic separations for hydrophobic synthetic polymers.

Another efficient way to separate neutral macromolecules is by size-exclusion electrochromatography (SEEC). In this mode, a strong EOF is generated that drives the polymer compounds through a capillary column typically packed with porous silica particles. Only a few researchers have investigated the possibility of SEEC for the separation of synthetic polymers [66-68]. Polystyrene standards were used as model polymers in most studies. The main advantage of SEEC is the possibility to obtain highly efficient separations of polymer molecules according to size. Plate numbers for monodisperse samples with electro-driven systems can be five times higher than with conventional SEC systems.

As most CE systems are equipped with a UV detector, direct and indirect photometric detection is used in the majority of electrokinetic separations of synthetic polymers. With this type of detection specific information on the chemical structure of polymer products and by-products cannot be obtained. Recently, the potential of non-aqueous CE in combination with electrospray ion trap MS was investigated for monitoring the production process of poly(N-e-trifluoroacetyl-L-lysine)
Chapter 1

{poly(TFA-Lys)} [69]. With this system the degree of polymerisation of the separated oligomers could be directly determined from the MS spectra. Also interesting was the information on the chemical structure of "dead" polymers and other impurities that could be obtained from MS-MS experiments. Detection limits for the polymers were of the same order as typically obtained with UV detection. Figure 1.12 shows a typical separation of poly(TFA-Lys) performed by non-aqueous CE in combination with MS.

![Graph showing separation of living and dead polymers](image)

Figure 1.12 Non-aqueous CE-MS analyses for monitoring the polymerisation process of poly(TFA-Lys) [69].

1.4 Scope of this thesis

Even polymer-analysis is dominated by liquid chromatographic techniques such as interactive liquid chromatography and size-exclusion chromatography capillary electrophoresis (CE) can be useful alternative for the characterization of synthetic polymers. The scope of this thesis is to demonstrate the potential of CE for the separation of water-soluble as well as more hydrophobic synthetic polymers. The ability to obtain detailed information on characteristics of macromolecules including their sizes, number of end-groups and charge densities is examined. A primary focus of the research is the understanding of the mechanism of the electrokinetic separations and detection of the synthetic polymers.

In Chapter 2, applications of capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) to the determination of the size of linear PEGs and PPGs are investigated. It is demonstrated that with a simple CZE system both types of polymeric compounds were separated based on the number of monomers in the polymer chains. In the free solution mode PEGs and PPGs with comparable chain lengths migrated by approximately similar electrophoretic mobilities. With MEKC different separation mechanisms for PEG- and PPG derivatives were found. Therefore, the method can be used to separate a blend of both polymers with a similar molar mass.
The output of the research on PEGs and PPGs is the starting point for the investigation of the potential of CE for the characterisation of glycerin-based polyols described in Chapter 3. The CZE method optimised for the characterization of EO-PO copolymers is also useful for the determination of the size of technical glycerin-based polyols. The key target of the study was the development of an analytical method for the determination of by-products in technical polyol samples. Research on the interaction distribution of PEGs, PPGs and copolymers with SDS surfactants in the buffer allowed to apply CE conditions so that the glycerin-based polyols and by-products were separated based on the number of end-groups. The optimised method is validated and quantities of by-products in technical sample are determined.

In Chapter 4 the use CE for the determination of the charge density of high molar mass CMC is examined. With an aqueous buffer CMC samples with different average charge densities were separated. Additional CZE experiments were performed to study the origin of the peaks and the influence of the size of the CMCs in the electrophoretic separations. It can be concluded that CZE provide detailed information on the average charge density and its dispersity of CMCs.

Chapter 5 describes a non-aqueous CE method optimised for the separation of metallo bis(terpyridine) diblock polymers to obtain information on the sizes and purity of the technical samples. Aspects of the type of background electrolyte, organic solvent and capillaries on the separation of the metallo containing polymers are studied. It is demonstrated that CZE is a useful tool for the characterization of the hydrophobic and charged polymers, while other separation techniques failed.

In Chapter 6, the potential of contactless conductivity detection (CCD) for monitoring the elution of macromolecules by a size-exclusion electrokinetic chromatography (SEEC) is investigated. Instrumental aspects, such as sensitivity, repeatability and robustness for the detection of polystyrene are tested. The origin of the CCD signal of polystyrene is investigated. The results indicated that the viscosity of the sample zone was not the basis for conductivity detection of neutral polymers.

Note on the text

The chapters in this thesis have been composed as articles for publication in international scientific journals and can be read independently. Consequently, some overlap may occur.
Chapter 1

References

General introduction

Chapter 1


