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Review

Nonaqueous and aqueous capillary electrophoresis of synthetic polymers

Hervé Cottet[a], Carolina Simó[b], Willy Vayaboury[a], Alejandro Cifuentes[b,*]

[a] *Organisation Moléculaire, Évolution et Matériaux Fluorés, UMR CNRS 5073, Université de Montpellier 2, Case Courrier 017, Place Eugène Bataillon, 34095 Montpellier, France*
[b] *Institute of Industrial Fermentations (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain*

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Abstract

In this work, the use of capillary electrophoresis (CE) to analyze synthetic polymers is reviewed including works published till February 2004. The revised works have been classified depending on the CE mode (e.g., free solution capillary electrophoresis, capillary gel electrophoresis, etc.) and type of buffer (i.e., nonaqueous, aqueous and hydro–organic background electrolytes) employed to separate synthetic macromolecules. Advantages and drawbacks of these different separation procedures for polymer analysis are discussed. Also, physicochemical studies of complex polymer systems by CE are reviewed, including drug release studies, synthetic polyampholytes, dendrimers, fullerenes, carbon nanotubes and associative copolymers.

Keywords: Review; Synthetic polymers; Nonaqueous capillary electrophoresis; Macromolecules; Micellar electrokinetic chromatography; Capillary gel electrophoresis; Capillary electrophoresis

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* Corresponding author. Tel.: +34 91 5622900; fax: +34 91 5644853.
E-mail address: acifuentes@ifi.csic.es (A. Cifuentes).

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1. Introduction

Nowadays, capillary electrophoresis (CE) has become widely used for the separation of biopolymers such as proteins, peptides or DNA fragments as a result of the good possibilities provided by this technique in terms of analysis speed, high efficiency and low sample consumption [1–3]. It is well known that CE allows separation of these substances according to their different molecular size, charge/mass ratio, isoelectric point, etc. Logically, these modes of separation can also be valuable for synthetic polymer characterization [4,5], mainly considering that properties like size and molecular dispersion of synthetic macromolecules have a great influence on their applications. CE has demonstrated to be a very valuable technique for the fast characterization of this kind of polymers. However, since synthetic macromolecules can have a wide variety of forms differing in shape (branched, cross-linked, linear, etc.), sizes (with molecular masses ranging from hundreds to over a million g/mol) and chemical properties (neutral, ionic, hydrophobic, hydrophilic, etc.), no one CE technique is universally applicable to all polymers. In order to deal with this different polymer nature, several CE modes have been used (e.g., free zone capillary electrophoresis, capillary gel electrophoresis, etc.) using nonaqueous, hydro–organic or aqueous separation buffers. Thus, one of the main characteristics of CE is that this technique makes possible the development of uniquely tailored separations, that can monitor very different polymers together with their degradation products or by-products formed during polymerization. Interestingly, such information can be in some cases complementary to that provided by other classical techniques based as size-exclusion chromatography (SEC) or RP-HPLC.

A description of the works that have been published up to now on CE analysis of synthetic polymers is given below.

2. Nonaqueous CE of synthetic polymers

2.1. Polymer analysis by nonaqueous free solution capillary electrophoresis (NAFSCE)

The principal applications of free solution capillary electrophoresis (FSCE) in polymer analysis are: (i) size-based separation of end-charged oligomers with low to moderate degrees of polymerization (DP, typically, $0 < DP < 50$), (ii) separation of evenly charged oligomers of low DP (typically, $DP < 10$), and (iii) separation of copolymers according to their charge density.

The first interest in using nonaqueous FSCE (NAFSCE) for polymer analysis is to increase the solubility of hydrophobic polymers that cannot be analyzed by aqueous or hydro–organic CE. Thus, NAFSCE allows one to extend the range of CE applications to non water-soluble polymers. Of course, the electrophoretic migration is only possible if the polymer solutes are ionized in the background electrolyte (BGE) or in interaction with a charged additive of the separation medium. Number of non-dissociating solvents currently used in polymer chemistry such as tetrahydrofuran (THF), dichloromethane, chloroform, dioxane, alkanes, etc. have very low dielectric constant (<10) and are usually not considered as potential candidates for performing CE. However, pioneering and more recent studies [6,7] reported the possibility of using non-dissociating solvents as a main component in a BGE for electrophoretic separations. Early work in 1950 [6] reported the possibility to ionize particles (carbon black) in nonconducting solvent such as kerosene or cetane. Electrophoretic mobilities of the particles were very low (in the order of 0.2×10^{-9} m^2 s^{-1} V^{-1}) but were high enough to be determined and could be increased up to 1.2×10^{-9} m^2 s^{-1} V^{-1} in cetane saturated with acetone.

In a recent study, Cottet et al. [7] demonstrated that a high content of non-dissociating solvent can be used as a main component of the electrolyte for polymer analysis. This approach was exemplified by the separation of highly hydrophobic *N*-phenylaniline oligomers, belonging to the family of conductive and electroactive polymers. Electrolytes based on binary solvent mixtures containing a high percentage (e.g., 75%, v/v) of non-dissociating solvents such as chloroform, THF, or dichloromethane and a moderate content of methanol (MeOH) (e.g., 25%, v/v) were used. Electrophoretic migrations of the oligomers were even reported in a THF–MeOH (95:5, v/v) mixture with supporting electrolyte. However, the electrophoretic mobilities were relatively low in this latter case. The ionization (protonation) of the solutes was obtained by the addition of 10 mM perchloric acid in the solvent mixture. The origin of the selectivity was not clearly elucidated but the electrophoretic mobility was a decreasing function of the DP. This observation suggested that the oligomers were not evenly charged since the electrophoretic mobility of evenly charged oligomers is usually an increasing function of the DP for low molecular masses (typically for $DP < 10$, although this limit depends both on the ionic strength of the electrolyte and on the polymer stiffness [8]). The main limitation for using non-dissociating solvents in NACE is their relatively strong UV absorbance (especially chloroform and dichloromethane in the solvents mentioned above).

Methanol and acetonitrile (ACN) are more UV-transparent than aforementioned solvents and, for this reason, they were extensively used in NACE of small molecules (see e.g., [9]). These solvents were also used for polymers or oligomers NAFSCE analysis. Bowser et al. [10] described the separation of porphyrin oligomers based on electrostatic ion–dipole interaction between analytes and a polyether surfactant in a methanolic electrolyte. It was proposed that the polyether complexes with the porphyrins through the carboxylic acid functional groups. These interactions could hardly take place in water-based electrolytes and are favored in methanol due to the low dielectric constant. The methanol-based electrolyte also reduced the aggregation of porphyrin oligomers. Partial separation of 60 peaks was performed in 30 min leading to a qualitative fingerprinting method for characterizing porphyrin samples.

Okada [11] described the separation of polyether oligomers according to their degree of polymerization in methanolic electrolyte by electron donor–acceptor interactions between the nonionic oligomer and the cation of the electrolyte. Polyethers were derivatized before analysis by 3,5-dinitrobenzoyl chloride for facilitating the detection. The longer the oligomer, the higher was the electrophoretic mobility due to higher complexation with the cations. Binding constant was determined for various cations. Such separations based on electron donor–acceptor interactions would not be possible in water-based electrolyte due to the weakness of these interactions in water. These two latter examples show that organic solvents can modify not only the solubility of the polymer but also the selectivity of the separation in comparison with water-based background electrolytes.

Recently, Cottet et al. [12] developed a nonaqueous FSCE method for the characterization of synthetic organic polypeptides. Poly(N_ε-trifluoroacetyl-L-lysine) were separated in a MeOH–ACN (87.5:12.5, v/v) mixture containing 1 M acetic acid and 20 mM ammonium acetate. This electrolyte allowed the separation of the oligomers according to two different polymer characteristics: the polymer functionality (end-groups) and the molecular mass (see Fig. 1). The separation of the polymer according to its functionalities was possible in this electrolyte in which amine groups were fully protonated (cationic) while carboxylic groups were partially deprotonated (anionic). In Fig. 1, the cationic polymers detected before the electroosmotic flow (e.o.f.) marker correspond to living polymers (i.e., polymers able to continue to react with the monomer) while polymers detected after the e.o.f. correspond to the so-called dead polymer. Simó et al. [13] coupled this NACE method with an ion trap mass-spectrometer using an electrospray interface. This coupling resulted in a powerful analytical tool for the characterization of complex mixtures of synthetic polymers and for the investigation

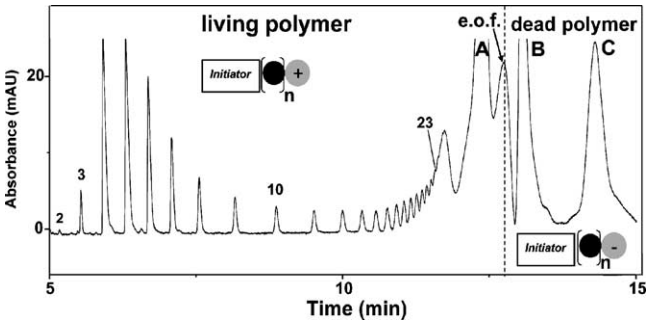

Fig. 1. Separation of poly(N_ε-TFA-L-lysine) according to its functionalities (end-groups) and according to the molecular mass by nonaqueous capillary electrophoresis. Fused silica capillary, 48 cm (39.8 cm to the detector) × 50 μm i.d. Running buffer: 1 M acetic acid, 20 mM ammonium acetate in a MeOH–ACN (87.5:12.5, v/v) mixture (I = 18 μA). Capillary temperature: 25 °C. Run voltage: +30 kV. Hydrodynamic injection: 17 mbar, 3 s. UV detection at 200 nm. Identification: number of monomeric units of the living (cationic) poly(N_ε-TFA-L-lysine) as indicated; (A) living polymer of high molecular mass; (B and C) dead polymers; e.o.f., electroosmotic flow. Redrawn from [12].

of the mechanisms of polymerization. The molecular structure of poly(N_ε-trifluoroacetyl-L-lysine) up to 38 monomers (m/z = 2156, z = 4) was confirmed by this NACE–multiple MS (MS^n) technique (see Fig. 2). The chemical nature of the end groups of the different polymer families separated in the capillary was identified. The detection and the identification of by-products (small molecules) by NACE–MS^n were also very informative on the polymerization mechanism [13]. To our knowledge, this work [13] was the first demonstration of the great possibilities of the combined use of NACE and MS to characterize synthetic polymers.

2.2. Polymer analysis by nonaqueous capillary gel electrophoresis (NACGE)

Capillary gel electrophoresis (CGE) is useful for the size-based separation of evenly charged polyelectrolytes. Indeed, the electrophoretic mobility of evenly charged polyelectrolyte is independent of the molecular mass (free draining behavior) above a certain limit in molecular mass that depends on the ionic strength of the polymer and on its stiffness [8]. Due to the high number of charges, polyelectrolytes are generally water-soluble polymers. For this reason, CGE or entangled polymer solution capillary electrophoresis are performed in water-based electrolytes. To our knowledge there is only one paper dealing with the size-based separation of synthetic polymers by CGE in nonaqueous buffers [14]. In this work, NACGE method has been developed for the analysis and characterization [molecular masses (M_r values)] of a series of synthetic, organic polymers, mainly polystyrenes and polymethylmethacrylates [14]. It is demonstrated that depending on the nature of the organic gel in the capillary and the buffer additives, polymers of known M_r values can be made to migrate through the capillary with time, generating a standard M_r values versus migration time calibration plot, analogous to what is derived for SEC analysis of the same polymers [14].

Although it deals with natural polymers, we found interesting to mention the work done by Lindberg and Roeraade [15] about the size-based separation of DNA fragments using a poly(dimethylacrylamide) matrix in *N*-methylformamide (NMF). The interest of this approach was to denaturate the DNA by the use of a nonaqueous electrolyte instead of using classical additives in water such as urea. The detection was performed by laser-induced fluorescence since UV detection in NMF is only possible for wavelengths above 250 nm. Gels in undiluted NMF were prepared and seemed to have similar denaturating ability as water-based gels containing 7 M urea. Nevertheless, the stability of the gel matrices was quite limited at high field strengths.

2.3. Polymer analysis by capillary electrochromatography

Recently, a new separation technique for macromolecular compounds based on capillary electrochromatography

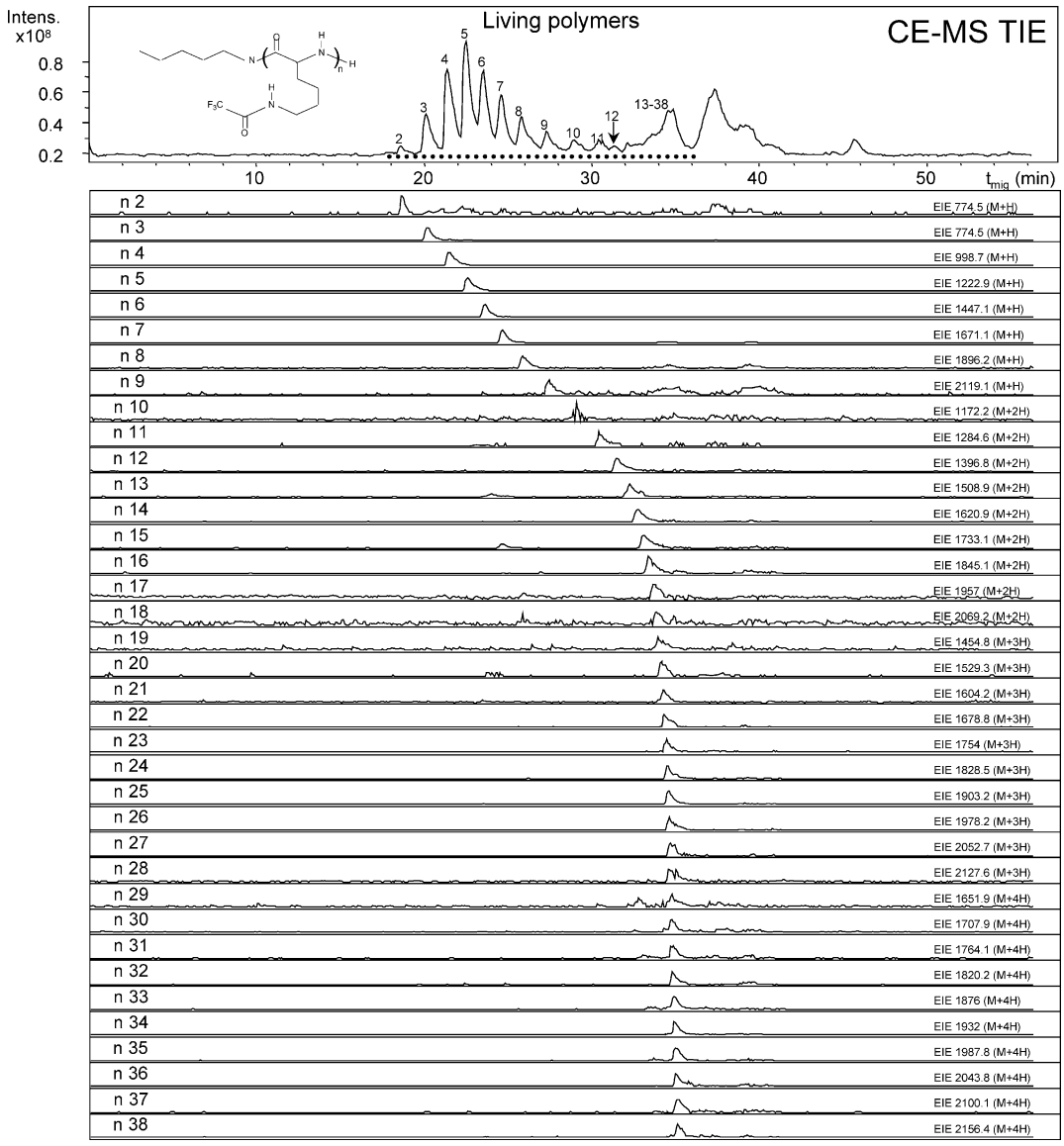

Fig. 2. CE-MS total ion electropherogram (TIE) and extracted ion electropherograms (EIE) of the living poly(N_ε-TFA-L-lysine), from $n = 2$ to $n = 38$ monomers under optimum CE-MS conditions. Fused silica capillary: 87 cm × 50 μm i.d. Sheath liquid composition: methanol–acetonitrile (87.5:12.5, v/v). CE separation buffer: 1 M acetic acid, 20 mM ammonium acetate in methanol–acetonitrile (87.5:12.5, v/v). Run voltage: +25 kV. Injection: 15 s at 0.5 p.s.i. (1 p.s.i. = 6894.76 Pa). ESI-MS conditions: nebulizer at 3 p.s.i.; dry gas at 4 L/min; dry temperature: 300 °C; sheath liquid flow: 0.3 mL/h (5 μL/min). Redrawn from [13].

has been developed [5,17,18]. The technique is called electrically driven size-exclusion chromatography (ED-SEC) or size-exclusion electrochromatography (SEEC). This technique employs capillary columns (typical i.d. of 30–100 μm) packed with bare silica particles (typically 3–10 μm), together with high dielectric constant solvents such as acetonitrile or dimethylformamide (DMF). Under these conditions, after applying the high voltage, a strong electroosmotic flow is generated and with it the macromolecules move within the capillary. Polymers are separated based on their different size due to the differential exclusion from different fractions of the mobile phase in the column. According to the authors, plate numbers in SEEC can be two to three times higher than in standard pressure-driven size-exclusion chromatography [5,17,18].

SEEC has been applied to the analysis of polystyrenes in packed capillaries with DMF as solvent [17]. In that work, an improvement of the efficiency was found for polystyrenes polymers by using SEEC compared to that obtained by standard pressure driven SEC analysis. Unfortunately, with SEEC the retention window seems to be smaller than under pressure conditions and separation appeared to depend strongly on the ionic strength of the mobile phase. This phenomenon was attributed to the occurrence of pore flow that was further studied in reference [18]. To do this, the applicability of SEEC for the separation of polystyrenes was investigated using capil-

lary columns packed with 5 μm particles with different pore sizes. It was found that under SEEC conditions, a significant intraparticle pore flow was generated. Besides, the relative intraparticle velocity with respect to the average interparticle velocity increased with the pore size and ionic strength. It was also observed that with increasing pore flows the plate height of polymers decreased considerably. On the other hand, the intraparticle velocity impaired the selectivity of the separation. These effects could be theoretically explained in that work [18].

Recently, it has been reported the use of rigid polymer monolithic capillary columns for the separation of polystyrenes by CEC [19]. However, the reported chromatogram shows an extremely low selectivity and only polymers with a very large difference in molecular mass could be separated by these columns.

3. Aqueous and hydro–organic CE of synthetic polymers

3.1. Polymer analysis by aqueous free solution capillary electrophoresis (FSCE)

One of the first application of FSCE has been the analysis of sulfated and carboxylated polystyrene nanospheres ranging from 39 to 700 nm [20]. Separation of these nanoparticles was achieved using a 50 μm internal diameter bare fused silica capillary [20]. In the same way, separation of chemically different latex particles (also bearing different numbers of attached carboxylate or sulphate groups) with higher particle size (from 30 to 1160 nm) has been also carried out by FSCE using a 75 μm internal diameter bare capillary [21]. In both applications, an UV absorption detector working at 254 nm and separation voltages of ca. 30 kV were used. These conditions provided analysis time in less than 5 min for most of the separations. In a later work, McCormick [22] demonstrated the separation of colloidal silica particles ranging in size from 5 to 500 nm by FSCE. The speed of these separations ($t < 20$ min) prevents analysis interferences due to aggregation reactions of the colloidal systems. On the other hand, efficiencies obtained during these separations were very low. A FSCE method was developed to separate composite particles formed by colloidal polyaniline (PANI) and poly-*N*-vinylpyrrolidone (PVP) from silica-PVP [23]. The possible aggregation between particles could be the responsible of the low reproducibility observed. Although separation of composite particles formed by colloidal silica and PVP, and the particles formed by polypyrrol (PPy) and poly(vinyl alcohol) (PVAL) could not be achieved, PANI–PVP was clearly separated from silica-PVP.

End-charged synthetic polymers can be separated by FSCE according to their molecular mass in the low molecular mass range (i.e., oligomers). In 1993, Bullock [24] developed a CE method for the separation of several Jeffamine ED series polymers using indirect UV detection. Optimal separations for the Jeffamine polymers of average molecular mass between 600 and 900 were obtained in less than 10 min. Several phthalate-derivatized poly(ethylene glycol) (PEG) and poly(oxyalkylene) amines (Jeffamine ED series) were analyzed by FSCE [25] using a polyacrylamide coated capillary in order to improve separation due to a reduction of the electroosmotic flow. Phthalic anhydride was used as derivatization reagent in order to provide charge and detectability. The potential of physically modified capillaries using polycations with different structures was studied for the characterization of positively charged poly(ethylene oxide) macromolecules [26]. Due to the lack of UV sensitivity of these compounds, indirect detection was used in this work. Fig. 3 shows the FSCE electropherograms obtained for Jeffamine 600 using different polycation fused-silica capillaries. As can be observed the electroosmotic mobility, that is characteristic of a given polycation, affected directly to the polymer separation. Better resolution was obtained for lower electroosmotic flow value as shown in Fig. 3C with a capillary modified by diethylaminoethyl dextran.

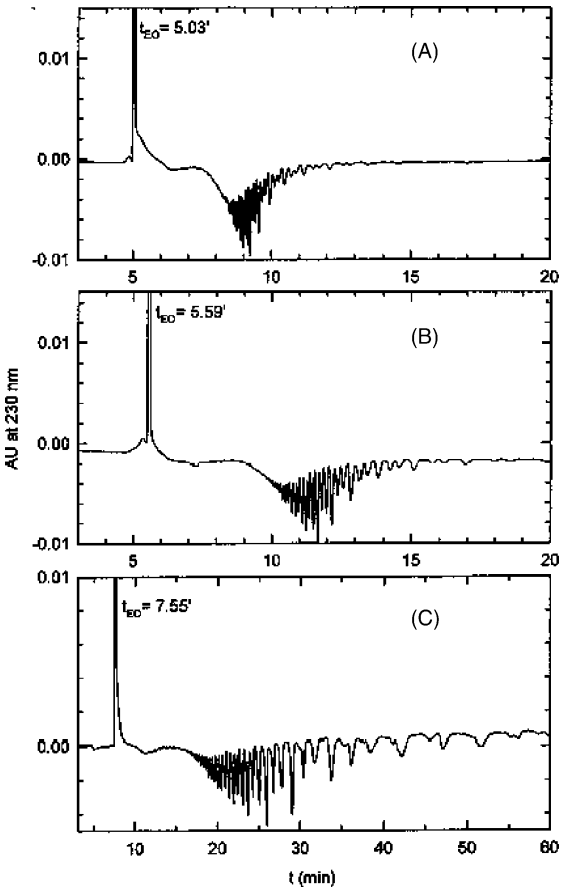

Fig. 3. Electropherograms of Jeffamine ED 600 as a function of the nature of the polycation used to modify the capillary surface. (A) Polydiethylaminoethyl methacrylate, (B) polydiallyldimethylammonium chloride, and (C) diethylaminoethyl dextran. Fused silica capillary: 57 cm l_t, 50 μm i.d. Running buffer: 25 mM creatinine at pH 4.8 (adjusted with acetic acid). Capillary temperature: 25 °C. Run voltage: −20 kV. Sample volume injected: 10.5 nl. UV detection at 230 nm. Redrawn from [26].

Vidil et al. [27] studied the potential of aqueous FSCE for the analysis of end charged oligomers of poly(D,L-lactic acid) as degradation by-products of poly(D,L-lactic acid). In this study, a basic borate buffer was used and the oligomers of poly(D,L-lactic acid) carried negative electrical charge via their carboxylate end group. Separation up to DP < 8 was obtained since oligomers with higher DPs were insoluble. Diastereoisomers of oligomers of poly(D,L-lactic acid) were also partially separated in a neutral phosphate buffer [28]. The use of FSCE with neutral phosphate buffer as the background electrolyte was applied to the analysis of other oligo(hydroxyacids), namely, oligomers of glycolic, D,L-3-hydroxybutyric and 6-hydroxyhexanoic acids [28,29]. Later, FSCE was used to study the hydrolytic degradation of gluconic–glycolic–lactid acid copolymer [30].

The separation of evenly charged polymers (polyelectrolytes) according to their molecular mass is not possible by FSCE above a certain value of molecular mass. Indeed, the friction of the counterion cloud is distributed all along the polyelectrolyte backbone. Thus, both charge and frictional coefficient are proportional to the polyelectrolyte molecular mass and the electrophoretic mobility is independent of the molecular mass. This is the so-called free-draining behavior of polyelectrolytes. Nevertheless, small evenly charged oligomers, with length smaller than or similar to Debye length, must be considered as small molecules and do not behave as polyelectrolytes. Cottet et al. [8] studied in detail the electrophoretic behavior in the crossover region between oligomers and polyelectrolytes. The first oligomers (DP < 10) of polystyrenesulfonates (PSSs) can be separated by FSCE due to the hydrodynamic coupling between monomers [8]. As shown in Fig. 4, their electrophoretic mobility is an increasing function of the DP [8,31]. Similar increase in mobility with increasing molecular mass has been observed for other synthetic oligomers such a polyphosphates [32], but also for natural polyelectrolytes such as polylysines [33], single-stranded DNA [34,35] and poly(deoxythymidines) [34,36]. For higher molecular masses, the electrophoretic mobility levels off or decreases slowly until it reaches a constant value corresponding to the free-draining behavior. It was demonstrated for polystyrenesulfonates [8] that the plateau in mobility was reached when the polyelectrolyte was long enough for being in a coil conformation, i.e., for DPs corresponding to more than a few persistence lengths (DP = 100 under 20 mM ionic strength, DP = 800 in pure water for PSSs). Similar results were reported for double stranded DNA for which the independence of the electrophoretic mobility was reached at about 170 base pairs (bp) (about one persistence length) in a 40 mM Tris–acetate–EDTA buffer [35]. To sum up, if high selectivity can be obtained for size-based separation by FSCE of relatively low molecular mass evenly charged oligomers, the peak assignment is not always straightforward since the variation of the electrophoretic mobility with DP is not monotonous (bell-shaped curve ending with a plateau, see Fig. 4). Similar bell-shaped curve was also reported for the separation of *N*-acetylneuraminic acid macromolecules for which adequate resolutions of polymers till a DP of 190 was achieved in the presence of a sieving matrix [37]. Moreover, in that paper [37], correlation between migration behavior and biological function of *N*-acetylneuraminic acid polymers was also described.

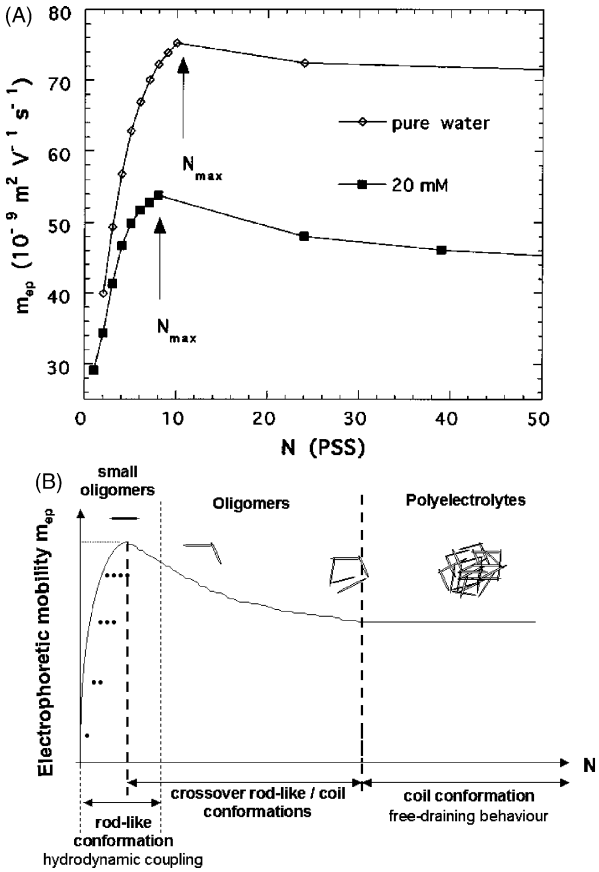

Fig. 4. Electrophoretic behaviour of evenly charged polyelectrolytes in FSCE as a function of the degree of polymerization. (A) Electrophoretic mobility of polystyrenesulfonates; (B) schematic representation of the conformational changes in correlation with the electrophoretic behaviour. Fused silica capillary, 33.5 cm (detector, 25 cm) × 50 μm i.d. Running buffer: 20 mM ionic strength borate buffer pH 9.2 or pure water. Capillary temperature: 27 °C. Run voltage: +7.5 kV. Hydrodynamic injection (5 kPa, 2 s). UV detection at 225 nm. The solid lines are guides for the eye. Redrawn from [8].

Polyphosphates are a good example of highly and evenly charged polymers that are used in many industries such as detergents, foods, and fertilizers. Glassy polyphosphate samples are very complex polymeric mixture with DP that can vary from ∼5 through at least 1000. Wang and Li [38] showed the separation of the first oligomers of polyphosphate and of some polycarboxylic acids by FSCE using adenosine 5′-triphosphate as a UV chromophore for indirect detection and cetyltrimethylammonium bromide as electroosmotic flow modifier. Better separation of polyphosphates by FSCE (up to about 30 monomers) was obtained in a buffer containing pyromellitic acid (for indirect UV detection), triethanolamine and hexamethonium hydroxide (for increasing the selectivity by ion-pairing) [39,40]. For more details on

the separation of phosphorus oxo anions and their polymers, the reader can refer to a recent review [40].

FSCE can also be very useful for the separation of polyelectrolytes varying in their charge density. This is for example the case when considering the separation according to the chemical composition of random copolymers containing both neutral and charged monomers. Hoagland et al. [41] demonstrated that the separation by FSCE in a basic phosphate buffer of acrylic acid/acrylamide copolymers according to the proportion of acrylic acid was possible up to 35–40% (in mol). Indeed, whatever the content in acrylic acid, as far as it is higher than 35–40%, the effective charge density of the copolymer is maintained constant due to the Manning's condensation effect. For example, a 100% acrylic acid polyelectrolyte has on average about 35% of its monomers that are effectively charged. The other 65% monomers are condensed by the counterions of the electrolyte, and thus, cannot be considered as effective charges. Thus, above 35% in nominal charge rate (acrylic acid proportion), the electrophoretic mobility is independent of the chemical composition of the copolymer. The critical nominal charge rate above which Manning's condensation occurs (about 35% in the case of a vinyl copolymer) depends on the average distance between successive monomers (for more details see for example [41]). Similar electrophoretic behavior was observed by Gao et al. [42] on random copolymer of 2-acrylamido-2-methylpropanesulfonate and acrylamide. The separation of three of these copolymers containing 10, 25, and 50% of charged monomers was shown. In summary, the separation of two variously charged polyelectrolytes having an hydrophilic backbone is possible by FSCE if their nominal charge densities are not both higher than the critical value given by the Manning's theory of condensation.

Cottet et al. [8] pointed out that the electrophoretic behavior of charged polyelectrolytes with hydrophobic backbone (variously sulfonated polystyrenesulfonates) was different from that observed for polyelectrolytes having hydrophilic backbone. Indeed, the electrophoretic mobility of PSSs increased steadily but slowly (in absolute value) with the sulfonation rate. The Manning behavior with a mobility plateau above a critical nominal charge rate was not observed. However, the separation of PSSs differing in their sulfonation rates was not possible in aqueous buffer because of very strong interactions between the PSSs when they were injected as a mixture. These interactions were hydrophobic and the use of an organic solvent (50% of MeOH) or of a surfactant (sodium dodecyl sulfate) in the electrolyte was required for suppressing it.

3.2. Polymer analysis by hydro–organic free solution capillary electrophoresis (FSCE)

The addition of organic solvents to a water-based BGE is in some cases necessary to increase the selectivity of the separation. This was exemplified by Bullock [24] on the separation of PEG (with molecular masses from 1000 to over 3500) derivatized with phthalic anhydride. In that work [24], addition of 30% of acetonitrile to a borate buffer containing 1,3-diaminopropane was necessary to increase the resolution of the PEG oligomers by diminishing the intensity of the electroosmotic flow (the anionic solute were migrating in counter-electroosmotic mode). Similar effect was observed after adding different quantities of acetonitrile or methanol to a 210 mM Tris aqueous buffer at pH 8.7, allowing nearly baseline separation of the oligomers from phthalate-derivatized PEGs and Jeffamines [25]. An example of the effect of acetonitrile on PEG resolution is shown in Fig. 5. As can be observed, addition of ACN reduces the electroosmotic flow, and consequently, resolution is increased.

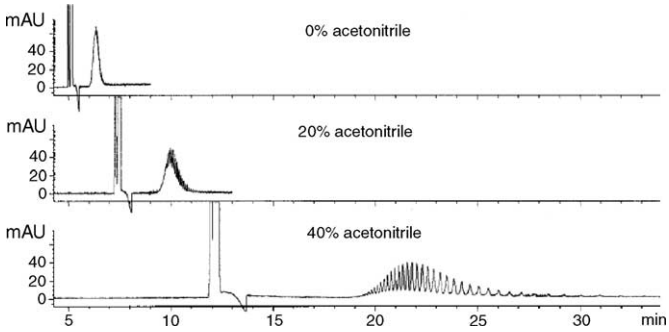

Fig. 5. Analysis of phtalate derivatized PEG 2000 (2 mM). Uncoated fused silica capillary: 67 cm l_t, 58.5 cm l_d, 50 μm i.d. Running buffer: 210 mM Tris at pH 8.7, with 0, 20, and 40% ACN. Capillary temperature: 25 °C. Run voltage: 30 kV. Injection at 50 mbar for 8 s. UV detection at 205 nm. Redrawn from [25].

Analysis of Jeffamine ED series derivatized with a fluorescent dye as 2,3-naphthalenedialdehyde (NDA) has also been demonstrated using a running buffer containing methanol [43]. By means of this procedure it was possible the separation in less than 15 min of 30 Jeffamines with average molecular mass of 600 differing in one $-CH_2-$ group between them. Oudhoff et al. [44] used a borate buffer containing 30% of an organic solvent (THF, MeOH or ACN) for complete oligomeric baseline separation of a phthalate-PEG 600. Although peak shapes were similar using the different organic additives (THF, MeOH or ACN), better resolutions between higher molecular mass oligomers were obtained using methanol and THF because of higher reduction of the electroosmotic flow velocity with these latter organic modifiers. PEG with higher molecular masses have also been separated using a sieving matrix (in aqueous electrolyte, see below) [45] or through the formation of DNA–PEG conjugates [46].

Fatty alcohol ethoxylates (FAEs) are the most important nonionic surfactants used in commercial formulations. They are produced by condensation of ethylene oxide with fatty alcohols. Separation of FAEs after their derivatization with phthalic anhydride was achieved by FSCE using a 150 mM borate buffer at pH 8.5 containing 50% MeOH [47].

Mengerink et al. [48] demonstrated that hydro–organic FSCE can also be used to separate low solubility linear

oligomers of polyamide-6. Polyamide-6 oligomers were solubilized and positively charged, via their amine end group, using an acidic phosphate buffer containing 65% (v/v) of hexafluoroisopropanol (HFIP). The oligomers were separated according to their charge-to-mass ratio, with the low molecular mass compounds migrating first. Due to the high price of HFIP, FSCE is an attractive alternative to other classical procedures, as e.g., size-exclusion chromatography, for the analysis of polymer (as e.g., the mentioned polyamides) because of the low consumption of solvents.

FSCE of commercial bisphenol A ethoxylate dimethacrylates (Bis-EMA) was carried out using 30 mM formic acid adjusted to pH 4.0 with ammonium hydroxide and 20% (v/v) acetonitrile as BGE. Prior to their FSCE analysis, Bis-EMA compounds were transformed into ionizable amines by derivatization. Formation of the derivatives was confirmed by electrospray ionization MS. FSCE was used to determine the different average number of ethoxy groups per Bis-EMA. For Bis-EMA with 30 ethoxy groups in average, about 23 homologues could be differentiated. The high-resolution power of FSCE enabled characterization of commercial dental composite material [49].

3.3. Polymer analysis by aqueous capillary gel electrophoresis (CGE)

Size-based separation of polyelectrolytes with different molecular masses can be obtained by CGE. A cross-linked gel capillary, or an entangled polymer solution, is used to generate the sieving effect. Covalently bonded gels (usually cross-linked polyacrylamide) were initially used in CE for size-based separations. However, their application to synthetic polymer analysis is very rare. Garcia and Henion [50] used a commercial gel-filled capillary column containing cross-linked polyacrylamide to achieve separation of a mixture of poly(acrylic acids) by CGE coupled to mass spectrometry through a liquid junction-interface [50]. Cross-linked polyacrylamide gel-filled columns were also used by Wallingford [45] for the separation of PEG oligomers, and ionic and nonionic ethoxylated surfactants. The neutral polymers were derivatized with phthalic anhydride in order to provide them charge and detectability by UV at 280 nm. Fig. 6 shows an example of the different selectivity that can be achieved by using FSCE and CGE for the separation of synthetic polymers [45]. CGE was successful to baseline resolve more than 54 oligomers of AP40P, a phosphated alkylphenol ethoxylate surfactant (Fig. 6B). The use of cross-linked polyacrylamide gel-filled columns showed two main drawbacks: long analysis time (almost 90 min) and low stability and durability of the gel-filled columns used. Currently these gels are being substituted by non cross-linked polymers (for example cellulose derivatives, polyacrylamide, poly(vinylalcohol) derivatives, dextran), since they have better stability and less reproducibility problems. In this way, a running buffer containing dextran was used as molecular sieving for 1,2,4-benzenetricarboxylic anhydride (BTA) derivatized-PEG and ethoxylated surfac-

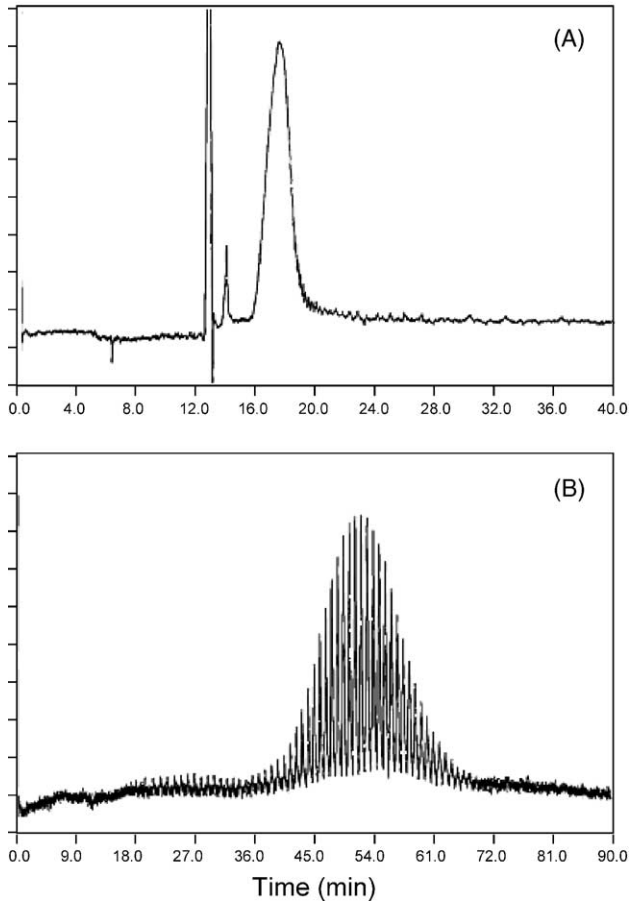

Fig. 6. (A) Hydro–organic FSCE separation of AP40P. Uncoated fused silica capillary: 58 cm, 75 μm i.d. Running buffer: 6 mM $Na_2B_4O_7$–10 mM $NaHPO_4$–20% CH_3CN–30% methanol, at pH 7. Run voltage: 30 kV. Gravity injection at 25 mm for 25 s. UV detection at 206 nm. (B) CGE separation of AP40P. Column μ-PAGE-3 without urea. Running buffer: Tris–borate at pH 8.3. Field strength −220 V/cm. Electrokinetic injection at −5000 V for 5 s. UV detection at 230 nm. Redrawn from [45].

tants analysis [51]. They found that under their separation conditions, migration time was linearly dependent on analyte molecular mass.

The first application of synthetic polymers analysis by CGE using an entangled polymer solution was presented by Poli and Schure [52]. They developed a method for CGE separation of eight poly(styrenesulfonates) (with molecular masses from 1.8×10^3 to 1.2×10^6), in less than 10 min, adding hydroxyethyl cellulose (HEC) to the running buffer. A comparison between CGE and size-exclusion chromatography for polymer characterization was shown in that work. It was observed that CGE provided better results in terms of resolution, efficiency and fractionating power. Moreover, it was demonstrated that CGE is three to five times faster than SEC.

In a later work, separation selectivity and migration regimes of PSSs were investigated in HEC solutions [53]. Cottet and Gareil [54] carried out a study of the electrophoretic behavior of PSSs (with molecular masses ranging from 16×10^3 to 9.9×10^5) with regard to the nature (HEC and polyethyleneoxide) and concentration of the separating

polymer, as well as the electric field, and the ionic strength and the nature of the buffer counterion. Later, the effect of the mesh size (or blob size) of the separating network as well as the effect of network dynamics on the size-based separation of PSSs were studied [55]. The mesh size is a decreasing function of the separating polymer concentration while the network dynamics only depends on the molecular mass of the separating polymer. Quantitative estimations of the mesh size and of the network dynamics as a function of the separating polymer characteristics (concentration and molecular masses) are given in [55]. It was demonstrated that, for a given mesh size (i.e., for a given separating polymer concentration), the molecular mass of the separating polymer (and thus, the network dynamics) had a large influence on the polyelectrolyte mobility below a threshold value. It was demonstrated that, at this threshold value, the lifetime of the mesh was of the same order of magnitude of the time taken by the PSS to migrate on the mesh (or blob) size. Above this threshold value, the molecular mass of the separating polymer had practically no influence on the PSS mobility at a given separating polymer concentration. Interestingly, the PSS electrophoretic mobility was close to be a universal function of the ratio of the mesh size to the radius of gyration. Cottet and Gareil [56] later demonstrated that the use of binary polymer mixtures of two different molecular masses but of same nature can be a convenient alternative to modulate the dynamics of the network. The influences of ionic strength, mesh size and electric field were also investigated concurrently to that of temperature [57]. Using the activation energy concept, this study allowed monitoring analyte and matrix deformations under given experimental conditions. For example, the increase of the ionic strength resulted in higher activation energies, and thus, in the PSS deformations being reduced, explaining why improvement in size resolution was achieved under increasing ionic strength. Mechanisms of separation of PSSs in dilute neutral polymer solution were also investigated by Starkweather et al. [58]. Namely, that work [58] examines how the molecular weight dependent mobility of PSS depends on the molecular weight and concentration of various dilute pullulans used in the background electrolyte. As a conclusion, three parameters (entanglement probability, average entanglement duration, and average entanglement displacement) are used to explain observed variations in electrophoretic mobility of PSS.

Other sieving media such as dextrans, with a good water solubility and low UV absorption at wavelengths higher than 200 nm, have been used for the analysis of anionic (PSS) and cationic [poly(2-vinylpyridines)] synthetic polyelectrolytes, using bare fused-silica capillaries and coated capillaries, respectively [59]. Welch and coworkers used pullulan solutions as sieving matrices [58,60,61] and added a cationic surfactant in the electrolyte for limiting the solute [poly(2-vinylpyridines)] adsorption [60]. Grosche et al. [62] showed that molecular mass averages and polydispersity indexes can be measured by CE using entangled polymer solutions. They also studied the possibility to perform indirect detection in the presence of a neutral entangled polymer solution [62]. Polydiallyldimethylammonium of different molecular masses were separated with pyridine as background electrolyte. However, high peak asymmetry of the high molecular mass polyelectrolytes was due to dispersion by electromigration.

CGE has also been used for particle analysis made of synthetic polymers. Thus, Radko et al. [63] used a buffer solution containing uncrosslinked polyacrylamide to achieve size-based separation of fluorescein isothiocyanate-derivatized polystyrene carboxylate (PSC) particles with sizes ranging from 2.8 to 10.3 μm. It is interesting to mention that in this case the limits of particle diameter lies close to 10 μm, as a result of the fluctuation of the fluorescence observed due to the progressive light scattering of the particle. CGE has also allowed the separation of highly charged inorganic polymers such as polymers of condensed phosphated [64] using linear polyacrylamide gel in the running buffer.

3.4. Polymer analysis by aqueous micellar electrokinetic chromatography (MEKC)

MEKC is a mode of capillary electrophoresis in which uncharged compounds are separated depending on their different hydrophobicity, through the addition of a surfactant to the running buffer. Surfactant, above a given concentration in the running buffer, forms micelles that act as pseudo stationary phase. Separation of solutes is obtained due to the different interactions that take place between micelles and analytes.

In 1999, Gallardo et al. [65] developed the first MEKC method for the characterization of high molecular mass copolymer systems. MEKC was used to monitor the preparation of copolymers from free radical polymerization of *N*-vinylpyrrolidone (VP) and 2-hydroxyethyl methacrylate (HEMA) at different conversion degrees [65]. It could be observed that this reaction showed a bimodal behavior in which copolymers rich in HEMA were initially formed, while after 24 h a second family of compounds appeared corresponding to copolymers rich in VP. These results were confirmed by the kinetic analysis of this reaction. A comparison between MEKC and SEC was made, observing that MEKC and SEC provided complementary information with respect to the average composition of copolymer chains and their macromolecular size and size distribution. These results allowed, in two following works, the application of MEKC for the control of cyclosporine released from VP–HEMA copolymer systems. These works proved, using in vitro [66] and in vivo [67] assays, the dependence of release velocity of the drug on copolymer composition.

Recently, aqueous MEKC has been used to monitor the radical copolymerization reactions of 2-hydroxyethylmethacrylate)–(2-acrylamide-2-methylpropanesulfonic acid (HEMA–AMPS), and *N*,*N*-dimethylacrylamide)–(2-acrylamide-2-methylpropanesulfonic acid (DMAA–AMPS) [68]. The effect of the conversion and chemical composition of HEMA–AMPS and DMAA–AMPS, on the distribution as well as on the molecular mass were analyzed by using this MEKC method. The large possibilities of MEKC for

obtaining information about synthesis progress, nature and composition of the formed ionic copolymers were demonstrated. Moreover, it was shown that capillary electrophoresis instrumentation can be used to monitor the electrical conductivity of the reaction product obtained at different copolymerization stages of the HEMA–AMPS system in order to clarify the polymerization mechanism.

Polyanionic macromolecules made of poly(*N*-vinylpyrrolidone-co-maleic acid) were separated by MEKC due to their hydrophobicity and charge/mass ratio [69]. Because of the similar charge/mass ratio of these macromolecules, addition of SDS was needed to reach their complete separation.

3.5. Polymer analysis by hydro–organic micellar electrokinetic chromatography (MEKC)

Micelles do not form in most nonaqueous solvents because solvophobic interactions are too weak for surfactant molecules to aggregate (see e.g., [16]). The weakness of the solvophobic interactions in nonaqueous solvents in comparison with water is due to low cohesion energy density of most of these solvents. Formamide is an exception. Micelles can form in formamide but at higher temperatures and higher surfactant concentrations than in water. However, to our knowledge, there is no publication reporting MEKC in formamide neither for small molecules or polymer analysis. On the other hand, the addition of an organic solvent in aqueous MEKC is very often used for controlling the partitioning of the solute between the liquid and the pseudo stationary phases. The solvent content may also help for optimizing the electroosmotic flow intensity and thus the apparent selectivity. Furthermore, the organic solvent can partially or completely break the micelles or aggregates of the electrolyte. The use of hydro–organic MEKC has been applied in several works for the separation of neutral surfactants [70]. Thus, Bullock [24] demonstrated by conductivity measurements that micelles were not formed when 35% ACN was added in a 25 mM boric acid electrolyte containing different concentrations in SDS. These experiments were further ratified by Cifuentes et al. [71] using directly a CE instrument as a conductimeter. For neutral surfactants, the interaction with the SDS was mainly located on the hydrophobic tail of the solute since the absolute value of the effective mobility was a decreasing function of the DP. Neutral surfactants as Triton X series oligomers (from 1 to 46 units) with significant hydrophobic character have been separated by hydro–organic MEKC in less than 20 min using a borate buffer with the addition of SDS and ACN. The migration of the Triton oligomers is thus obtained by solvophobic association between solute and surfactant molecules [24]. In a later work, sulfate and sulfonate surfactants additives and high concentrations of organic solvents were used for the separation of nonionic surfactants of the alkylphenol polyoxyethylene type as ethylene oxide homologues [72].

Bile salts such as sodium deoxycholate (SDC) and sodium cholate (SC) were used to separate octyl- and nonylphenol ethoxylates (OPEs and NPEs), important nonionic surfactants used in numerous industrial processes. Although resolution was not modified by addition of *n*-propanol or acetonitrile, electropherograms with slightly less noisy background were obtained. The optimized method was used for the analysis of commercial samples with different quantities of industrial NPE [73].

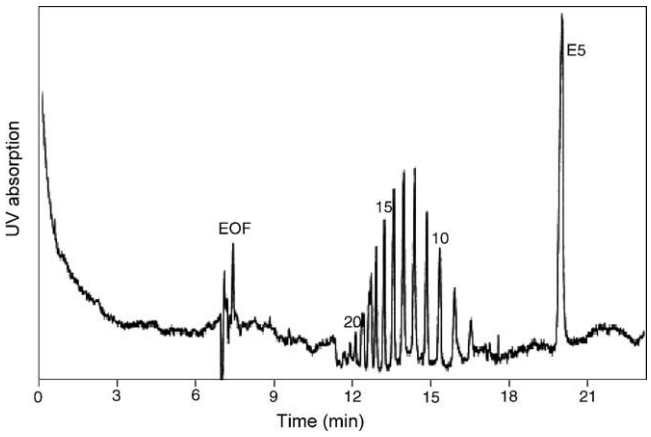

Fig. 7. Hydro–organic MEKC analysis of a cosmetic product containing PEG with chain lengths between 8 and 21. Uncoated fused silica capillary: 58 cm l_t, 44 cm l_d, 50 μm i.d. Running buffer: 20 mM borax, 50 mM SDS, 20% THF. Run voltage: 25 kV. Injection at 50 mbar for 6 s. UV detection at 234 nm. Peak E5: internal standard. Redrawn from [44].

Different volume percentages of methanol in the background electrolyte (10 mM sodium dodecylsulfate, 100 mM borate–50 mM phosphate buffer, pH 7.0) were used for the MEKC separation of monomeric constituents of ethoxylated bisphenol A (Bis-EMA) with between 2 and 15 ethoxy groups per phenol in the molecule. The conditions allowed the differentiation of the lower from the higher Bis-EMA homologues and of isomers, and enabled the characterization of commercial dental composite materials [74].

Recently, phenyl isocyanate derivatized mixtures of polypropylene glycol (PPG) and PEG of similar molecular masses (about 400) were separated in a 20 mM borate buffer containing 80 mM SDS and 30% ACN [44]. Contrary to the PEG electrophoretic behavior, the PPG electrophoretic mobility was an increasing function of the DP, what could be explained by PPG chain interactions with SDS. An example of an hydro–organic MEKC separation of PEG oligomers of low M_r is shown in Fig. 7, where a 20 mM borate buffer containing 50 mM SDS and 20% THF was used for the analysis of a cosmetic product containing this polymer.

4. Application of CE for characterization of special properties and physico-chemical parameters of complex polymer systems

4.1. Monitoring polymer drug delivery systems (PDDS)

Monitoring a polymer drug delivery system (PDDS) requires frequently the development of a separation method

able to follow both the drug release and the polymer degradation (or solubilization) during in vitro or in vivo assays. In this way, a more complete picture about the release (and/or degradation of the polymeric device) can be obtained. Ideally, this information should be derived from a single analysis. This requirement has increased the need for more reliable analytical methodologies able to characterize simultaneously these materials. Hopefully, drug and polymer usually differ in thousands of Da, and show very different physicochemical properties that can make easier their separation.

Recently, a review about the use of CE to monitor PDDS has been published [75] in which CE is demonstrated to be a powerful analytical tool that can provide useful information about the chemical properties of these complex devices [66,67,76–85]. Thus, fast, reproducible, and efficient separations can be obtained for both polymers and drugs in a single analysis by using CE methods. One of the main characteristics of CE is that this technique makes possible the development of uniquely tailored separation procedures to monitor PDDS of very different nature. Interestingly, such information can be in some cases complementary to that provided by other classical techniques as HPLC.

The main advantages of CE are the high speed of analysis (generally under 10–20 min per run, which, given the large number of samples that have to be analyzed during any in vitro or in vivo experiment, deserves to be taken into account), high separation efficiency (usually in the interval from 10^5 to 10^6 theoretical plates per meter of column, depending on the type of analyte and the separation conditions) and the small volumes of samples required (only a few nanoliters are injected, which becomes a very interesting property for in vivo experiments). Moreover, it is important to mention that CE allows the simultaneous monitoring of both the polymer used in the PDDS and the released drug in a single run. This interesting property can make possible for instance the study of the control of the resorption rate of the polymeric delivery systems (and the influence of the composition percentages of the different monomeric unities) on the drug release [66,76]. As an example, FSCE was used for monitoring growth hormone (GH) released from different copolymeric devices (i.e., films and slabs) made of vinylpyrrolidone–hydroxyethyl methacrylate (VP–HEMA). It was observed that GH released rate is controlled by copolymer composition, and it was possible to simultaneously monitor by FSCE the release of GH and the polymer dissolved during experiments. Interestingly, it was demonstrated that both data were connected [76]. In the same way, MEKC was used for monitoring cyclosporine released from VP–HEMA [66].

The main effort of the current and future drug delivery systems (DDS) will be focused on the delivery of non-traditional drugs or active compounds as genes, peptides, proteins as enzymes, hormones, vaccines, etc. The gene therapy field is especially remarkable, and great efforts are being directed to the development of efficient non-viral vectors, mainly based on cationic polymer systems. Improvements in chronopharmacokinetics, in the preparation of nano-DDS, in the optimization of mucoadhesive devices, in the use of clean technologies based on supercritical fluids, and in the development of self-regulator DDS for the delivery of insulin and other therapeutics, will probably be also very relevant issues within the DDS field.

4.2. Synthetic polyzwitterions and polyampholytes

Bohrisch et al. [86,87] studied, by short-end injection FSCE, the electrophoretic behavior of polycarboxybetaines (highly dipolar polyelectrolytes) that bear one permanent cationic charge via a quaternized ammonium group and a weak acidic group (carboxylate). The variation of the electrophoretic mobility in the pH range 1.8–4.0 was studied as a function of the chemical nature of the substituents of the ammonium group and as a function of the flexibility and the length of the spacer groups between the opposite charges. Lower mobilities were measured when the intramolecular association between the carboxylate group and the quaternary ammonium group was facilitated (shielding of the ammonium by bulky substituents or flexibility of the spacer group). Both intra- and intermolecular associations between different chains of polycarboxybetaines were also pointed out by injection of mixtures [62,86].

Synthetic polyampholytes are polymers containing both weak acid and basic groups. Similar to proteins, the sign of the electrophoretic mobility of such polymers depends on the pH of the electrolyte. Thus, it is possible to use aqueous CZE to determine the isoelectric point (p*I*) by measuring the effective electrophoretic mobility as a function of the pH of the electrolyte at a constant ionic strength. The ionization point is determined by extrapolation of the effective mobility to zero value. Recently, Martin and Engelhardt [88,89] applied this methodology for the determination of the p*I* of synthetic polycarboxybetains and poly(alkylphenyl maleamide acid-alt-diallyl alkyl amines). They compared the p*I* experimental values (from 4.5 to 5.5) determined by CZE with the values derived from capillary isoelectric focusing (CIEF) experiments. Both methods gave similar results but the CZE approach was more time-consuming and CIEF method can help to detect impurities or heterogeneities of the polymer.

4.3. Separation of dendrimers

Pesak and Moore [90] showed the separation of phenylacetylene dendrimers terminated with *tert*-butyl esters on their periphery from phenylacetylene dendrimers terminated with carboxylic acids. These latter dendrimers were obtained from a last step thermolysis reaction able to convert *tert*-butyl ester end groups to carboxylic acids. CZE was able to characterize the heterogeneities of the dendrimer resulting from the incomplete thermolysis reaction since the charge of the dendrimer depended on the number of carboxylic groups. Separation of dendrimers of different generations was also obtained. The information provided by FSCE was very helpful to finally prepare more homogeneous dendrimers. Broth-

ers et al. [91] reported the separation of polyamidoamine (PAMAM) dendrimers by CZE and slab-gel electrophoresis. Five full-generations PAMAM dendrimers (positively charged) were separated in a 0.1 M phosphate buffer pH 2.8. The use of a phosphate buffer and the relatively high ionic strength restricted the adsorption of the dendrimers onto the fused silica capillary wall. Under these electrophoretic conditions, the lower generations have higher effective mobilities (in absolute value) than the higher generations. The electrophoretic behavior of dendrimers is not yet clearly understood and the origin of the selectivity in the separation of the different generation remains unclear. Half generation PAMAM dendrimers with neutral ester end groups were hydrolyzed in alkaline conditions into carboxylate dendrimer derivatives. CZE provides a useful analytical strategy for separating various degrees of terminal group substitution (from the ester to the carboxylate in [91]). Similar separations of PAMAM dendrimers were also published by Ebber et al. [92].

4.4. Separation of fullerenes and carbon nanotubes

Empty fullerenes (C_{60}, C_{70}, and C_{84}) and several acidic, neutral or basic derivatives of C_{60} were separated by Wan et al. by NACE [93]. Even if fullerenes may not be considered as polymers, they constitute a good example of high molecular mass non water-soluble analytes that can be separated by NACE. The cationic pyrrolidine derivative of C_{60} was first separated from the neutral C_{60} fullerene in a nonaqueous electrolyte constituted of 68% ACN, 20% chlorobenzene, 10% MeOH, 1% acetic acid, 1% trifuoroacetic acid, and 20 mM ammonium acetate. Chlorobenzene was added to increase solubility and trifluoroacetic acid was added to maintain the ability to protonate in solvent of low polarity. Simultaneous analysis of both acidic (carboxylic group) and basic fullerene derivatives was reported in an electrolyte where pyrrolidine derivative was partially protonated and succinic acid derivative was partially deprotonated. Separation of neutral fullerenes was obtained through solvophobic interactions between the analytes and tetraalkyl ammonium salts. The separation of the three fullerenes (C_{60}, C_{70}, and C_{84}) was demonstrated in a nonaqueous electrolyte containing 100 mM tetra-*n*-decylammonium bromide and 50 mM tetraethylammonium bromide. The long-chain tetraalkylammonium salt induced the electrophoretic migration of the neutral solutes by solvophobic interaction while the short-chain tetraalkylammonium salt mainly reduced the e.o.f.

Treubig and Brown [94] proposed a strategy based on MEKC for the separation of neutral fullerenes. C_{60}- and C_{70}-SDS complexes were separated in a basic phosphate buffer containing 100 mM of SDS.

Tamisier-Karolak et al. [95] described the electrophoretic behavior of dendro-60-fullerene, a highly anionic water-soluble fullerene derivative, in hydro-aqueous electrolyte containing various content of methanol. The e.o.f. intensity was reduced by the addition of hydropropylcellulose (from 0.075 to 0.125%) to decrease the analysis time.

Recently, Doorn et al. [96] demonstrated the possibility of separating carbon nanotubes by CZE. Samples were dissolved in an aqueous solution containing 0.5% SDS and were separated in 50 mM Trizma-base electrolyte containing 0.5% SDS. The nanotubes (typically, 0.1–10 μm in length) are too large to reside in the intramicellar region. However, the hydrophobic SDS tail interacts with the nanotube surface, providing it with a negative charge. Moreover, the electrostatic repulsion between tubes stabilizes them against van der Waals attraction. The nanotubes were either detected by UV absorbance at 360 nm (commercial apparatus) or by Raman detection (lab-built CE apparatus) observing very sharp peaks. Run-to-run changes in the number of observed peaks and their position were likely due to the heterogeneous nature of the nanotube suspension. The differences in mobility between fractions were due to differences in nanotube lengths and/or diameter [96,97]. The separation mechanism was likely based on alignment of the nanotube along the electric field.

4.5. Characterization of associative copolymers

CZE is also a valuable tool for studying changes in polymer structures. A nice example was reported by Morishima [98] for the characterization of hydrophobically-modified random copolymers. Such copolymers may form unimolecular flower-like micelles (second-order structure) for low content in hydrophobic monomers. As the number of hydrophobic monomers on the polymer chain is increased, the second-order structure may collapse into a third-order yielding to a highly compact assembly. The collapse of the second-order structure into the third-order structure can be observed by aqueous CZE. The peak corresponding to the copolymer became much broader as the content of hydrophobic monomers f_{hydro} increased up to 30% in mol. When f_{hydro} was increased to 40% in mol, the band abruptly became very narrow indicating the collapse of the second-order structure into the third-order structure.

Collet at al. [99] demonstrated that hydrophobically modified poly(acrylic acids) containing 0–10% in mol of hydrophobic monomers (dodecyl acrylamide monomer) can be separated according to their hydrophobicity by FSCE in the presence of a nonionic or a zwitterionic surfactants. The separation mechanism was interpreted as an expansion of the polymer coil in the presence of micelles and subsequent change of its frictional properties.

Amphiphilic diblock copolymers generally form micelles in aqueous solutions. In aqueous media, the unimer–micelle equilibrium is kinetically frozen when micelles are formed by long hydrophobic blocks such as high molecular mass polystyrene (PS). Thus, the injection of a mixture of PS–poly(methacrylic acid) (PS–PMA) micelles with PS–polyethyleneoxide (PS–PEO) micelles led to two distinct and well-separated peaks in CZE (borate buffer) [100]. The first peak corresponded to the neutral PS–polyethyleneoxide micelles and the second peak was assigned to PS–poly(methacrylic acid) micelles. The presence

of two peaks was due to the absence of exchange of the copolymers between the two micelles. Conversely, the electropherogram corresponding to hybrid micelles prepared by dialyzing a mixture of PS–PMA and PS–PEO copolymers from a dioxane–water (80:20, v/v) mixture into an alkaline borate buffer, led to only one peak. This peak corresponded to hybrid micelles. The electrophoretic mobility of the hybrid micelles depended on the proportion of PS–PMA content in the micelle. Thus, CZE is a valuable analytical tool that can give information on the kinetic of exchange of the copolymers between different micelles, but also on the composition of the micelles via the electrophoretic mobility value.

Cottet at al. [101] also studied the interest of aqueous and hydro-aqueous CZE for the characterization of associative diblock copolymers. Diblock copolymers were composed of a sodium poly(styrene sulfonate) hydrophilic block and a poly(ethylene propylene) or a poly(*tert*-butylstyrene) hydrophobic tail. The electropherograms of such copolymers showed two peaks. The major peak was attributed to the copolymer micelle while the second was assigned to the free copolymer unimer. The detection of two well-defined peaks implied that the kinetic of exchange of the copolymer between the micelle and the free states was very slow in comparison with the time-scale of the electrophoretic process. The relatively important proportion of unimers was likely due to the chemical heterogeneities of the block copolymers despite their synthesis were performed by anionic polymerization. The unimers detected by FSCE were likely enriched in hydrophilic monomers in comparison with the copolymer micelles, and thus did not associate. In this case, CZE is an effective separation technique amenable to quantify the quantity of unimers in the sample. The increase in the kinetic rate of exchange and/or the breaking of copolymer micelles by the addition of an organic solvent was also monitored by CZE.

4.6. Determination of kinetic constants relative to polymer systems

Vidil et al. [27] (see Section 3.1) used FSCE for monitoring the late stages of the hydrolytic degradation of high molecular mass poly(L,D-lactic acid). They were able to determine the rate constant and the activation energy of lactoyllactate (dimer) hydrolysis into lactate.

A kinetic study of the polymerization of α-aminoacid *N*-carboxyanhydrides (NCA) in aqueous solution was performed by Plasson et al. [102] using FSCE. Kinetic constants relative to the hydrolysis of NCA and its coupling with aminoacid and some synthetic oligopeptides were determined, taking L-valine as model compounds.

5. Conclusions and future outlooks

The complexity of the chemical composition of synthetic macromolecules has increased the need for more reliable analytical methodologies for characterizing these materials. CE has emerged as a powerful analytical tool able to provide useful information about the physicochemical properties of these complex molecules. Interestingly, information obtained by CE is often complementary to that provided by other classical techniques. For instance, characterization according to the polymer functionalities or separation according to the chemical composition can bring complementary information in comparison with that obtained by the currently used size-exclusion chromatography. In this review, it has been demonstrated that CE is being used in their different modes (mostly, FSCE, CGE, and MEKC) together with buffers of very different nature (nonaqueous, aqueous, and hydro–organic BGE) to successfully face the tremendous diversity that can be found during synthetic polymers analysis. The use of non-aqueous electrolytes enlarges considerably the field of applications of CE for the analysis of synthetic polymers. This new opportunity together with the complementarity and the versatility of CE techniques constitute a new important analytical tool for the polymer chemist. For these reasons, we believe that the number of CE applications in the field of polymer chemistry will rapidly increase in the non-distant future.

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