Review Article

Separation and Characterization of Synthetic Polyelectrolytes and Polysaccharides with Capillary Electrophoresis

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The development of macromolecular engineering and the need for renewable and sustainable polymer sources make polymeric materials progressively more sophisticated but also increasingly complex to characterize. Size-exclusion chromatography (SEC or GPC) has a monopoly in the separation and characterization of polymers, but it faces a number of proven, though regularly ignored, limitations for the characterization of a number of complex samples such as polyelectrolytes and polysaccharides. Free solution capillary electrophoresis (CE), or capillary zone electrophoresis, allows usually more robust separations than SEC due to the absence of a stationary phase. It is, for example, not necessary to filter the samples for analysis with CE. CE is mostly limited to polymers that are charged or can be charged, but in the case of polyelectrolytes it has similarities with liquid chromatography in the critical conditions: it does not separate a charged homopolymer by molar mass. It can thus characterize the topology of a branched polymer, such as poly(acrylic acid), or the purity or composition of copolymers, either natural ones such as pectin, chitosan, and gellan gum or synthetic ones.

1. Introduction to CE and Limitations of Size-Exclusion Chromatography (SEC/GPC)

Free solution capillary electrophoresis (CE), or capillary zone electrophoresis, is a robust polymer separation method. CE differs from the commonly known slab electrophoresis or capillary gel electrophoresis as the capillary does not contain any stationary phase: it is just filled with a buffer (also named background electrolyte). CE does not require tedious sample preparation, not even filtration (e.g., see later in Section 3.2.2). It has several advantages over traditional separation techniques for the characterization of polyelectrolytes which will be outlined in this review. The most commonly used method for the separation and characterization of polymers is size-exclusion chromatography (SEC, also known as GPC). SEC is relatively quick and affordable in obtaining data regarding the size or molar mass of a polymer with good repeatability [1]. Among SEC’s main limitations is its poor reproducibility in terms of molar mass analysis: round-robin tests often show poor accuracy of the values of the determined molar mass [2]. This is detailed in Berek’s recent critical review [3]. The review linked the common accuracy issue to the difficulties in obtaining a pure size-exclusion separation: secondary retention mechanisms, side processes, parasitic processes, osmotic effects, secondary exclusion, concentration effects, preferential interactions, and SEC band broadening. For the ultrahigh molar masses, the sample is generally thought to be degraded by shear [4], although a change of conformation of the polymer chains may also take place, leading to a new separation mechanism [5]. In addition, even in ideal conditions (pure size-exclusion mechanism, no degradation), SEC separates by hydrodynamic volume not by molar mass [6]. Apparent molar masses determined by SEC (e.g., polystyrene-equivalent molar masses) thus have a variable and sometimes limited accuracy [7, 8]. Different topologies
(branching) or compositions of the polymer sample influence the hydrodynamic volume and the separation is then incomplete in terms of molar mass when a range of branching structures or of compositions is present in a sample [9–11]. This can render the simple determination of molar mass using Mark-Houwink-Sakurada parameters inaccurate, like in the case of most poly(alkyl acrylates) [12, 13]. Up to 100% error in the determination of the molar mass of branched polymers has been measured using multiple detection SEC (light scattering and viscometry) [14, 15].

We recently discussed the SEC of branched polymers and polysaccharides in a review [16]. Composition of copolymers, branching, and purity are often overlooked in polymer characterization, since SEC has a quasi-monopoly and is not suited for these types of characterization. However, alternative methods are being developed, especially alternative liquid chromatography methods [17]. Liquid chromatography in critical conditions (or at the critical conditions) [18] is one of the most prominent alternative chromatography technique: the critical conditions for one homopolymer correspond to the absence of separation by molar mass for this homopolymer, allowing for separation solely by its topology if branched [19] or solely by its composition if copolymerized [20]. These critical conditions are, however, tedious to establish and low recoveries have been observed [21, 22]. CE offers an alternative and the objective of this review is to present and discuss the potential of CE for synthetic polymers and polysaccharides.

CE (defined here as free solution capillary electrophoresis) involves separation in a capillary filled with only buffer (no stationary phase) under high voltage [23]. The use of only a buffer and no stationary phase prevents the common problem of adsorption onto the stationary phase (and of degradation or deformation of the ultrahigh molar mass chains) commonly faced in SEC. The velocity of different analytes is proportional to the electric field: the proportional constant is named the electrophoretic mobility, \(\mu_{ep}\). The selectivity of CE separation relates to the difference in electrophoretic mobility of the analytes (see Figure 1 for the experimental determination of \(\mu_{ep}\)). The electroosmotic flow (EOF) is created by the movement of the ions of the background electrolyte through the capillary under electric field. The EOF is contributing to the migration of all molecules, even neutral ones. At a high pH the silanol groups of the glass layer of the capillary are completely ionized. This generates a strong zeta potential and an electrical double layer of silanolate groups and positive ions from the background electrolyte. The higher the pH, the higher the density of the electrical double layer which increases the EOF [23].

Successful applications of CE to polymer characterization have been the object of a number of publications, especially by Cottet's group, and the earliest works have been reviewed [24]. Building on these advances, using CE, our group was able to reliably characterize several natural and synthetic polymers, especially polysaccharides and poly(acrylic acid) as discussed in this review.

2. Free Solution Capillary Electrophoresis (CE)

Characterization of polymers by CE can be divided into at least four categories: separation of monomer units after depolymerization (see Section 2.1), separation of oligoelectrolytes (see Section 2.2), and separation of longer polyelectrolytes (see Section 3). The fourth category is the separation of polymers bearing a single charge or no charge. For the latter category, the reader is referred to the pioneering work of the groups of Cottet [25, 26] and Cifuentes [24, 27].

2.1. Average Composition of Polysaccharides

2.1.1. Robust Separation of a Mixture of Monosaccharides. A number of polysaccharides, such as hemicellulose [28, 29], have highly complex chemical structures: they are composed of several different monomer units, mainly monosaccharides. The analysis of these polysaccharides is extremely difficult. The average composition can be determined after depolymerization (hydrolysis) and quantification of the different resulting monosaccharides. Currently high performance liquid chromatography (HPLC) is used to separate carbohydrates using different modes; however, this technique and the different modes used have limitations in regard to coelution [30], tedious sample preparation, and short column life [31]. The detection of monosaccharides is another difficulty. CE is most easily and classically applied to analytes that are charged and possess chromophores. The pKa of most mono- and disaccharides is around 12 [32, 33] and separation in CE was obtained at high pH [34] but initially indirect UV detection, conductivity detection, or derivatization was required for detection. Rovio et al. showed that different hemicelluloses can be characterized not only with CE but also with direct UV detection [32, 33]. This method was applied to plant fiber samples without any derivatization: CE achieved a high-resolution separation of the depolymerized fiber samples (Figure 1) and was compared to the various common HPLC methods and IC (HPAEC) [30, 32]. The CE separation can be optimized at minimal cost by changing the capillary length, buffer counter-ion, and/or the buffer concentration [35]. The main advantage of CE is the robustness of the technique, especially the minimum sample preparation that is required. The precision of the peak identification and the quantification were greatly improved with the use of an electroosmotic flow (EOF) marker and an internal standard [30]. Figure 1 shows electropherograms when raw data (migration time) are compared to corrected data (electrophoretic mobility). Figure 1 also gives the equation used to perform this transformation. In the equation, \(\mu_{ep}\) is electrophoretic mobility, \(V\) is voltage, \(L_d\) is the length to the detector, \(L_t\) is the total length of the capillary, \(t_m\) is the time of migration, and \(t_{null}\) is the migration of a neutral species. Using electrophoretic mobility (thus correcting for EOF variations) allows easy visual comparison of results, for example, to allow identification of trace sugars in ethanol fermentation [36].

CE was able to resolve and quantify mannose, galactose, and xylose. The CE quantification of these sugars results in larger amounts when compared to the HPLC results. This might indicate incomplete recovery in HPLC possibly due
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Figure 1: Separation by CE at high pH (12.6) and with direct UV detection of a depolymerized plant fiber sample plotted as a function of electrophoretic mobility (a) and of migration time (b). The sample contains (1) cellulose, (2) galactose, (3) glucose, (4) rhamnose, (5) arabinose, and (6) xylose (the molecular structures are given for the sole purpose of identification, and they are in equilibrium with a number of linear and charged forms) [30].

to adsorption onto the stationary phase, which is a common problem associated with the HPLC of samples in complex matrices. There were weaknesses in the direct UV detection in CE which have been addressed recently (see Section 2.1.2).

2.1.2. Direct Detection due to the Photooxidation of Sugars. Rovio et al. [33] showed that the detection of monosaccharides was possible at 270 nm at pH 12.6. Sarazin et al. [43] suggested the method of detection was due to a photooxidation reaction occurring at the detection window. We confirmed that the detection is by photooxidation using a combination of simulation, multidimensional CE migration, and NMR spectroscopy analysis [30, 37]. The detection occurs without the electric field (i.e., in pressure mobilization instead of CE) but the electric field enhances the sensitivity of the detection. The photooxidation is initiated either by hydroxyl radicals formed by minimal but sufficient water decomposition or by direct decomposition of the carbohydrates under UV irradiation [35]. The diode-array detector (DAD) emits UV light down to 190 nm. These wavelengths are not leading to any known sample degradation, except for the photooxidation of carbohydrates at high pH. The photooxidation is a type of in situ derivatization. If one wishes to avoid the photooxidation reaction taking place, then the lowest wavelengths need to be filtered out: on commercial equipment this simply means using UV detection and not a DAD. Even by using a DAD, most of the sugar molecules are not photooxidized (in the timeframe of the detection). The UV-absorbing species are intermediates in the photooxidation process [37]. These intermediates are present at low concentration but have a high UV absorption coefficient. The final products do not absorb UV and are likely obtained after reacting with oxygen. NMR spectroscopy used as offline detection after CE migration allowed for the identification of a number of carboxylated compounds in the final products. This CE method is ideally suited for the separation of mono- and disaccharides in complex matrices. Direct detection has the advantage of simplicity and of using the most common detector in CE (diode-array detection). The detection of the CE was found to have a limit of detection 10–100x better than HPLC and a better selectivity of detection. Direct detection in CE is not as sensitive as more convoluted methods based on derivatization or pulsed-amperometric detection in ion chromatography [36]. The method will thus need further improvement to be used for trace detection. Preliminary results showed that using a radical photoinitiator can increase the sensitivity of the direct UV detection [37].

The CE method (Figure 2) is useful not only to determine the average composition of complex polysaccharides, but also to monitor carbohydrates, for example, in a fermentation process. The latest developments showed that fermentation products such as ethanol can also be determined [35]. Ethanol is inhibiting the photooxidation process and this leads to indirect detection of ethanol in the presence of a sugar, such as sucrose. This indirect detection was successfully applied to monitoring lignocellulosic fiber fermentation in terms of both ethanol and sugars alcohols [36].

2.2. Oligoelectrolytes. Cottet and Gareil have shown that oligo(styrene sulfonate)s can be separated by their molar mass up to a degree of polymerization of 9 [44]. Oligo(sodium acrylates)—oligoAAs—are used in the paint and coating industries to stabilize emulsions [45]. Controlled polymerization methods such as reversible addition-fragmentation chain transfer (RAFT) allow the controlled synthesis of oligoAA. CE can separate oligoAAs (Figure 3) at a higher resolution than that ever obtained with SEC (for oligomers) [38] even using optimal SEC conditions [46, 47].

CE was able to separate and quantify the residual RAFT agent used to obtain the oligoAAs as well as the species of degrees of polymerization (DP) of one, two, and three. The identification of these peaks was obtained by the online coupling of CE with ESI-MS-TOF (electrospray ionization-mass spectrometry-time of flight) [48]. MS analysis showed that the oligomers are separated not only according to their
Figure 2: Mechanism of direct UV detection in CE of carbohydrates owing to a photooxidation reaction [37].

Figure 3: Electropherograms in lithium borate for two different oligoAAs, AA5 and AA15, produced by RAFT polymerization, where 5 and 15 correspond to the degree of polymerization obtained at the maximum of the mass spectrum from ESI-MS-TOF (electrospray ionization-mass spectrometry-time of flight) direct infusion (adapted from [38]). AA15 is not separated by molar mass; it is thus in the critical conditions. The bottom electropherogram is of the RAFT agent, that is, the control agent for the polymerization.

degree of polymerization and end-group, but also according to their tacticity. The shortest oligoAAs were shown to contain 50% of unreacted RAFT agent, while the direct infusion in ESI-MS estimated that the sample contained only 2% of unreacted RAFT agent. This large discrepancy is due to the known issue of the bias of the ionization towards low degrees of polymerization and hydrophilic species in MS analysis [49]. CE was shown to be a relevant and fast method in the study of kinetics of polymerization of RAFT. It has also been used to shed light on the kinetics and mechanism of ring opening polymerization of either 2-oxazoline [50] or N-carboxyanhydrides [26].

Most importantly, the high-resolution separation of CE by molar mass is limited to oligoelectrolytes, with degrees of polymerization below about 10. For large polyelectrolytes, no separation by molar mass is obtained, which corresponds to the "critical conditions" described below.

3. CE in the Critical Conditions

3.1. Explanation of “Critical Conditions”. The first example of analysis of synthetic polyelectrolytes by electrophoresis dealt with poly(4-vinyl-N-n-butylpyridinimum bromide) more than half a century ago [51]. The authors concluded that “the electrophoretic behavior of polyvinylbutylpyridinium is not very sensitive to molecular weight.” CE in the “critical conditions” differs from the CE undertaken in the separation and characterization of polyelectrolytes such as oligoAA. Critical conditions refer to the conditions sought in liquid chromatography (LC) in which a homopolymer is not separated by molar mass (see Section 1). While these critical conditions are of no use to characterize simple (homo)polymers, most, if not all, polymers are not simple in the sense that they possess a distribution of molar masses as well as different end-groups, distribution(s) of compositions for copolymers, distribution of branch molar masses and of positions of branching points for branched polymers, and so forth. Polymeric samples are multidimensional: the critical conditions thus enable the characterization of complex polymers through the
simplification of a multidimensional problem. While a lot of research has been devoted to LC and critical conditions, the method remains tedious and plagued with low accuracy and recovery [21]. Applications to hydrophilic and/or charged polymers are very limited. CE is an alternative to LC in this specific, but important, case of complex polyelectrolytes. The molecular reasons behind critical conditions in LC and CE are completely different and are not widely accepted in any case. The electrophoretic mobility always depends on the charge to friction ratio. It does not depend on the ratio of the charge to the size in the case of polyelectrolytes, since the friction is not only hydrodynamic in this case. The critical conditions do not correspond to the free draining model as proposed by Flory, in which the solvent penetrates the polymer chains freely [52]. Electrostatic friction, however, screens the hydrodynamic friction [53, 54] and leads to the electrophoretic mobility having a very weak dependence on molar mass for a degree of polymerization generally above 15–20 [44, 55, 56]. Thus, CE leads to migration independent from molar mass for polyelectrolytes and this corresponds to the critical conditions sought in LC-CC. CE has been used in the critical conditions outside of our group in the separation of pectins [57] and carboxymethylcellulose [58] according to their composition. In our group using CE in the critical conditions (CE-CC) has allowed the investigation of the composition of natural polymers as well as of synthetic polymers. Further, we have also been able to look at the degree of branching of synthetic polymers.

3.2. Separation by Composition

3.2.1. Pectin and Carboxymethylcellulose (CMC). CE-CC effectively separates the polysaccharide pectin by composition. Several studies reported the separation of pectin by its degree of substitution (DS, which may include either esterification or methyl-esterification) [57, 59–62]. Within one sample, pectins macromolecules with different degrees of esterification (DE) could be separated. It was later hypothesized that the shape of the peaks could additionally be used to indicate a distribution of methyl esters of pectin within samples [60]. Guillotin et al. [62] established a protocol in which pectin’s degree of amidification, degree of methyl-esterification, and subsequently the degree of substitution could be determined.

Other research involved the use of capillary electrophoresis to determine the DS of carboxymethylcellulose [58]. The study showed the possibility of not only determining the average DS but also determining the heterogeneity/distribution of the compositions of CMC.

3.2.2. Gellan Gum. Gellan gum is a natural polymer which is widely distributed in the environment. Due to its rheological properties it is viewed as a possible stabilizing agent in various industries [63]. Gellan gum’s monomer unit structure contains n-glucuronic acid, n-glucose either with or without acyl substituents, and l-rhamnose. The proportion of acyl chains attached to the glucose and the distribution of these acyl chains along the polysaccharide vary from sample to sample. Gellan gum is often characterized by its degree of acylation, which affects its desired properties. CE allowed some separation of gellan gum oligomers according to their degree of polymerization and separation of polymers by their degree of acylation (composition) [39]. CE gave a unique separation of gellan gums that could not be attained with any other existing separation methods. CE could characterize not only a low acyl gellan gum but also a high acyl gellan gum (Figure 4). The latter sample was a turbid dispersion: while obtaining a true solution was not possible, the characterization of this dispersion is relevant for its applications such as the stabilization of carbon nanotubes [64]. Characterization of the high acyl gellan gum showed the presence of aggregates forming during the dissolution of the gellan gum samples and appearing as very narrow peaks due to their very low diffusion coefficients. This illustrates the robustness of the method as it did not require sample filtration (while the background electrolyte still requires filtration). Filtration would have changed the nature of this colloidal sample and should thus not be performed for a meaningful characterization. Complementing the CE separation with a simple pressure mobilization analysis (a qualitative version of Taylor dispersion analysis [65]; see 3.4), the presence of oligomers of gellan gum was confirmed while they had never been identified previously in these gellan gums. Through the CE separation, the oligomers could be separated and quantified. Separation and characterization of this dispersion could only be obtained by CE or field flow fractionation (FFF). In the most common form of FFF, flow FFF, the oligomers would have been lost through the membrane. The low acyl samples contained more oligomers than the high acyl samples suggesting the occurrence of some degradation during the deacylation process. The electrophoretic mobility is also sensitive to the conformation of gellan gum and complementary to light scattering characterization. A high mobility peak, present in the high acyl sample and becoming more intense in the presence of potassium borate ions, suggests the possibility
of a double-helix conformation. This peak differs in mobility and, thus, also suggests that the rest of the macromolecules are in a random coil conformation.

In order of increasing mobility, the electropherograms show the presence of gellan gum oligomers, then gellan gum polymer chains containing different degrees of acylation (random coil conformation), then aggregates of gellan gum chains, and finally gellan gum polymer chains in a helix conformation. The gellan gums studied in this work are copolymers containing both repeating units (not necessarily forming blocks). The top molecular structure represents fully acetylated gellan gum and the bottom one represents fully deacetylated gellan gum. The monomer unit is constituted from left to right of one D-glucose with two acyl substituents (one acetyl and one glycerate bearing a diol or glycol), one D-glucuronic acid, one D-glucose without substituents, and one L-rhamnose.

The study undertaken on gellan gum is an example of the robustness of the CE technique. Whilst allowing the successful separation of complex samples by composition, it also provides information on the conformation of the polymer chains and the presence of aggregates.

3.2.3. Chitosan. Chitosan is a polysaccharide produced from the N-deacetylation of chitin. Chitin is the main component of the shells of crabs and shrimps and can also be found in the cell wall of fungi. It is a renewable resource that it is a large waste product. Chitosan's structure contains N-acetyl-D-glucosamine as well as D-glucosamine units. The composition of this copolymer is quantified by the degree of acetylation (DA), which is the fraction of N-acetyl-D-glucosamine. Chitosan is receiving extensive research interest due to its inherent properties. It is biocompatible, antimicrobial, antifungal, biodegradable, and pH-responsive [66, 67]. However, one limitation of chitosan is in the incomplete characterization by current methods; while being a natural product, there is a large variation among samples. Chitosan is often characterized by its average degree of acetylation (DA) [40]. Chitosan samples are, however, not composed of polymer chains with all the same DA, but they contain a distribution of DAs. The complexity and importance of the distribution of DAs have been revealed recently through a coupling of SEC with $^1$H NMR spectroscopy [68]; however, it still has not been measured. $^1$H NMR spectroscopy can determine number-average as well as weight-average DAs [40]. The measurements are accurate, but precise results are time-consuming and alternative methods are often considered. Chitosan is often characterized by only one of its average DAs, which is implicitly and incorrectly assuming the sample is homogeneous in terms of DA, that is, does not have a distribution of DAs.

CE-CC separates chitosan by its degree of acetylation (Figure 5) [40]. Chitosan macromolecules with a lower DA have a higher mobility (at low pH below the glucosamine monomer unit pKa) since they have a higher number of free amino groups which increase their charge and therefore their electrophoretic mobility. Another important attribute revealed in the CE separation is the broadness and shape of the peaks of the chitosan samples. The broadness corresponds to the distribution of DAs and some samples have broader distributions than others (Figure 5). These differences in distributions will likely affect functional properties such as adhesion, biodegradability, and bacteriostaticity [68]. With proper calibration, the CE separation will allow the determination of the distribution of DAs. The distribution can be calculated from the UV signal taking into account the nonlinear relation between electrophoretic mobility and migration, as it is done to calculate molar mass distributions in SEC [10].

We have also used CE to assist in the grafting of synthetic polymers, poly(sodium styrene sulfonate) and poly(methyl methacrylate-co-acrylonitrile), onto a chitosan backbone [41].

![Figure 5: Separation of chitosan samples by their DA with CE (sodium phosphate, pH 3): electropherogram shown as a function of (a) migration time and (b) apparent electrophoretic mobility. Samples with different degrees of acetylation (weight-average DA determined by NMR spectroscopy) are shown: black solid line (4%), green dashed line (4.3%), blue dashed-dotted line (16.5%), red dashed line (18.7%), pink dashed line (19.8%), brown dotted line (22.4%), and black dotted line (23.6%) [40].]
3.3. Separation by Topology (Branching). There is a large interest in the characterization of water-soluble polyacrylates as their use has increased to include a range of applications from industrial protective coatings to food packaging. The poly(sodium acrylate)s, PNaA, studied by our group were produced by nitroxide-mediated polymerisation (NMP) [70].

The aim of the study was to characterize the branching in PAA using CE-CC. Different topologies of the PAA samples, linear, hyperbranched, and three-arm star, were separated within 15 min [42]. Figure 7 presents the CE results both as raw data, as a function of migration time and as EOF corrected data, as a function of electrophoretic mobility. This highlights the importance of converting the results to electrophoretic mobility plots as a trend is not seen in the migration time results due to the variation in EOF between injections. When the EOF correction is made, it can be seen that the hyperbranched polymer exhibits the lowest electrophoretic mobility followed by three-arm star and finally the linear ones. The differences in electrophoretic mobility can be explained by a decrease in the effective charge of the branched samples when compared to the linear samples. The results obtained were highly repeatable and reproducible with relative standard deviations (RSD) values below 1.6%. The separations were also successfully reproduced in a different buffer and whilst they produced different electrophoretic mobility values (as expected, due to the different counterions), the electrophoretic mobility remained lower for the more branched structures. This study highlights also the accuracy (related to the reproducibility of the separation) of the technique.

The CE results obtained also provided information regarding the homogeneity of the branching topology. These samples are expected to have a controlled molar mass owing to the reversible termination with the nitroxide but the broadness of the peaks obtained suggests some heterogeneity in the branching structure. The broad range of electrophoretic mobilities is attributed to a broad range of branching topologies produced in the polymerization.

The CE-CC separation was also shown to be influenced by end-groups. In Figure 8 the red solid line represents PAA with a SG1 [N-tert-butylnitroxide][N-tert-butyl-N-(1-diethylphosphono-2,2-dimethylpropyl)nitroxide] moiety as the end-group. There is a marked difference between this sample and the sample with the PAA that has been heated in the presence of thiophenol to replace the SG1 end-groups with hydrogen. The electrophoretic mobility increases with the removal of the SG1 end-group, as expected, since the bulky SG1 molecule would contribute to the hydrodynamic friction of the PAA chains more than hydrogen (and neither contributes to its charge). The result also suggests heterogeneity of the sample in terms of branching. The thiophenol treatment was also applied to a PAA sample obtained from the hydrolysis of poly(t-butyl acrylate) and CE-CC showed that the hydrolysis of poly(t-butyl acrylate) did not just cleave the targeted t-butyl groups, but also likely lead to some degradation of the SG1 end-group. This led to a greater heterogeneity of the sample and is expressed in the broadness of the peak.

Through CE-CC, we were able to separate polymers by their topology (branching) as well as by their end-groups. The ability of CE-CC to separate based on the presence of SG1 (control agent used for nitroxide-mediated polymerization)
Figure 7: Separation of linear (purple line), three-arm star (black line), and hyperbranched (red line) poly(sodium acrylate) by capillary electrophoresis in sodium borate buffer (pH 9.2) shown as a function of (a) migration time and (b) electrophoretic mobility, which is a more reproducible quantity than the former [42].

Figure 8: Electrophoretic mobility distributions of a PNaA obtained by nitroxide-mediated polymerization of acrylic acid initiated by the monofunctional initiator Monams (red solid line) followed by cleavage of the SG1 end-group by treatment with thiophenol (blue dashed line) in sodium borate (pH 9.2) [42].

reveals information regarding the “livingness” of the obtained sample (allowing continuing reacting through NMP) [42, 71]. This allows the optimization of the method used to produce the sample and provides information regarding further functionalization of the PAA.

3.4. Size Determination with TDA. One limitation of CE for polymer characterization, compared to multiple detection SEC [16], is the limited number of detectors, especially the lack of molar mass sensitive detectors. The group of Cottet is, however, bridging this gap rapidly by demonstrating that the CE separation can be coupled to Taylor dispersion analysis (TDA). TDA is a method that does not involve separation but allows the determination of the diffusion coefficient/hydrodynamic radius of a sample. TDA has been looked at previously to obtain diffusion coefficients in liquid systems [72, 73]. Further it has proven to be practical in the size characterization of macromolecules and particles of virtually any molar mass [65, 74]. TDA has several advantages including that it is an absolute method, meaning that no calibration is required. The group of Cottet has shown that a CE instrument is particularly well suited to carry out TDA [65, 75]. Le Saux and Cottet [76] coupled CE and TDA. A copolymer mixture of 2-acrylamido-2-methylpropanesulfonate/acrylamide and DNA was injected into a fused silica capillary. The mixture was separated in CE conditions (with an electric field) and then pressure was used to push the samples to the detection window where the analysis took place. The experiment proved that the coupling of CE and TDA allowed not only a complete separation of the copolymer from the DNA in the mixture, but also the successful determination of the diffusion coefficient of both the copolymer and the DNA. A successful coupling of CE with TDA allowed a combination of a high performance and throughput method with an absolute method for the calculation of diffusion coefficients [77]. The diffusion coefficient can then be related to the hydrodynamic volume of the macromolecule as it is classically done in light scattering by the following equation:

\[
D = kT6\pi \eta r,
\]

where \(k\) is the Boltzmann constant, \(T\) is the temperature, \(\eta\) is the viscosity of the solvent, and \(r\) is the hydrodynamic radius of the macromolecule [78].

The relation of the hydrodynamic radius to the molar mass is complex and is influenced by branching and copolymer composition as discussed in Section 1 for SEC [16].
4. Conclusions and Future Directions

Characterization by capillary electrophoresis involves both separation and characterization of complex polymers. This review outlined the broad range of samples that can be analyzed with CE. The characterization of complex polymers is significant for various industries including food, biomedical, energy/fuel, and materials (such as paint and bioplastics) industries. The robustness of the method, especially the minimal sample preparation, is one of the main strengths of the method (shared with field flow fractionation). The continual development of the methods in CE and their coupling with other techniques such as TDA widens the scope and depth of the possible characterization and meets the ever-growing needs of progressively increasing complex macromolecular structures for increasingly advanced applications. CE in the critical conditions (CE-CC) has the most potential and can be applied to a wide variety of charged polymers to characterize their topology, composition, or end-groups. The method is complementary to SEC.

Future directions will look into further characterizing complex polyelectrolytes, for example, in terms of determination of the molar mass distribution of one block in block copolymers, or in terms of sugars quantification in food samples. The different distributions related to branching will also be studied. CE coupled with TDA is a very useful and simple technique that will definitely be examined further as it is able to provide extremely valuable information regarding the size and shape of sample molecules by the calculation of their diffusion coefficient. Its simplicity and being an absolute method mean that it can be applied to a variety of samples investigated by our research group and other polymer research groups.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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