ELSEVIER

Journal of Chromatography A, 966 (2002) 1–13

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Copolymer solutions as separation media for DNA capillary electrophoresis

Benjamin Chu*, Dehai Liang

Chemistry Department, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, USA

Received 29 January 2002; received in revised form 6 May 2002; accepted 17 May 2002

Abstract

A review on copolymers used as DNA separation media in capillary electrophoresis is presented. Copolymers can combine the desirable properties of different monomers, yielding many attractive features, such as high sieving ability, low viscosity, self-assembly behavior and dynamic coating ability. Copolymers with different molecular architecture, including block copolymers, random copolymers, and graft copolymers, have been developed and tested as DNA separation media with unique and tailored properties that cannot be achieved easily by using only homopolymers.

Keywords: Reviews; Polymeric separation media; DNA; Polymers

Contents

1. Introduction

Capillary electrophoresis (CE) has been proven to be a powerful method for DNA analysis. It offers great advantages over conventional slab gel electrophoresis in terms of higher resolution, shorter analysis time, minimal sample requirement, negligible waste of toxic chemicals, high detection sensitivity and ease of automation. Moreover, recent development on capillary array electrophoresis (CAE) [1–17] and microfabricated chips [18–25] has significantly increased its throughput.

*Corresponding author. Tel.: +1-631-632-7928; fax: +1-631-632-6518.

E-mail address: bchu@notes.cc.sunysb.edu (B. Chu).

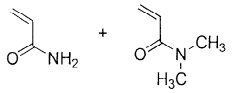
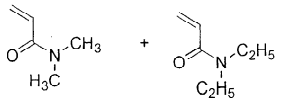
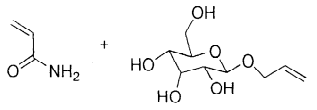
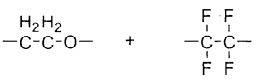
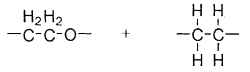
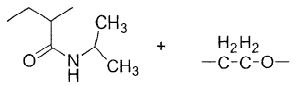
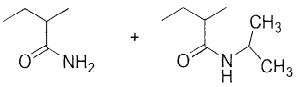
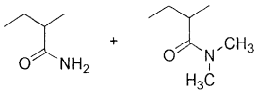
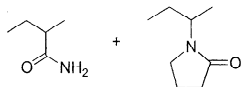
0021-9673/02/$ – see front matter
PII: S0021-9673(02)00702-1

In the above strategies, the separation medium has played an important role because it determines both the DNA migration behavior and the resolution. Cross-linked polyacrylamide gel, being widely used in slab gel electrophoresis, was employed in early CE [26]. Despite the successful performance, i.e., sequencing up to 700 bases with a resolution of 0.5 in about 230 min [27], it became less popular mainly due to bubble formation, gel inhomogeneity, short column life and low reproducibility. As an alternative, non cross-linked polymer solutions have been widely used in recent CE applications. A range of hydrophilic homopolymers have been developed and tested as DNA separation media, including linear poly(acrylamide) (LPA) [28–44], poly(ethylene oxide) (PEO) [45–47], poly(vinyl pyrrolidine) (PVP) [48–50], poly(*N*,*N*-dimethyl acrylamide) (PDMA) [14,51–57], cellulose and its derivatives [58–68], polysaccharides [57,69–71], and poly(*N*-acryloylaminopropanol) [72,73]. Among them, LPA showed the best sequencing performance, producing 1000 bases in 1 h [31] and 1300 bases in 2 h [33] with optimized polymer molecular mass distribution, matrix formulation, sample preparation and clean-up, and base calling algorithms. However, LPA has some limits in CE. The polymer solution usually has a high viscosity, especially for high-molecular-mass polymer solutions in order to increase the DNA read length. High polymer solution viscosity usually results in a more difficult medium replacement. Furthermore, the silica capillary needs pretreatment of coating with poly(vinyl alcohol) (PVA) [30,31] or LPA [74] that is covalently attached to the inner capillary wall by bifunctional reagents in order to suppress electroosmosis and sample adsorption. This kind of treatment demands in situ synthesis and often gives rise to problems such as capillary fouling, coating inhomogeneity and limited shelf life. Dynamic coating, which takes advantage of the interactions between the silanol group of the capillary inner wall and the separation medium, is a promising alternative approach. However, only several polymers, such as PEO, PDMA and PVP, have the dynamic coating ability in a sequencing buffer.

The development of a separation medium, which possesses high sieving ability, low viscosity and dynamic coating ability, will facilitate the automation of CE, CAE and microfabricated chips and further enhance their performance. Other properties, such as non-toxicity and ease of preparation, are also desirable but not crucial. It is difficult for a homopolymer solution to possess all the above properties, because the physical nature of the polymers is essentially determined by the chemical composition of the monomer segments. However, copolymers, which can combine the desirable properties of different monomers, can offer the possibility to tune the desirable properties by appropriate adjustments in molecular architecture and chemical composition. A range of copolymers have been developed and tested as DNA separation media as shown in Table 1. They can be divided into three categories according to the type: (i) random copolymers, e.g., copolymer of LPA and PDMA [P(AA–co-DMA)] [75]; (ii) block copolymers, e.g., amphiphilic triblock copolymer $E_{99}P_{69}E_{99}$ (with E and P denoting oxyethylene and oxypropylene, respectively) [76–81]; (iii) graft copolymers, e.g., poly(*N*-isopropylacrylamide)–g-PEO [82,83]. The copolymers could not only combine the desirable properties of its components, some specific features, which would be absent in the homopolymers, could also be achieved. For example, the ability to form a micellar structure renders an amphiphilic triblock copolymer, $E_{99}P_{69}E_{99,}$ a temperature-dependent viscosity adjustable property, that is not available to either polyoxypropylene or PEO [76,78].

Viovy [84] has written a review on the physical mechanisms of electrophoresis of DNA and other polyelectrolytes. From his viewpoint of a physicist, the mechanisms responsible for the electrophoretic separation of polyelectrolytes depend on polyelectrolyte architecture, applied electric field, and properties of the separation medium. The present review deals with how we can change the physical properties of the separation medium by changing its chemical composition and molecular architecture. We restrict our attention to size fraction on the above three types of copolymers [(i)–(iii)] used as DNA separation media. Moreover, the use of (iv) polymer mixtures and (v) interpenetrating networks (IPNs), has almost the same effects as those of copolymers. Thus, these applications are also covered briefly in this review.

Table 1
Copolymers used as DNA separation media

Name of copolymers	Chemical structure	DNA sample
(i) Random copolymers		
P(AA–co-DMA)		DNA sequencing, dsDNA [75,85]
P(DMA-co-DEA)		Oligonucleotides, DNA sequencing [88–90]
P(AA–co-AG)		Oligonucleotides, DNA ladder [86,87]
(ii) Block (or end-capped) copolymers		
$E_{99}P_{69}E_{99}$ (F127)		Oligonucleotides, dsDNA [76–81]
PEG end-capped with fluorocarbon		DNA sequencing [91]
n-Dodecane–PEO–*n*-dodecane		Oligonucleotides [92]
(iii) Graft copolymers		
PNIPAM–g-PEO		dsDNA [82,83]
PAA–g-PNIPAM		DNA standard [95]
(iv) Polymer mixtures		
Mixture of PAA and PDMA		DNA sequencing [101]
$B_{10}E_{270}B_{10}+B_6E_{46}B_6$		dsDNA [102,103]
(v) IPN		
IPN of LPA and PVP		dsDNA [97]

2. Random copolymers

In designing a random copolymer as a separation medium for DNA sequencing analysis, acrylamide (AA) is a desirable first choice as one of the monomer segments because polyacrylamide is al-

ready well known for its high sieving ability. The choice of the other monomer segment is to address the additional requirements; i.e., to improve the dynamic coating ability or/and to decrease the polymer solution viscosity.

Random copolymers of P(AA–co-DMA) [75] developed in our laboratory is based on the above consideration. According to Madabhushi [53], PDMA has the best coating ability when compared with other reported separation media, such as PVP and PEO. Its combination with LPA should be able to render the copolymer a dynamic coating ability without losing the high sieving ability of LPA. Random copolymers with AA to DMA ratios of 3:1, 2:1 and 1:1 were synthesized by free-radical solution polymerization. To make a better comparison, LPA was also prepared following the same procedure. ^{1}H-Nuclear magnetic resonance (NMR) results showed that the real AA to DMA ratio was very close to the initially designed ratio. All the copolymers including LPA had a comparable molecular mass of about 2.2 *M*. Single-colored DNA sequencing sample was used to test the separation performance in uncoated capillary (except for LPA). It was found that all the random copolymers had enough dynamic coating ability in the sequencing buffer. However, the separation varied at different AA to DMA ratio. The calculated resolution and efficiency showed that P(AA–co-DMA) (3:1) had the best separation performance and P(AA–co-DMA) (1:1) had the worst. Under optimized electrophoresis conditions for longer read length DNA sequencing, one base resolution of 0.55 up to 699 bases and 0.30 up to 963 bases were achieved within 80 min at ambient temperature by using 2.5% (w/v) P(AA–co-DMA) 3:1 as the separation medium. This performance has exceeded that of LPA with comparable molecular mass and under the same conditions but in coated capillary [75]. Recently, the applications of copolymer of P(AA–co-DMA) in the separation of double-stranded (ds) DNA fragments and single strand conformation polymorphism analysis have been reported [85]. The molecular mass of such copolymers was controlled at about 40 000 by using isopropanol as a chain transfer agent in aqueous solution. Successful single strand conformation polymorphism analysis was achieved by using 5% P(AA–co-DMA) with AA to DMA ratio of 7:3 at 20 °C [85].

Chiari and co-workers [86,87] developed low viscosity random copolymers containing acrylamide and sugar monomers. One of the sugar monomers that had been studied in detail was ally-β-D-glucopyranoside (AG) [86]. Such a monomer was obtained by enzymatic synthesis of D-glucose and allyl alcohol in water. Random copolymers with AA to AG ratios of 1:1, 1:0.5 and 1:0.3 were synthesized by free-radical solution polymerization. With the hydroxyl groups in AG acting as a chain transfer agent, the copolymers could not reach a high molecular mass like that of LPA homopolymers. The P(AA–co-AG) molecular mass was below 400 000 and inversely related to the AG to AA ratio for the same reason. Random copolymers of P(AA–co-AG) had a much lower viscosity when compared with LPA of the same molecular mass, e.g., the viscosity of a 6% LPA with a molecular mass of 320 000 was 1000 cP, a value three times larger than that of P(AA–co-AG) having the same molecular mass. Good performance on both dsDNA and oligonucleotides separation by UV detection was observed by using 10% (w/v) P(AA–co-AG) (1:1) as the separation medium with 0.05% (w/w) PDMA acting as the dynamic coating reagent. Besides AG, allyamide gluyconic acid (AAG) and allyamide lactobionic acid (AAL) [87] were also used to copolymerize with acrylamide. Low viscosity and good separation were maintained. Moreover, P(AAG–co-AA) showed self coating ability and could be used in untreated capillary. The addition of a small amount (1.4%) of oxirane groups in P(AAG–co-AA) further suppressed electroosmosis to a negligible level.

Random copolymers of DMA and *N*-diethylacrylamide (DEA) developed by Sassi et al. [88] used another strategy to decrease the viscosity: lower critical solution temperature (LCST) behavior. This separation medium showed a worse separation performance than that of LPA and PDMA mainly due to the higher hydrophobicity of the diethyl group in DEA. However, P(DMA–co-DEA) has a significant temperature-dependent viscosity-adjustable property. At 20 °C, the viscosity of 2% P(DMA–co-DEA) was about 100 cP. It sharply decreased to about 5 cP when the solution was heated to 50 °C. Such a

temperature-dependent viscosity behavior is thermally reversible, facilitating easier medium replacement. Moreover, P(DMA–co-DEA) also showed good dynamic coating ability. By using 7.35% P(DMA–co-DEA) with a molecular mass of 4.0 M and DMA to DEA mass ratio of 47:53, DNA sequencing up to 463 bases in 78 min with 97% base-calling accuracy was achieved [89]. Copolymers of P(DMA–co-DEA) with different DEA to DMA ratios were also used to study the hydrophobicity effect on DNA separation [90]. It was found that the DNA sequencing read length was decreased with increasing DEA content.

3. Block copolymers

Block copolymers in selective solvents can self-assemble to form micelle structures. The driving force comes mainly from the hydrophobicity in one of the blocks. When compared with linear polymers, block copolymers could provide an alternative strategy to make transient networks with appropriate mesh sizes by using hydrophobic blocks as cross-linking points.

The amphiphilic triblock copolymer, $E_{99}P_{69}E_{99}$ (F127) [76–81], could demonstrate many advantages as a DNA separation medium in terms of dynamic coating ability, viscosity-adjustable property, commercial availability and non-toxicity. At temperatures below 15 °C, 25% (w/v) $E_{99}P_{69}E_{99}$ had a viscosity below 50 cP, providing an easy means to inject the separation medium into the capillary. At room temperature, a sharp increase in the viscosity was observed and a gel-like structure was formed. Scattering techniques (laser light, X-ray and neutron) [78] showed that, at concentrations above the critical micelle concentration (CMC), $E_{99}P_{69}E_{99}$ formed a star-like micelle in 1×TBE (89 mM Tris/89 mM boric acid/2.5 mM EDTA) buffer at room temperatures with the P blocks forming the core and the E blocks dangling outside forming the corona. At the concentration where electrophoresis was performed (above 20%), the aggregation number was above 60 and the micelles were highly packed to form a face centered cubic structure [78].

Due to the high block copolymer concentration (above 20%) and the closely packed cubic structure, the polymer network showed good sieving ability for small size DNA fragments, especially oligonucleotides [76,79–81] as shown in Fig. 1. It also allowed the use of very short columns. Fig. 2 shows that the 11 DNA fragments in ΦX174/Hae III digest can be clearly separated within 2 min by using an 8-mm column operating at 300 V/cm [laser-induced fluorescence (LIF) detection] [77]. However, $E_{99}P_{69}E_{99}$ was not very suitable for the separation of large size DNA fragments due to its small mesh size. More importantly, it could not be used for DNA sequencing analysis since the DNA denaturing reagents in the sequencing buffer also affected the micellar structure.

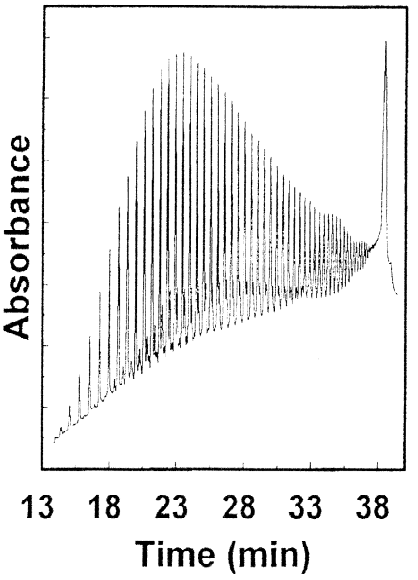


Fig. 1. Single nucleotide resolution of components of poly(U) by capillary electrophoresis. Electrophoresis was performed in 25% Pluronic F127 in TBE buffer at 25 °C with an applied voltage of 18 kV (500 V/cm). Reprinted from Ref. [79], with permission.

Menchen et al. [91] developed a block copolymer consisting of polyethylene glycols (PEGs) end-capped with fluorocarbon (C_nF_{2n+1}) tails. In aqueous solutions above the CMC, such copolymers formed a flower-like micelle structure with the hydrophobic fluorocarbons forming the core and PEG dangling outside forming the hydrophilic corona. This self-assembled structure is different from that formed by $E_{99}P_{69}E_{99}$ due to the difference in molecular ar-

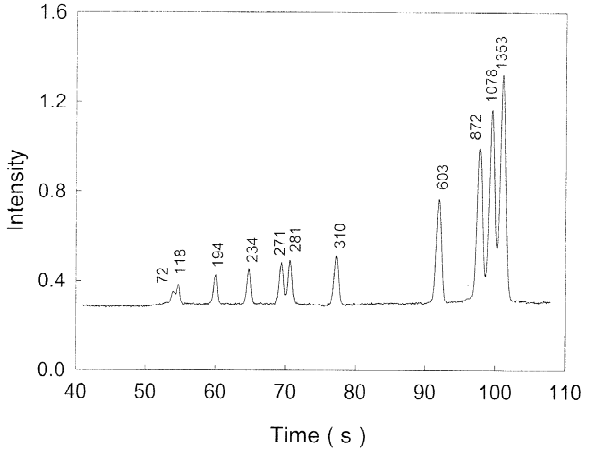


Fig. 2. Electropherogram of ΦX174/Hae III digest by using 25% $E_{99}P_{69}E_{99}$ in 1×TBE buffer. Conditions: 8 mm effective capillary length×50 μm I.D.; running electric field strength 300 V/cm; electrokinetic injection at 300 V/cm for 1 s. Peak identification from left to right in bp are: 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, 1353. Reprinted from Ref. [77], with permission.

chitecture. With increasing polymer concentration, the micelles could further aggregate to form a network structure with the PEG blocks functioning as bridges to link the micelles together. Such bridging invariably increased the viscosity and allowed the copolymer to be used at lower polymer concentrations (below 6%) for DNA separation. The block length effect on the DNA separation was investigated by using copolymers of $(C_6F_{13})_2$PEG8000 (8000 denoting PEG molecular mass), $(C_6F_{13})_2$PEG35000, and $(C_8F_{17})_2$PEG35000 with two significant figures for all molecular masses. It was found that the end groups had subtle effects on DNA sequencing since they formed the micellar core as well as the cross-linking points that affect the mesh size as mentioned briefly before. The PEG chain length, which partially governed the mesh size, showed a major effect, with short chains PEG (molecular mass 8000) leading to a catastrophic resolution loss for DNA fragments larger than 100 bases. It was also found that the optimum formulation was a 6% mixture of $(C_6F_{13})_2$PEG35000 and $(C_8F_{17})_2$PEG35000 (1:1).

Another triblock copolymer, *n*-dodecane–PEO–*n*-dodecane (POEM10), was synthesized and used in the separation of oligonucleotide by Magnusdottir et al. [92]. The PEO block had a molecular mass of 9.9 K. In aqueous solution, such a copolymer formed the same micelle structure as that of polyethylene glycols end-capped with fluorocarbon tails. Oligonucleotide of poly-d(A) 25–30 and 40–60 was used to test the performance of POEM10. Baseline resolution was essentially achieved under a concentration between 5.1% to 10.1% with UV detection. Such separation was much better than that of PEO having the same molecular mass and under the same conditions.

4. Graft copolymers

Based on the mechanism that DNA fragments could be separated in size by using a static sieving matrix, graft copolymer solutions should have a higher sieving ability than those solutions formed by entangled linear polymer chains. At a concentration above C^* (overlap concentration), the linear polymer chains entangled with one another to form a transient network with a certain mesh size (pore size) as schematically shown in Fig. 3a. If the DNA fragment has a size larger than the mesh size, it will adjust itself to migrate through like a snake as described by the reptation model [93]. On the other hand, the network would also make an ''adjustment'' to enlarge the mesh size by changing the positions of the

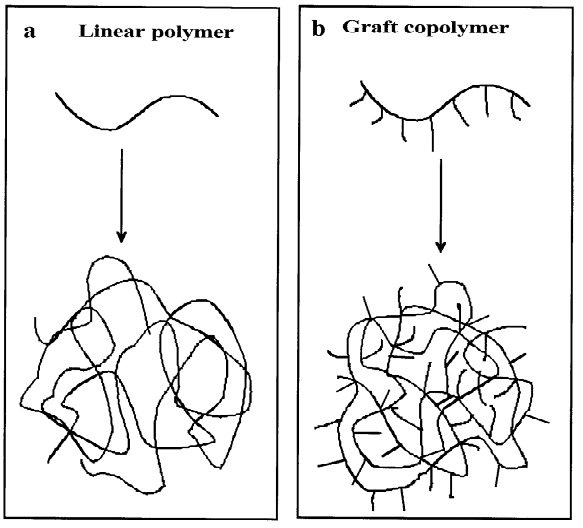


Fig. 3. Schematic pictures showing the network formation by (a) linear homopolymers, and (b) graft copolymers.

entanglements by a slide of the chains, resulting in a lower sieving ability. From another viewpoint, the entanglement time is relatively short in flexible linear polymer chains. When the residence time of a DNA fragment in the mesh exceeds the polymer relaxation time, the resolution could deteriorate [44,94]. However, such a situation can be improved by using graft copolymers, where the short-chain branches attached to the backbone chains could lock up the entanglements and prevent the chains from sliding away from each other as schematically shown in Fig. 3b. Such entanglements should increase the polymer chain entanglement time.

Copolymers with long-chain poly(*N*-isopropylacrylamide) (PNIPAM) as the backbone and densely grafted short PEO chains (22 repeat units) was synthesized and tested as a DNA separation medium in our laboratory [82,83]. PNIPAM–g-PEO has a temperature dependent ''coil-to-globule'' conformational change due to the ''coil-to-globule'' transition behavior of PNIPAM. At low concentrations and room temperatures, both PNIPAM and PEO are hydrophilic and the copolymer has a random coil conformation. When the temperature is above 31 °C, PNIPAM begins to shrink and collapse. A globule state with PNIPAM as the core and the hydrophilic PEO branches on the surface is eventually formed upon further increase in temperature [83]. The physical properties of the separation medium showed a direct relationship with its performance on DNA separation. A good resolution was observed at room temperature where a uniform and stable network was formed by PNIPAM–g-PEO in the random coil state. At elevated temperatures, the formation of globule destroyed the network with an appropriate mesh size and thus the separation ability. pBR322/Hae III DNA digest was used to test its sieving ability at room temperatures. A resolution of 1.4 on the separation of 123/124 base pairs (bp) was achieved within 12.5 min by using 8% (w/v) PNIPAM–g-PEO in 1×TBE buffer with an effective column length of 10 cm and an applied electric field strength of 200 V/cm with LIF detection as shown in Fig. 4. Moreover, it could also be used for high-speed separation. Fig. 5 shows that the 11 fragments in *Φ*X174/haeIII digest have been clearly separated within 24 s with a 1.5 cm capillary at 800 V/cm [82]. As a separation medium, PNIPAM–g-PEO also showed a dynamic coating ability but only weak viscosity-adjustable property with temperature.

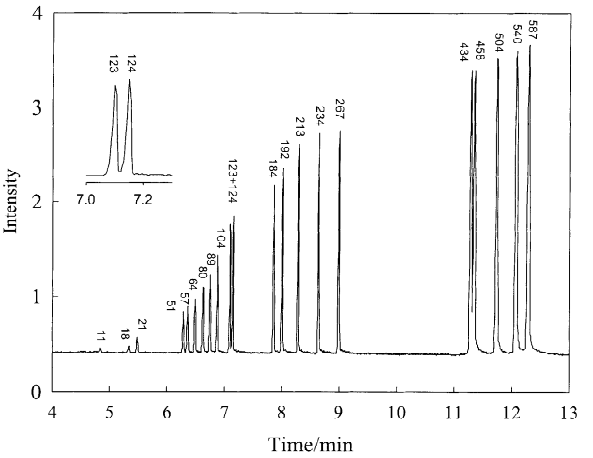


Fig. 4. Electropherogram of pBR322/Hae III digest by using 8% (w/v) PNIPAM–g-PEO in 1×TBE buffer. Conditions: 10 cm effective length×100 μm I.D. capillary; running electric field strength: 200 V/cm; electrokinetic injection at 300 V/cm for 1 s; peak identification from left to right in bp are: 11, 18, 21, 51, 57, 64, 80, 89, 104, 123, 124, 184, 192, 213, 234, 267, 434, 458, 504, 540, 587. Reprinted from Ref. [82], with permission.

PNIPAM–g-PEO has a backbone with a LCST and short hydrophilic branches. Another graft copolymer with an opposite design was reported by

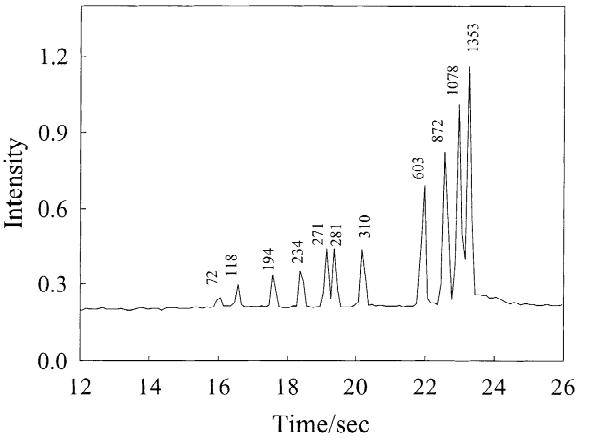


Fig. 5. Electropherogram of ΦX174/Hae III digest by using 8% (w/v) PNIPAM–g-PEO in 1×TBE buffer. Conditions: 1.5 cm effective length×100 μm I.D. capillary; running electric field strength 800 V/cm; electrokinetic injection at 300 V/cm for 1 s. Peak identification as in Fig. 2. Reprinted from Ref. [82], with permission.

Sudor et al. [95]. They proposed a copolymer with polyacrylamide as the backbone and PNIPAM as the branches. The copolymers were characterized in terms of size of polymer backbone, size of side chains, and weight fraction of PNIPAM. Rheological behavior and separation performance were systemically studied. All the copolymers showed a marked thermoviscosifying effect, with a large plateau at high temperatures. It was found that a long backbone ($1.5–2\cdot10^6$) of polyacrylamide grafted with a small fraction (less than 10%) of relatively short side chains (around 10 000) of PNIPAM seemed to be the best choice. Without complete optimization, a resolution of 0.5 could be achieved for DNA segments of around 800 bases differing by 1 base in less than 1 h in a commercial ABI 310 apparatus. However, it is not applicable to evaluate the read length on real DNA sequencing sample, because the base-calling software on the commercial instrument of ABI 310 could not provide a way to adjust the mobility shifts [95].

With graft copolymers having appropriate density and length of short grafted chains, the resultant extension of the polymer backbone chains and increase in backbone chain entanglement time can improve DNA separation when compared with the corresponding linear homopolymer backbone chain.

5. Interpenetrating network

An interpenetrating network (IPN) usually consists of two distinct networks with different properties or compositions. The two networks intertwine on a molecular level without covalent cross-links. However, the network itself is usually cross-linked, preventing the two distinct networks from macroscopic phase separation. The synthesis of IPN usually involves two polymerization steps. The first step is to form a network of polymer A, typically with cross-linking reagent added. The second step is to soak the network A into a solution containing monomer B, cross-linking reagent and buffer, to be followed by the polymerization and cross-linking of B. Boyd et al. [96] synthesized neutral and slightly charged polyacrylamide-based IPN to improve the DNA separation by using high-frequency alternating-cross-field slab gel electrophoresis.

A typical IPN is not suitable for DNA capillary electrophoresis since it is cross-linked and not replaceable. An IPN consisting of LPA and PVP but without cross-linking points was developed and tested as a DNA separation medium in our laboratory [97]. By taking advantage of the low viscosity of PVP, the IPN was synthesized in one polymerization step. After carefully mixing the monomer of acrylamide with the PVP (molecular mass about $1\cdot10^6$) matrix and followed by a degassing stage, the polymerization was started by the addition of initiators and allowed to proceed until completion. The formation of IPN was confirmed by dynamic light scattering experiments [97]. The molecular mass of LPA inside the IPN was estimated to be $4\cdot10^5$. In comparison with LPA, PVP has a low viscosity and good dynamic coating ability but relatively poor separation performance. A combination of these two polymers to form an IPN seems to be very attractive. However, their mixtures showed a weak sieving ability mainly due to the incompatibility of these two polymers. IPN is one of the approaches to alleviate the polymer immiscibility since the entanglements between the two interpenetrating networks prevent the macroscopic phase separation over a sufficiently long time period. IPNs with different concentrations of LPA (0.5, 1, 2, 4, 6%) and PVP (0.5, 1, 2, 3, 4, 5, 6%) were synthesized and used for dsDNA separation. It was found that a total concentration of LPA and PVP of less than 6% (w/v) was able to provide superior separation than 6% (w/v) LPA. The separation speed increased with decreasing total concentration. With a concentration combination of as low as 2% (w/v) PVP + 1% (w/v) LPA, the 22 fragments in pBR322/Hae III digest, including the doublet of 123/124 bp were successfully separated within 6.5 min with LIF detection as shown in Fig. 6 [97]. Under the same separation conditions, similar resolution could only be achieved by using LPA (molecular mass $=4\cdot10^5$) with concentrations higher than 6% (w/v), and could not be achieved by using only PVP (molecular mass = 1 M) with a concentration as high as 15% (w/v). It is noted that the IPN of LPA and PVP also has the dynamic coating ability.

6. Mixtures of polymers

The mixing of two polymers is another approach

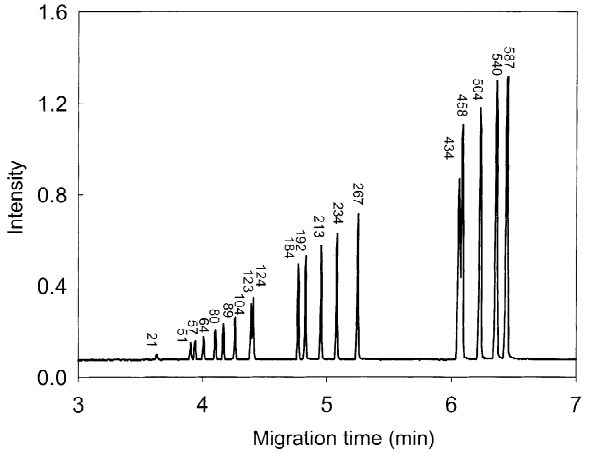


Fig. 6. Electropherogram of pBR322/Hae III digest by using 2% (w/v) PVP+1% (w/v) LPA 1×TBE buffer. Conditions: 10 cm effective length×50 μm I.D. capillary; running electric field strength: 200 V/cm; electrokinetic injection at 50 V/cm for 3 s; peak identification as in Fig. 4. Reprinted from Ref. [97], with permission.

to combine the desired properties of different components. The Yeung and co-workers used mixtures of PEO with different PEO molecular masses for the separation of both dsDNA [98] and single-stranded (ss) DNA [45,46]. It was found that low-molecular-mass polymers provided higher efficiency for smaller DNA fragments, while high-molecular-mass polymers favor the larger DNA fragments. In other words, polymer mixtures were beneficial to the separation of a broader range of DNA fragments. Mixtures of hydroethylcellulose (HEC) [99], LPA [31,32], and HEC with agarose [100] have been successfully applied to DNA separation. Zhou et al. [33] has employed a polymer mixture, consisting of a very-high-molecular-mass LPA and a small amount of low-molecular-mass LPA, to achieve a DNA sequencing up to 1300 bases within a short time period without significant increase in viscosity.

The above mixtures use the same types of polymers, i.e., the components are totally compatible. The mixture of polymers with different chemical compositions have also been tried by Kim and Yeung [47], where a mixture of PEO and hydroxypropylcellulose (HPC) was used for DNA sequencing analysis. However, the separation was very poor. The failure was attributed to the incompatibility of the two polymers. Based on their behaviors in random copolymers, LPA and PDMA were mixed and tested in DNA separation by CE in our laboratory [101]. 2.5% (w/v) LPA (2.2 M) was mixed with PDMA with different molecular masses (8000, 106 000, 1.1 M) and concentrations (0.2, 0.5, 1%). It was found that the incompatibility of the two polymers increased with increasing molecular mass and concentration and thus gave rise to a worse separation on DNA analysis. By incorporating low concentrations (0.2%) and low-molecular-mass (8000) PDMA in high-molecular-mass LPA solution, good dynamic coating ability of PDMA and good separation ability of LPA has been successfully combined. One base resolution of 0.5 up to 730 bases has been achieved within 80 min at ambient temperatures.

Mixtures of block copolymers of $B_{10}E_{270}B_{10}$ (B denoting polyoxybutylene) and $B_6E_{46}B_6$ were another type of DNA separation media developed in our laboratory [102,103]. Both $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ form flower-like micelle in aqueous solution, similar to the behavior of polyethylene glycols end-capped with fluorocarbon tails [89] and POEM10 [91] as has been discussed above. $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ have the same chemical composition and they are compatible. Light scattering results showed that they formed a uniform micelle in their mixture over a broad range of ratio in aqueous solution. Small angle X-ray scattering showed that no ordered packing was observed at elevated concentrations ($>2\%$) [102]. The physical properties of their mixtures are similar to those formed by the same triblock polymer with different chain length and molecular mass. However, the performance on DNA separation is quite different. Neither $B_{10}E_{270}B_{10}$ nor $B_6E_{46}B_6$ was a good separation medium. Poor resolution on dsDNA separation was observed when $B_{10}E_{270}B_{10}$ of up to 8% was used as the separation medium. $B_6E_{46}B_6$ had no sieving ability at all mainly due to its smaller size. However, the mixture of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ showed very promising results in dsDNA separation. The mass ratio of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ was determined by employing two series of experiments with one keeping the content of $B_{10}E_{270}B_{10}$ constant and the other keeping the content of $B_6E_{46}B_6$ constant. By taking into account of the desired properties on viscosity, speed and resolution, an optimal mass ratio was found to be 3% (w/v) $B_{10}E_{270}B_{10}$+5% (w/v) $B_6E_{46}B_6$. Under optimal

conditions, a resolution of 1.3 was achieved on the separation of 123/124 bp in the pBR322/HaeIII digest within 20 min by using a 10 cm column of 75 μm ID with LIF detection [102]. It is noted that the mixture of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ has all the advantages of F127 as a DNA separation medium in terms of dynamic coating ability, viscosity-adjustable property, and non-toxicity.

7. Summary

Copolymers used as DNA separation media attract growing interests due to its versatility in design and ability to provide new features. This capability should be even more important when dealing with new substrates (other than quartz) that are suitable for microchip fabrications. A range of copolymers with different molecular architecture and morphology have been developed and tested as DNA separation media with many promising features. Although LPA has remained the best separation medium to-date, the validation of our concept has endowed copolymers with great potential and promise to surpass LPA.

The design of the copolymer dominates its performance on DNA separation. Current designs rely partially on the knowledge of homopolymers with a purpose to improve its performance, e.g., all the copolymers reported today contain monomer segments of AA, E or DMA which are well-known separation media for DNA analysis. In fact, the concept of copolymers as DNA separation media is beyond the demonstration stage. According to Soane and others [44,93,104], cross-linked polymer gels have a higher resolution for large DNA fragments than that of linear polymer solutions because the relaxation time of the mesh is longer in gels, i.e., polymer gels form a network with the mesh size much more stable than that in polymer solution. Nevertheless, due to the drawbacks in column preparation, polymer gels have almost been replaced completely by polymer solutions in DNA capillary electrophoresis. Its advantage in the network formation could not be transplanted to homopolymer solutions since the entanglements in homopolymer network are transient in nature. However, it can at

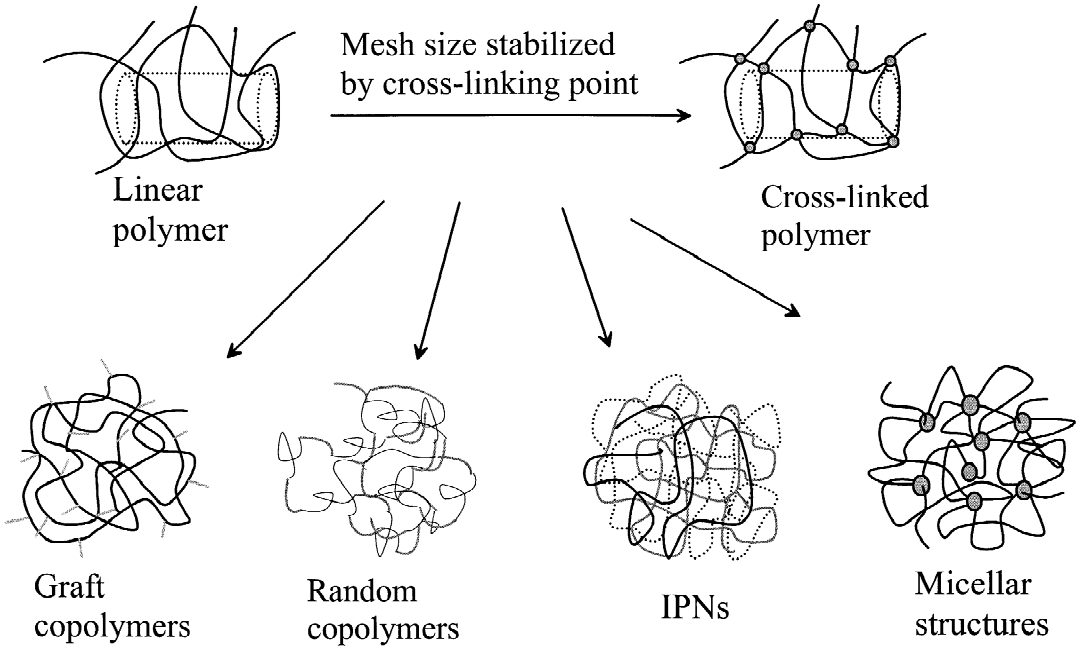


Fig. 7. Schematic pictures showing the stabilized mesh size in cross-linked polymer gels and the structures formed by copolymers with different molecular architectures.

least partially be maintained by using copolymers as schematically shown in Fig. 7. The (partial) incompatibility of the two components in copolymers is the driving force to keep the mesh size stabilized. As shown in Fig. 7, the branches in graft copolymers not only slow down the movement of the entangled chains, their tendency to aggregate provides a way to form transient cross-links [95]. Furthermore, with short side chains, the backbone chains should become more extended. Thus, random copolymers could provide another way to extend the polymer chains. The formation of IPN without cross-links decreases the amount of polymer chains needed to form the same mesh size. The hydrophobic cores in the micellar structure act like cross-linking points. All of the above copolymers conceptually provide an approach to increase the sieving ability. The degree of incompatibility, resulting from the properties of the monomer segments, plays a key role in the design of copolymers. For example, high incompatibility could result in a phase separation and ruin the whole performance, while low incompatibility may not provide strong enough driving force to enhance the sieving ability substantially.

In brief, copolymers with appropriate design should provide a practical way to exploit new DNA separation media with attractive features such as low solution viscosity, dynamic coating ability, and high sieving ability. The tailored separation media will not only improve the overall DNA sequencing throughput and reduce the cost-per-base rates but also benefit the general DNA analysis, e.g., for substrates other than quartz capillaries.

Acknowledgements

B.C. gratefully acknowledges the support of this work by the National Human Genome Research Institute (NIH 2R01HG01386-07) and the National Science Foundation (DMR-9984102).

References

[1] J. Zhang, M. Yang, X. Puyang, Y. Fang, L.M. Cook, N.J. Dovichi, Anal. Chem. 73 (2001) 1234.
[2] X.H. Huang, M.A. Quesada, R.A. Mathies, Anal. Chem. 64 (1992) 967.
[3] X.H. Huang, M.A. Quesada, R.A. Mathies, Anal. Chem. 64 (1992) 2149.
[4] I. Kheterpal, J.R. Scherer, S.M. Clark, A. Radhakrishnan, J. Ju, C.L. Ginther, G.F. Sensabaugh, R.A. Mathies, Electrophoresis 17 (1996) 1852.
[5] J.R. Scherer, I. Kheterpal, A. Radhakrishnan, W.W. Ja, R.A. Mathies, Electrophoresis 20 (1999) 1508.
[6] T. Irie, T. Oshida, H. Hasegawa, Y. Matsuoka, T. Li, Y. Oya, T. Tanaka, G. Tsujimoto, H. Kambara, Electrophoresis 21 (2000) 367.
[7] S. Takahashi, K. Murakami, T. Anazawa, H. Kambara, Anal. Chem. 66 (1994) 1021.
[8] T. Anazawa, S. Takahashi, H. Kambara, Anal. Chem. 68 (1996) 2699.
[9] T. Anazawa, S. Takahashi, H. Kambara, Electrophoresis 20 (1999) 539.
[10] Y. Zhang, H. Tan, E.S. Yeung, Anal. Chem. 71 (1999) 5018.
[11] H. Tan, E.S. Yeung, Anal. Chem. 70 (1998) 4044.
[12] S.X. Lu, E.S. Yeung, J. Chromatogr. A 853 (1999) 359.
[13] M.A. Quesada, S. Zhang, Electrophoresis 17 (1996) 1841.
[14] M.A. Quesada, H.S. Dhadwal, D. Fisk, F.W. Studier, Electrophoresis 19 (1998) 1415.
[15] E.S. Mansfield, J.M. Robertson, M. Vainer, A.R. Isenberg, K. Ferguson, S. Chow, D.W. Harris, D.L. Barker, P.D. Gill, B. Budowler, B.R. McCord, Electrophoresis 19 (1998) 101.
[16] S. Behr, M. Matzig, A. Levin, H. Eickhoff, C. Heller, Electrophoresis 20 (1999) 1429.
[17] M. Neumann, D.P. Herten, J. Dietrich, W.M. Sauer, J. Chromatogr. A 871 (2000) 299.
[18] D. Schmalzing, A. Adourian, L. Koutny, L. Ziaugra, P. Matsudaira, D. Ehrlich, Anal. Chem. 70 (1998) 2303.
[19] D. Schmalzing, N. Tsao, K. Lance, D. Chisholm, A. Srivastava, A. Adourian, L. Linton, P. McEwan, P. Matsudaira, D. Ehrlich, Genome Res. 9 (1999) 853.
[20] A.T. Woolley, R.A. Mathies, Proc. Natl. Acad. Sci. USA 91 (1994) 11348.
[21] Y. Shi, P.C. Simpson, J.R. Scherer, D. Wexler, C. Skibola, M.T. Smith, R. Mathies, Anal. Chem. 71 (1999) 5354.
[22] J.W. Simpson, M.C. Ruiz-Martinez, G.T. Mulhern, J. Berka, D.R. Latimer, J.A. Ball, J.M. Rothberg, G.T. Went, Electrophoresis 21 (2000) 135.
[23] A.T. Woolley, G.F. Sensabaugh, R.A. Mathies, Anal. Chem. 69 (1997) 2181.
[24] S. Liu, Y. Shi, W.W. Ja, R.A. Mathies, Anal. Chem. 71 (1999) 566.
[25] P.C. Simpson, D. Roach, A.T. Woolley, T. Thorsen, R. Johnston, G.F. Sensabaugh, R.A. Mathies, Proc. Natl. Acad. Sci. USA 95 (1998) 2256.
[26] A.S. Cohen, D.R. Najarian, A. Paulus, A. Guttman, J.A. Smith, B.L. Karger, Proc. Natl. Acad. Sci. USA 85 (1988) 9660.
[27] M. Kamahori, H. Kambara, Electrophoresis 17 (1996) 1476.
[28] K. Kleparnik, Z. Mala, P. Bocek, Electrophoresis 22 (2001) 783.
[29] M.C. Ruiz-Marinez, J. Berka, A. Belenkii, F. Foret, A.W. Miller, B.L. Karger, Anal. Chem. 65 (1993) 2851.

[30] E. Carrilho, M.C. Ruiz-Martinez, J. Berka, I. Smirnov, W. Goetzinger, W. Miller, A.D. Brady, B.L. Karger, Anal. Chem. 68 (1996) 3305.
[31] O. Salas-Solano, E. Carrilho, L. Kotler, A.W. Miller, W. Goetzinger, Z. Sosic, B.L. Karger, Anal. Chem. 70 (1998) 3996.
[32] W. Goetzinger, L. Lotler, E. Carrilho, M.C. Ruiz-Martinez, O. Salas-Solano, B.L. Karger, Electrophoresis 19 (1998) 242.
[33] H. Zhou, A.W. Miller, Z. Sosic, B. Buhholz, A.E. Barron, L. Kotler, B.L. Karger, Anal. Chem. 72 (2000) 1045.
[34] S. Carson, A.S. Cohen, A. Belenkii, M.C. Ruiz-Martinez, J. Berka, B.L. Karger, Anal. Chem. 65 (1993) 3219.
[35] K. Kleparnik, F. Foret, J. Berka, W. Goetzinger, A.W. Miller, B.L. Karger, Electrophoresis 17 (1996) 1860.
[36] C. Wu, M.A. Quesada, D.K. Schneider, R. Farinato, F.W. Studier, B. Chu, Electrophoresis 17 (1996) 1103.
[37] N. Best, E. Arriaga, D.Y. Chem, N.J. Dovichi, Anal. Chem. 66 (1994) 4063.
[38] J.Y. Yan, N. Best, J.Z. Zhang, H.J. Ren, R. Jiang, J. Hou, N.J. Dovichi, Electrophoresis 17 (1996) 1037.
[39] J.Z. Zhang, Y. Fang, J.Y. Hou, H.J. Ren, R. Jiang, P. Roos, N.J. Dovichi, Anal. Chem. 67 (1995) 4589.
[40] Y. Fang, J.Z. Zhang, J.Y. Hoq, H. Lu, N.J. Dovichi, Electrophoresis 17 (1996) 1436.
[41] T. Manabe, N. Chen, S. Terabe, M. Yohda, I. Endo, Anal. Chem. 66 (1994) 4243.
[42] N. Chen, T. Manabe, S. Terabe, M. Yohda, I. Endo, J. Microcol. Sep. 6 (1994) 539.
[43] M. Kamahori, H. Kambara, Electrophoresis 17 (1996) 1476.
[44] S.L. Pentoney, K.D. Konrad, W. Kaye, Electrophoresis 13 (1992) 467.
[45] E.N. Fung, E.S. Yeung, Anal. Chem. 67 (1995) 1913.
[46] N. Iki, E.S. Yeung, J. Chromatogr. A 731 (1996) 273.
[47] Y. Kim, E.S. Yeung, J. Chromatogr. A 781 (1997) 315.
[48] Q. Gao, E.S. Yeung, Anal. Chem. 70 (1998) 1382.
[49] J.M. Song, E.S. Yeung, Electrophoresis 22 (2001) 748.
[50] C. Barta, Z. Ronai, M. Sasvari-Szekely, A. Guttman, Electrophoresis 22 (2001) 779.
[51] M.A. Quesada, Curr. Opin. Biotechnol. 8 (1997) 82.
[52] B.R. Rosenblum, F. Oaks, S. Menchen, B. Johnson, Nucleic Acids Res. 25 (1997) 3925.
[53] R.S. Madabhushi, Electrophoresis 19 (1998) 224.
[54] Y. Xiong, S.-R. Park, H. Swerdlow, Anal. Chem. 70 (1998) 3605.
[55] C. Heller, Electrophoresis 21 (2000) 593.
[56] C. Heller, Electrophoresis 20 (1999) 1962;
C. Heller, Electrophoresis 20 (1999) 1978.
[57] C. Heller, Electrophoresis 19 (1998) 3114.
[58] J. Bashkin, M. Marsh, Appl. Theor. Electrophoresis 6 (1996) 23.
[59] V. Dolnik, W.A. Gurske, Electrophoresis 20 (1999) 3373.
[60] A.E. Barron, D.S. Soane, H.W. Blanch, J. Chromatogr. A 652 (1993) 3.
[61] A.E. Barron, H.W. Blanch, D.S. Soane, Electrophoresis 15 (1994) 596.
[62] A.E. Barron, W.M. Sunada, H.W. Blanch, Electrophoresis 15 (1994) 64.
[63] A.E. Barron, W.M. Sunada, H.W. Blanch, Electrophoresis 17 (1996) 744.
[64] Y. Baba, N. Ishimaru, K. Samata, M. Tsuhako, J. Chromatogr. A 653 (1993) 329.
[65] P.D. Grossman, D.S. Soane, Biopolymers 31 (1991) 1221.
[66] L. Mitnik, L. Salome, J.L. Viovy, C. Heller, J. Chromatogr. A 710 (1995) 309.
[67] H.E. Schwartz, K. Ulfelder, F.J. Sunzeri, M.P. Busch, R.G. Brownlee, J. Chromatogr. A 559 (1991) 267.
[68] M. Strege, A. Lagu, Anal. Chem. 63 (1991) 1233.
[69] T. Izumi, M. Yamaguchi, K. Yoneda, T. Isobe, T. Okuyama, T.J. Shinoda, J. Chromatogr. A 652 (1993) 41.
[70] K. Ganzler, K.S. Greve, A.S. Cohen, B.L. Karger, A. Guttman, N.C. Cooke, Anal. Chem. 64 (1992) 2665.
[71] V. Dolnik, W.A. Gurske, A. Padua, Electrophoresis 22 (2001) 707.
[72] E. SimoAlfonso, C. Gelfi, R. Sebastiano, A. Citterio, P.G. Righetti, Electrophoresis 17 (1996) 723;
E. SimoAlfonso, C. Gelfi, R. Sebastiano, A. Citterio, P.G. Righetti, Electrophoresis 17 (1996) 732.
[73] C. Gelfi, E. SimoAlfonso, R. Sebastiano, A. Citterio, P.G. Righetti, Electrophoresis 17 (1996) 738.
[74] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[75] L. Song, D. Liang, J. Kieleczawa, J. Liang, E. Tjoe, D. Fang, B. Chu, Electrophoresis 22 (2001) 729.
[76] C. Wu, T. Liu, B. Chu, Electrophoresis 19 (1998) 231.
[77] D. Liang, B. Chu, Electrophoresis 19 (1998) 2447.
[78] C. Wu, T. Liu, B. Chu, D. Schneider, V. Graziano, Macromolecules 30 (1997) 4574.
[79] R.L. Rill, B.R. Locke, Y. Liu, D.H. Van Winkle, Proc. Natl. Acad. Sci. USA 95 (1998) 1534.
[80] R.L. Rill, Y. Liu, D.H. Van Winkle, B.R. Locke, J. Chromatogr. A 817 (1998) 287.
[81] J.D. Epperson, J. Dodge, R.L. Rill, N.L. Greenbaum, Electrophoresis 22 (2001) 771.
[82] D. Liang, L. Song, S. Zhou, V.S. Zaitsev, B. Chu, Electrophoresis 19 (1999) 2856.
[83] D. Liang, S. Zhou, L. Song, V.S. Zaitsev, B. Chu, Macromolecules 32 (1999) 6236.
[84] J.L. Viovy, Rev. Mod. Phys. 72 (2000) 813.
[85] J. Ren, Z. Fang, J. Chromatogr. B 761 (2001) 139.
[86] M. Chiari, F. Damin, A. Melis, R. Consonni, Electrophoresis 19 (1998) 3154.
[87] M. Chiari, M. Cretich, S. Riva, M. Casali, Electrophoresis 22 (2001) 699.
[88] A.P. Sassi, A. Barron, M.G. Alonoso-Amigo, D.Y. Hion, J.S. Yu, D.S. Soane, H.H. Hooper, Electrophoresis 17 (1996) 1460.
[89] B.A. Buchholz, E.A.S. Doherty, M.N. Albarghouthi, F.M. Boghan, J.M. Zahn, A.E. Barron, Anal. Chem. 73 (2001) 157.
[90] M.N. Albarghouthi, B.A. Buchholz, E.A.S. Doherty, H. Zhou, A.E. Barron, Electrophoresis 22 (2001) 737.
[91] S. Menchen, B. Johnson, M.A. Winnik, B. Xu, Electrophoresis 17 (1996) 1451.
[92] S. Magnusdottir, J.L. Viovy, J. Francois, Electrophoresis 19 (1998) 1699.

[93] L.S. Lerman, H.L. Frisch, Biopolymers 21 (1982) 995.
[94] Y.C. Bae, D.J. Soane, J. Chromatogr. A 652 (1993) 17.
[95] J. Sudor, V. Barbier, S. Thirot, D. Godfrin, D. Hourdet, M. Millequant, J. Blanchard, J.L. Viovy, Electrophoresis 22 (2001) 720.
[96] B.M. Boyd, J.M. Prausnitz, H.W. Blanch, Electrophoresis 19 (1998) 3137.
[97] L. Song, T. Liu, D. Liang, D. Fang, B. Chu, Electrophoresis 22 (2001) 3688.
[98] H.T. Chang, E.S. Yeung, J. Chromatogr. B 669 (1993) 113.
[99] A.R. Isenberg, B.R. McCord, B.W. Koons, B. Budowle, R.O. Allen, Electrophoresis 17 (1996) 1505.
[100] B.A. Siles, D.E. Anderson, N.S. Buchanan, M.F. Warder, Electrophoresis 18 (1997) 1980.
[101] L. Song, D. Liang, Z. Chen, D. Fang, B. Chu, J. Chromatogr. A 915 (2001) 231.
[102] D. Liang, T. Liu, L. Song, B. Chu, J. Chromatogr. A 909 (2001) 271.
[103] T. Liu, D. Liang, L. Song, V.M. Nace, B. Chu, Electrophoresis 22 (2001) 449.
[104] P. Lindberg, M. Stjersnstrom, J. Roeraade, Electrophoresis 18 (1997) 1973.