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Review

Capillary electrophoresis of proteins and nucleic acids in gels and entangled polymer solutions

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Abstract

Slab gel electrophoresis is still the predominant technique for the separation of proteins and nucleic acids. Capillary electrophoresis, however, has potentially many advantages over the traditional slab gels and in the last few years there has been a steady transition from the slab to the capillary format. Early attempts to separate biological macromolecules were based on gel-filled capillaries, but soon it was discovered that the use of entangled polymer solutions offers several advantages over gels. It has now become possible to separate almost all species of macromolecules in such transient networks.

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1. Introduction

Electrophoresis in gels (e.g., polyacrylamide or agarose) is a powerful tool for separating biological macromolecules such as DNA and proteins and a variety of different electrophoretic techniques have been developed. These separations are effected in slab or rod-like gels at low electric fields. However, pouring and running the gel can be slow and time consuming. Detection and quantification are only possible by using post-separation procedures, e.g., densitometric or image analysis.

In capillary electrophoresis (CE), separation takes place in a thin fused-silica capillary, with on-line detection by absorbance or fluorescence. During the last 10 years, this technique has been developed into a powerful analytical method and has permitted breakthroughs in the separation of small organic and inorganic ions. These developments have also made it possible to exploit the potential of this method for the separation of large biopolymers.

In principle, capillary electrophoresis has several advantages over slab gel electrophoresis. Because of the small diameter of the capillaries, heat dissipation is very effective and band broadening due to Joule heating is minimized. Strong electric fields (up to 400 V/cm and more) can be used, therefore reducing the run time and diffusion. Capillaries are available in a variety of diameters (about 10–300 μm) and their length can be chosen in a wide range. The sensitivity is very high and minute amounts of sample can be analysed. Because of the reduced bandwidth, capillary electrophoresis has potentially a very high separation efficiency (several million theoretical plates per metre) and we should expect a better performance compared with slab gel systems. In contrast to slab gels, only one sample can be loaded at a time, but capillary arrays have been proposed to circumvent the problem [1–3].

In summary, capillary electrophoresis offers the possibility of rapid and automated analysis with high reproducibility and improved quantification.

2. The separation matrix

2.1. Gels versus polymer solutions

In traditional slab gel electrophoresis, the gel has two functions: it serves as an anti-convective medium and also as a "sieving" matrix that provides separation. Because of the low convection, CE permits a gel-free separation at high voltages, and thus yields unprecedented resolution and speed. This is important if ions or native proteins with different charge-to-mass ratios and therefore different electrophoretic mobilities are to be separated.

However, the situation is different for biopolymers such as RNA, double-stranded DNA (restriction fragments or PCR products) single-stranded DNA (sequencing fragments or oligonucleotides) or sodium dodecyl sulfate (SDS)-denatured proteins, which have a constant charge-to-size ratio. In this case, no separation occurs in free solution, and some sort of sieving matrix still has to be used (however, the anti-convective property of the matrix is no longer needed).

Early attempts to apply CE to the size separation of biomolecules, especially for the separation of small molecules, were based on gel-filled capillaries (e.g., cross-linked polyacrylamide) [4–6]. The gels are prepared in the same manner as slab gels, by adding the catalysts to the monomer solution, which is then pumped into the capillary, where the polymerization takes place. The capillary is previously treated with 3-methacryloxypropyltrimethoxysilane, which fixes the gel to the inner wall and prevents it from being extruded by electroosmotic forces.

However, gel-filled capillaries have several disadvantages. First, the filling of the capillary has to be done with great caution in order to avoid the introduction of air bubbles. Shrinkage of the gel during polymerization can also be a source of bubbles [7]. The capillary gel may also break subsequently during manipulation, because of the differences in compressibility modulus of the capillary wall and enclosed water. Gels, and in particular acrylamide, can

suffer from degradation by hydrolysis, particularly at the alkaline pH commonly used to separate biopolymers. Even if these different problems could be solved, e.g., by high-pressure polymerization and the use of more stable monomers [8], the difficulty would remain at the entrance of the gel, as this zone receives all particles and impurities with charge of the same sign as the species to be separated, and is therefore very prone to clogging. This is especially a problem in DNA sequencing, where the sample also contains the high-molecular-mass template DNA and the polymerase. It has also been observed that during repeated use, bubbles can form at the sample-injection end of the capillary [9,10]. Drying out of the gel at the ends can also occur [9]. These problems can be solved to a limited extent by periodically trimming the ends of the capillary [11], but this is at the expense of reproducibility and automation. Currently, the reported lifetime of gel-filled capillaries for DNA sequencing is only about four injections [2]. Considering the price of capillaries and the difficulty of casting in-capillary gels, this leads to an unacceptable cost.

A solution to these problems is to replace the gel with a polymer solution, e.g., linear (uncrosslinked) polyacrylamide. The idea of using polymer solutions to separate biopolymers is not new, as it was proposed years ago in the pioneering work of Bode [12,13]. However, it became particularly popular in combination with CE, because only the very efficient anti-convective and heat dissipation properties of thin capillaries permit separations in fluids without loss of resolution.

2.2. Theory of polymer solutions

Three regimes of polymer solutions have to be distinguished: dilute, semi-dilute and concentration. In dilute solutions, the polymer chains are hydrodynamically isolated from each other and their properties are that of a single chain (see Ref. [14] for a review).

When the concentration of the polymer solution is increased, the transition from the dilute to the semi-dilute regime occurs (see Fig. 1): the polymer chains become entangled, forming a transient network of obstacles. It should be pointed out that the interactions between the chains are purely topological, in contrast to gels, where the chains are irreversibly (so-called "chemical gel", e.g., cross-linked polyacrylamide) or reversibly (so-called "physical gel", e.g., agarose) cross-linked (for a detailed discussion, see Ref. [15]).

The entanglement takes place above the overlap threshold, or entanglement threshold, c^*. For a given polymer, this threshold can be determined experimentally by measuring the viscosity of the polymer solution at different concentrations and finding the point of departure from linearity on the viscosity vs. concentration plot [16]. However, Viovy and Duke [14] have argued, that this criterion is ambiguous, since it depends very much on the accuracy of the experimental data. Instead, they propose to use the following definition, derived from polymer physics [17]:

$$c^* \approx 3 M_w / 4 \pi N_A R_g^3 \quad (1)$$

where R_g and M_w are the radius of gyration and the molecular mass of the polymer, respectively, and N_A is Avogadro's number.

Eq. 1 is the geometrical definition of the entanglement threshold, i.e., c^* is the concentration at which the polymer chains (modelled as coils) touch each other. Therefore, in order to determine this threshold, the radius of gyration has to be known. A simple way to obtain this value is to measure the intrinsic viscosity (or limiting viscosity number) of the polymer solution in the dilute regime [18]:

$$[\eta] \approx 2.5 R_g^3 N_A / M_w \quad (2)$$

The intrinsic viscosity $[\eta]$ itself can be measured experimentally or can be approximately calculated for a large number of polymers by using the empirical Mark–Houwink equation:

$$[\eta] \approx K M_w^a \quad (3)$$

where K and a are characteristic constants for a

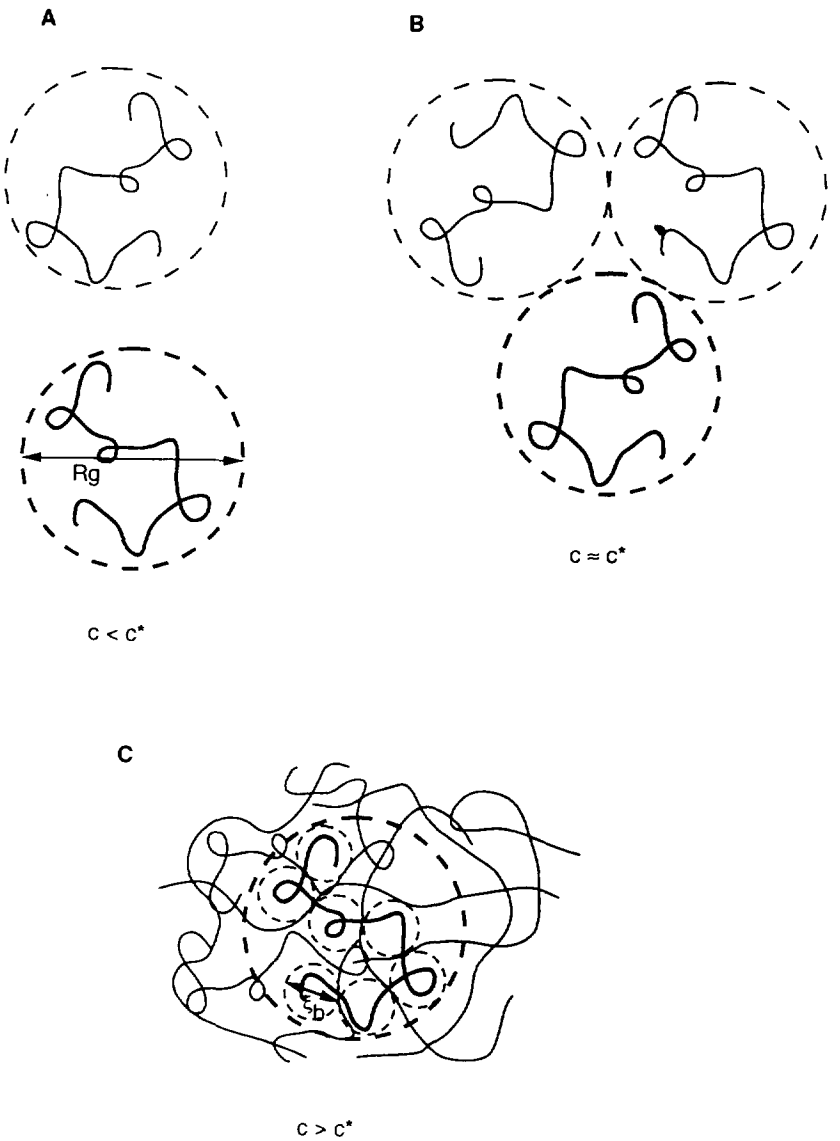

Fig. 1. Schematic representation of the entanglement process from a dilute to a semi-dilute solution. (A) Dilute solution; (B) $c = c^*$; (C) semi-dilute solution. One chain is drawn as a thick line for easier visualization. R_g = radius of gyration; ξ_b = "blob size".

given polymer–solvent system [19]. Combining Eqs. 1–3 then gives a simple expression for the overlap threshold:

$$c^* \approx 0.6\,[\eta]^{-1} \approx (0.6/K)\,M_w^{-a} \qquad (4)$$

This approach has the advantage that it avoids undertaking time-consuming viscosity measurements, as the intrinsic viscosity of numerous polymer–solvent systems is already known.

An important parameter in the theory of entangled polymer solutions is the screening length ξ [18], which can qualitatively be regarded as an average distance between polymer chains. Grossman and Soane [16] proposed to use this parameter as the effective "pore size" of

the transient network. However, Viovy and Duke [14] argued that the so-called "blob size", ξ_b (see Fig. 1 for illustration), should be used instead, but it is related to ξ by a universal prefactor [17]:

$$\xi_b = 2.86\,\xi = 1.43\,R_g\,(c/c^*)^{-3/4} \quad (5)$$

2.3. Choice of the polymer

It should be pointed out that, in contrast to the overlap threshold [which is dependent on the molecular mass (Eq. 1)], the "pore size" of an entangled polymer solution (i.e., if $c > c^*$) is not dependent on the degree of polymerization but only on its concentration. In other words, two solutions of the same type of polymer and with the same concentration but different molecular mass will have same "pore size" as long as they are entangled. However, their viscosity is dependent on molecular mass (Eq. 3), which has important consequences for the choice of the appropriate polymer for capillary electrophoresis.

For instance, for polyacrylamide (PAA) in water, the characteristic constants have been determined to be $K = 6.3 \cdot 10^{-3}$ ml/g and $a = 0.8$ [19], and therefore the entanglement threshold can be calculated for a given molecular mass:

$$c^*_{PAA}(\text{g/ml}) \approx 95\,M_w^{-0.8} \quad (6)$$

Similarly, the effective "pore size" of a semi-dilute PAA solution can be expressed as

$$\xi_{PAA}(\text{nm}) = 0.69\ c^{-3/4}, \text{ for } c > c^* \ (c \text{ in g/ml}) \quad (7a)$$

and

$$\xi_{PAA} = \infty \text{ for } c < c^* \quad (7b)$$

These relationships are presented graphically in Fig. 2 for a number of commercially available PAA preparations. It means that if one wants to perform a separation in, for example, a 6% PAA solution (0.06 g/ml), a PAA preparation with a molecular mass higher than 10 000 has to be used. Above that limit, all degrees of polymerization would give the same pore size. However, it is sensible to use the smallest possible molecular mass (e.g., 80 000) in order to keep the viscosity as low as possible.

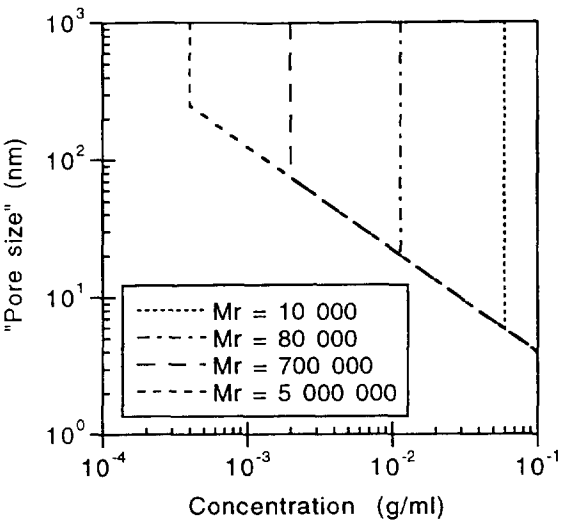

Fig. 2. Estimated "pore size" of some commercially available polyacrylamide solutions with different relative molecular masses as a function of concentration, calculated using Eq. 7 (see text). The entanglement thresholds are 0.059 g/ml ($M_r = 10\,000$), 0.011 g/ml ($M_r = 80\,000$), $1.99 \cdot 10^{-3}$ g/ml ($M_r = 700\,000$) and $4.1 \cdot 10^{-4}$ g/ml ($M_r = 5 \cdot 10^6$).

3. Separation mechanism

There are several theoretical approaches to describe the molecular mechanisms of the size separation of polyelectrolytes in gel electrophoresis. As a detailed discussion is beyond the scope of this paper, the main theories are recalled only very briefly (detailed information can be found in, e.g., Refs. [15 and 20]). Small molecules are separated by a sieving mechanism [21,22] and their mobility is proportional to $\exp(-M_w)$. With increasing molecular mass, they become larger than the pore size of the gel and they begin to "reptate" [23–25]. As a consequence, their mobility becomes inversely proportional to their size. Both regimes give a good band separation. With further increase in molecular mass, however, the mobility reaches a plateau and separation fails. As is known from theoretical considerations [23–25] and confirmed

by experimental studies on double-stranded DNA in agarose [26,27], this effect is due to orientation of the molecules. This upper separation limit, which decreases with increasing electric field, may be overcome by periodically changing the electric field [28,29]: suddenly switching the field direction forces the DNA to reorient and separation is achieved as the reorientation time is size dependent. In agarose gels, this method has been very successful for the separation of DNA molecules from a few kilobasepairs (kbp) up to several megabasepairs (Mbp) (see, e.g., Refs. [28–33]).

Most theoretical work has been done to explain the separation of double-stranded DNA, but essentially the same ideas can be applied to single-stranded DNA or to SDS-denatured proteins, taking into account the differences in persistence length, friction and charge.

Grossman and Soane [16] were the first to extend these theories to the separation of DNA in entangled polymer solutions. This attempt was later improved upon by Viovy and Duke [14]. They also postulated that another mechanism, called "constraint release", might play a role in the separation of large molecules.

Recently, Barron et al. [34,35] showed that double-stranded DNA fragments can even be separated in dilute polymer solutions, i.e., at concentrations below the entanglement threshold. They explained this astonishing behaviour with a "transient entanglement coupling" between the polymer and the DNA, i.e., the large DNA molecules drag the small polymer molecules with them, which results in a decrease in electrophoretic mobility. Separation takes place as the probability of DNA–polymer interaction increases with increasing DNA size.

4. Applications

4.1. Separation of oligonucleotides and DNA sequencing products

As was shown a few years ago, capillary electrophoresis can also be used to separate oligonucleotides [6,36–38] and DNA sequencing reaction products [11, 39–41]. For labelling and detection, the same sequencing chemistries are used as for the automated slab gels (see Ref. [42] for a review). However, despite a number of publications concerning this technique, to our knowledge, it has not yet been used for a larger sequencing project. One obvious reason for the low acceptance of this method for DNA sequencing is the lack of a suitable, commercially available apparatus. Several prototypes have been described [41,43–46], but a "ready-to-use" system with corresponding software is not yet on the market. However, there is rapid development in this field, which makes it possible that capillary electrophoresis might soon become a real alternative to the conventional method.

The sensitivity is very high and minute amounts of sample can be analysed, which probably could make the amplification of the template (in vivo or in vitro) unnecessary and offer the opportunity to sequence DNA directly isolated from plaques or colonies.

For separating oligonucleotides and sequencing reaction products, the same matrix as in slab gels (i.e., cross-linked polyacrylamide) can be used. Gel concentrations vary from 3 to 6%T and 3 to 5%C (%T = total concentration of acrylamide monomers; %C = concentration of cross-linker as a percentage of total concentration) and the same buffer and denaturants as in slab gels are used. Prefilled capillaries have also become commercially available.

For the reasons described above, gels are now increasingly being replaced by polymer solutions. For separating oligonucleotides and sequencing reaction products, solutions of about 8–10% PAA were successfully used (see, e.g., Refs. [2,44,47]). However, such solutions, produced by polymerization of monomer solutions of the respective concentrations, are highly viscous and the capillaries cannot be filled with the existing CE devices. Manual injection with a special syringe has been reported [48], whereas Chiari et al. [49] proposed to perform the polymerization within the capillary, a method that is now widely used. Again, the sample can only be injected electrokinetically and, as the capillaries cannot be rinsed or refilled, they have a limited lifetime. Therefore, regular rinsing after each run would still be desirable. So far, this can only be done

with less concentrated PAA solutions (<3%) as they are in use for the separation of double-stranded DNA fragments, but owing to their larger "pore size", they fail to separate oligonucleotides (see, e.g., Fig. 2 in Ref. [48]).

This problem has recently been addressed by Heller and Viovy [50]. Taking into account the consequences of the theory of polymer solutions (see above), they proposed to use a PAA preparation with a low degree of polymerization. Indeed, by using a linear PAA with a relative molecular mass of 80 000, they achieved a very good separation of oligonucleotides, comparable to that obtained using in situ polymerization with or without cross-linking (see Fig. 3). This short PAA has a low viscosity even at a concentration of 10%, which means that CE with replenishable capillaries can also be applied to oligonucleotide separation and DNA sequencing.

A similar approach was used by Ruiz-Martinez et al. [45], who produced a replaceable linear PAA matrix by adding high concentrations of catalyst (ammonium persulfate or N,N,N',N'-tetramethylethylenediamine) to the polymerization reaction. This leads to the initiation of a

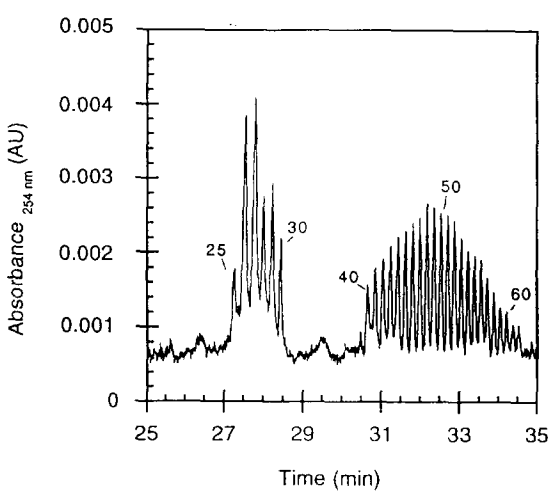

Fig. 3. Electrophoretic analysis of poly-dA_{25-30} mixed with poly-dA_{40-60} (2 mg/ml each) in a polyacrylamide solution. Conditions: injection by electromigration at 5 kV for 50 s; separation in 1 × TBE (89 m*M* Tris, 89 m*M* boric acid, 2.5 m*M* Na_2EDTA) containing 10% PAA (M_r = 80 000) at 200 V/cm in a 57 cm × 100 μm I.D. capillary (50 cm to the detector). The peaks of poly-dA_{25}, poly-dA_{30}, poly-dA_{40}, poly-dA_{50} and poly-dA_{60} are indicated.

greater number of polymer chains, but shorter in length. Adding 2-propanol to the polymerization reaction is another possibility for controlling the molecular mass of the synthesized PAA [51].

Using PAA with a low degree of polymerization may soon become the method of choice for the routine analysis of oligonucleotides. Polymer solutions of moderate viscosity could also replace the polyacrylamide gels currently used in sequence analysis with capillary electrophoresis and facilitate the automation of DNA sequencing.

For DNA sequencing, electric fields between 100 and 465 V/cm have been used. Apparently, the readability does not change very much with the electric field, but seems to get worse above 400 V/cm.

At such high electric fields, there is an early loss of separation owing to the molecular orientation of the molecules. Therefore, there is a trade-off between the speed of separation and the length of sequence reading. So far, a value of about 200–250 V/cm has been found to be a good compromise [52]. Under these conditions, up to 300–350 bases can be read in only a 30–60-min run time (see, e.g., Refs. [41 and 45]). Recently, however, Dovichi [53] reported even better separations, varying from 500 bases in 50–90 min at 200 V/cm to 200 bases in less than 5 min at 1200 V/cm.

In fact, for large-scale sequencing, it is the separation step that is currently the "bottleneck" (i.e., time-limiting step) in the whole sequencing procedure. Therefore, separations at high speed would be very useful. Together with the development of capillary arrays (100 and more capillaries), this would mean a several-fold enhancement in throughput, which means that capillary electrophoresis could soon replace the classical slab gels for DNA sequencing.

4.2. Separation of double-stranded DNA fragments

The first electrophoretic separation of DNA fragments in capillaries was reported in 1988, using urea and SDS as buffer additives [54], but the separation mechanism remains unclear. Early attempts to separate DNA in viscous

buffer solutions failed and cross-linked gels had to be used instead [55]. However, soon afterwards, successful separations in solutions of methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC) [56], in addition to linear polyacrylamide [47], were reported.

Since then, a large number of publications have appeared describing the separation of double-stranded DNA fragments in a variety of non-cross-linked polymer networks. The materials currently used are MC [57–59], hydroxyethylcellulose (HEC) [16,60,61], hydroxypropylcellulose (HPC) [58], HPMC [58], poly(vinyl alcohol) (PVA) [61], glucomannan [62], linear PAA [47,61,63–68], linear poly(N-acryloylaminoethoxyethanol) (AAEE) [69] and liquified agarose [70–73].

As the separation of restriction fragments and polymerase chain reaction (PCR) products requires larger pore sizes than the separation of oligonucleotides, less concentrated solutions of polymers can be used. Typical polymer concentrations are about 0.1–1% (up to 6% in the case of PAA) and most existing CE devices are now able to fill and empty the capillaries with such solutions automatically.

In contrast to agarose slab gels, the small fragments are eluted later than the larger fragments owing to the strong electroosmotic effect. However, by coating the inner wall of the capillary with polyacrylamide [74,75] or hydrophilic polymers [76], the electroosmosis can be strongly reduced. In this case, so-called "reverse polarity" (i.e., cathode on the injection side) has to be used and the DNA fragments arrive in the well known order. A variety of coated (e.g., with polyethylene glycol or polysiloxane coating) or deactivated capillaries is also commercially available. The polymeric additives themselves (especially the cellulose derivatives) also provide dynamic coating of the capillary walls.

It was also found that the addition of intercalating agents such as ethidium bromide can improve the resolution and lead to a stronger UV absorbance [63,77]. However, the electrophoretic mobility of the DNA fragments is decreased, owing to the positive charge of ethidium bromide and the increased molecular mass of the complex.

When carefully operated, CE shows a high reproducibility, allowing accurate size determination of DNA fragments (see, e.g., Ref. [78]) after calibration with a length standard. For higher precision, the standard can be co-injected with the unknown sample.

Consequently, the separation of PCR products and DNA restriction fragments in CE is now becoming routine (see [57,66,68] and Fig. 4, for example).

However, these data also show the limits of capillary electrophoresis under constant-field conditions: above about 2 kbp, molecular orientation begins to occur and separation fails. To overcome these limitations, a periodically inverted electric field must be used, in analogy to field inversion gel electrophoresis (FIGE) used for slab gels. To obtain a net forward migration, asymmetric pulses are applied, i.e., the amplitude of the "forward" pulse is different from that of the "backward" pulse, by keeping the duration of the pulses constant. Using field inversion, the separation above 2 kbp can be dramatically increased. For example, the larger fragments (1.3–12 kbp) of the kilobase ladder

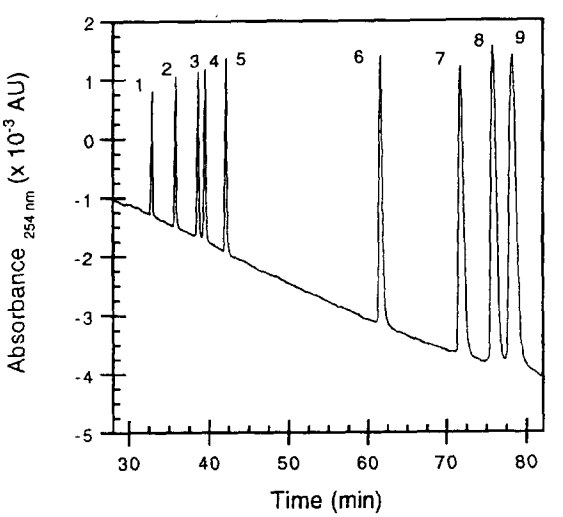

Fig. 4. Separation of PhiX-174/HaeIII fragments in 1% HPC, 1 × TBE (89 m*M* Tris, 89 m*M* boric acid, 2.5 m*M* Na_2EDTA) at 80 V/cm in a 37 cm long capillary (30 cm to the detector). The peaks can be identified as the following fragments: 1 = 194, 2 = 234, 3 = 271, 4 = 281, 5 = 310, 6 = 603, 7 = 872, 8 = 1078 and 9 = 1353 bp.

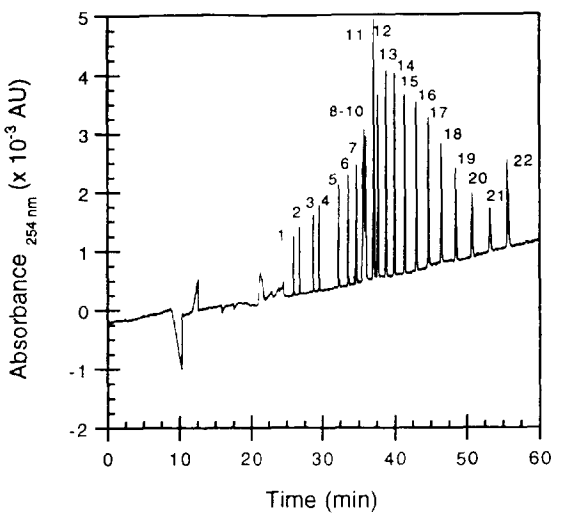

Fig. 5. Separation of the kbp ladder (BRL) in 1% HPC, 1 × TBE (89 m*M* Tris, 89 m*M* boric acid, 2.5 m*M* Na_2EDTA) with field inversion in a 37 cm long capillary (30 cm to the detector) with E_1 = 243 V/cm, E_2 = 81 V/cm and a frequency of 50 Hz. The peaks represent the following DNA fragments: 1 = 134, 2 = 154, 3 = 201, 4 = 220, 5 = 298, 6 = 344, 7 = 396, 8 = 506, 9 = 517, 10 = 1018, 11 = 1636, 12 = 2036, 13 = 3054, 14 = 4072, 15 = 5090, 16 = 6108, 17 = 7126, 18 = 8144, 19 = 9162, 20 = 10 180, 21 = 11 198 and 22 = 12 216 bp.

which migrate together under constant field conditions (not shown) are baseline resolved when using a non-stationary field (Fig. 5).

4.3. *Separation of proteins*

For the molecular mass determination and purity control of proteins, SDS–polyacrylamide gel electrophoresis (PAGE) is the method of choice. The first separations of proteins by SDS-PAGE in capillary format were published in 1987 by Hjertén et al. [4] and by Cohen and Karger [5]. Four years later, Tsuji [79] presented separations up to a molecular mass of 200 000. These separations were done in cross-linked gels, but soon afterwards, liquid non-linear polyacrylamide was used instead [80]. The use of linear polyacrylamide for the separation of proteins had already been demonstrated much earlier by Bode [12,13], but it is only in combination with capillaries that this technique offers a real advantage over gels.

For separating proteins, PAA solutions with a concentration of about 8–16% are needed [80,81]. As explained above, in situ polymerization of acrylamide monomer at such high concentrations leads to solutions of very high viscosity and the matrices cannot be easily replaced. Again, the use of low-molecular-mass PAA might solve this problem.

In addition, the absorbance of PAA at 214 nm is fairly high [81], so that detection has to take place at 280 nm, where the molar absorptivity of proteins is low.

To overcome these limitations, a UV-transparent matrix is needed. There are several types of polymers that are ideal candidates for this purpose. These are, for example, different preparations of dextran (M_r between $7.2 \cdot 10^4$ and $2 \cdot 10^6$), poly(ethylene glycol) (PEG), ($M_r = 10^5$) and poly(ethylene oxide) (PEO) ($M_r = 10^5$). In all instances, the absorbance at 214 nm is low and they also have a low viscosity at the concentrations needed for protein separation (3% for PEG and PEO, 10–15% for dextran). Using these polymers, proteins with a molecular mass range of 20 000–200 000 have been successfully separated with high speed and high reproducibility [81–84] (Fig. 6). Recently, Hjertén et al. [73] used a low-melting-point agarose as a replaceable matrix. In the meantime, ready-to-use polymer solutions have also become commercially available and have been successfully used for the separation of proteins (see, e.g., Refs. [85–88]).

As for the separation of DNA, coated capillaries are generally used. This suppresses the electroosmotic flow and the negatively charged SDS–protein complexes migrate towards the anode, with the small molecules eluting first.

5. Developments in instrumentation

Capillary electrophoresis is, of course, an instrument-based technique and much of its future performance will largely depend on developments on the instrumentation side. Therefore, in the following, we describe some current and future modifications in capillary electrophoresis.

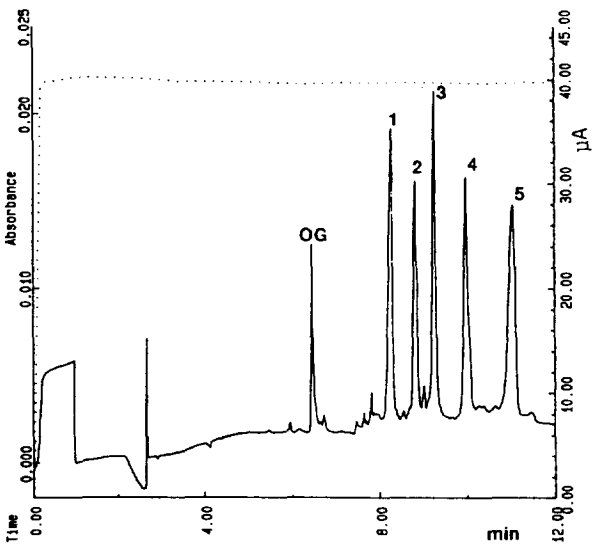

Fig. 6. Separation of proteins in a PEO-filled capillary in the presence of SDS. Peaks: OG = Orange G as tracking dye, 1 = α-lactalbumin (M_r = 14 200); 2 = soybean trypsin inhibitor (M_r = 20 100); 3 = carbonic anhydrase (M_r = 29 000); 4 = ovalbumin (M_r = 45 000); 5 = bovine serum albumin (M_r = 66 000). From Ref. [83].

5.1. Detection

The classical method to detect biopolymers is to measure UV absorbance. Therefore, the commercially available devices are all equipped with a photometer, with either single-wavelength or multi-wavelength detection (diode-array or fast-scan monochromator). However, for some applications, laser-induced fluorescence detection is advantageous or necessary, in order to obtain a higher sensitivity. This is especially the case for DNA sequencing, but also for the detection of small amounts of double-stranded DNA. For DNA sequencing, suitable covalently bound tags are used, whereas for double-stranded DNA fragments an intercalating dye such as thiazole orange or one of the recently developed dimeric dyes [89] should be the label of choice (see, e.g., Refs. [90–92]).

5.2. Capillary arrays

Capillary electrophoresis offers a number of advantages over classical slab gels, but there is one important drawback. In slab gels, parallel processing is possible, whereas in capillaries only one sample can be analyzed at a time. In order to restore the possibility of parallel processing, the introduction of multiple capillaries, i.e., capillary arrays, would be highly desirable. This is especially of importance for large-scale DNA sequencing, but also for the routine analysis of DNA fragments and proteins. A number of prototypes have been described (e.g., Refs. [1–3]), but there is no commercially available instrument yet.

5.3. Pulsed field electrophoresis

As described above, the limit for separating double-stranded DNA in CE is currently comparable to the limit in agarose gel electrophoresis. For slab gels, the separation limit could be increased by about two orders of magnitude with the introduction of pulsed field gel electrophoresis [28]. Therefore, it would be obvious to adapt this technique also to capillary electrophoresis. Indeed, the first attempts to modulate the electric field with square-wave [47,93] or sine-wave [94] signals have shown slight improvements in the resolution of DNA fragments. Pulsed fields were also shown to change the mobility and resolution of polysaccharides and poly(styrene sulfonates) [95,96]. Using asymmetric field inversion, Heller et al. [97] achieved very high resolution up to 23 kbp and more (see Ref. [97] and Fig. 5), whereas Sudor and Novotny [98] even reported separations up to a DNA size of 1 Mbp.

5.4. Blotting

For some applications it would be desirable to recover the separated compounds for further identification (e.g., immunochemical staining, amino acid analysis, hybridization). Fraction collection is possible (e.g., Refs. [6,36,99]), but the sample is further diluted, as the outlet vial has to contain a minimum of electrolyte solution. An alternative approach for sample collection is to move a membrane or a filter-paper along the capillary outlet. The separated molecules are then blotted on-line on to the matrix as they are

eluted from the capillary. Slightly different systems have been developed, using a drum, a disc or a plotter to move the membrane [100–102]. In fact, this development is analogous to the "direct blotting" technique that is successfully used for the on-line transfer of biomolecules from slab gels on to membranes [103–105].

6. Outlook

In the future, substantial advances can be expected for capillary electrophoresis. Because of numerous problems with cross-linked gels in capillaries, there will be a move towards replaceable polymer networks. As described above, such polymer solutions can now be used for the separation of proteins and nucleic acids in a broad size range. However, some more experimental and theoretical work is needed in order to understand fully the mechanism of separation. Such understanding will help to improve further the performance of capillary electrophoresis, e.g., by optimizing the electrophoresis conditions or by developing new polymers.

Together with the development of multiple capillary instruments, this technique should revolutionize the separation of biological macromolecules, as it offers high speed, high throughput and the possibility of complete automation together with high sensitivity.

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