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Star-shaped polymers for DNA sequencing by capillary electrophoresis

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ABSTRACT

The separation and sequencing of DNA are the main objectives of the Human Genome Project, and this project has also been very useful for gene analysis and disease diagnosis. Capillary electrophoresis (CE) is one of the most common techniques for the separation and analysis of DNA. DNA separations are usually achieved using capillary gel electrophoresis (CGE) mode, in which polymer gel is packed into the capillary. Compared with a traditional CGE matrix, a hydrophilic polymer matrix, which can be adsorb by the capillary wall has numerous advantages, including stability, reproducibility and ease of automation. Various water-soluble additives, such as linear poly(acrylamide) (PAA) and poly(*N*,*N*-dimethylacrylamide) (PDMA), have been employed as media. In this study, different star-shaped PDMA polymers were designed and synthesized to achieve lower polymer solution viscosity. DNA separations with these polymers avoid the disadvantages of high viscosity and long separation time while maintaining high resolution (10 bp between 271 bp and 281 bp). The influences of the polymer concentration and structure on DNA separation were also determined in this study; higher polymer concentration yielded better separation performance, and star-like polymers were superior to linear polymers. This work indicates that modification of the polymer structure is a potential strategy for optimizing DNA separation.

1. Introduction

Capillary electrophoresis (CE) is the most common separation and analysis technique for DNA because of its high separation efficiency and resolution, high analysis speed, ease of automation, and low sample consumption. Polymer-modified CE has become an attractive alternative to slab gel electrophoresis for DNA analysis because it possesses numerous advantages, including better separation performance and better efficiency in terms of time and labor. This technology has been successfully applied to the detection of mutations, genotyping, DNA sequencing, and gene expression [1–5]. In this type of CE, the separation performance is primarily affected by the separation medium, which influences both DNA fragment migration and electroosmotic flow (EOF). In early DNA CE separation methods, cross-linked poly(acrylamide) (PAA) gel was widely used. Although separation of some DNA fragments was successfully carried out using this gel, several problems, including false signals due to bubbles, inhomogeneity of the capillary wall, limited column life and poor reproducibility, still hinder its application. To overcome these problems, some non-crosslinked gels have been introduced for DNA CE separation. Several synthetic hydrophilic polymers have been developed and applied as DNA separation media, including linear poly(acrylamide) (PAA) [6–8], poly(*N*,*N*-dimethylacrylamide) (PDMA) [9–12], poly(*N*-acryloylaminoethoxyethanol) (PAAEE) [13], poly(acryloylaminopropanol) (PAAP) [14], poly(acryloylaminoethoxy ethyl glucopyranoside) (PAEG) [9], poly(ethylene glycol) (PEG) [15,16], and poly(vinylpyrrolidone) (PVP) [17]. Fused-silica capillaries are widely used in this method; however, the silanol groups on the surface of the capillary exist as sites for electrostatic interaction between the capillary surface and biomolecules, such as protein and DNA, and these interactions may result in poor resolution and reproducibility. Although covalent bonding to the silanol groups with hydrophilic polymers has been developed to solve this problem, this modification increases the cost of analysis and causes other problems, including capillary deterioration and coating inhomogeneity [18]. The use of a hydrophilic polymer matrix, which can be adsorb by capillary wall is an alternative way to obtain CE separation for DNA and other molecules. The polymer adsorbs to the inner capillary surface in a non-covalent manner; this coating is easier to utilize than the covalent coating method and obtains better reproducibility. Different types of hydrophilic polymers, such as PEG [15,16], PVP [17], PDMA [12] and some copolymers [19–22], have been used to form an adsorption layer on the capillary inner surface that can suppress the EOF and interactions between DNA fragments and the capillary inner surface.

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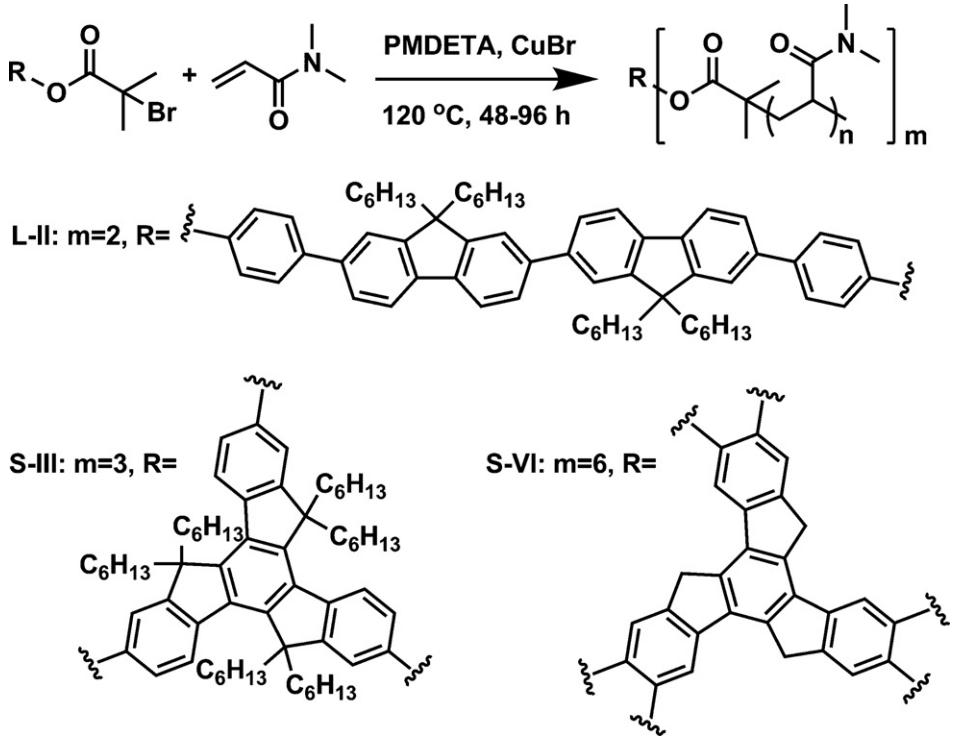

Scheme 1. Synthesis of polymers **L-II**, **S-III**, and **S-VI**.

CGE requires that the polymer solution reach a relatively high concentration, which usually leads to high viscosity of the polymer solution. High viscosity reduces the separation speed and limits column refreshing. In general, the viscosity of the polymer solution depends on the polymer molecular weight, which is also an important factor in the separation. Although there are methods to reduce the molecular weight of the polymer without sacrificing its sieving ability, the viscosity of a conventional polymer solution is still high. In fact, the concentration of polymer solutions applied in practice cannot exceed 6.5% [9], and the separation always requires thermo-control [23] or other complex devices [24,25]. There are a few reports on reducing viscosity by controlling the polymer structure. For example, a core-shell type of globular nano-sphere was developed to perform DNA separation at low viscosity [26]. However, the application of these polymers is limited because of their complicated synthesis.

In this paper, we report the design and synthesis of a series of PDMA polymers with linear and star-shaped topologies through atom transfer radical polymerization (ATRP). The main factors that affect DNA separation, such as viscosity and sieving ability, were studied. The separation performances of the PDMA polymers with different topologies were investigated and compared.

2. Experiment

2.1. Reagents and materials

2-Bromoisobutyryl bromide (97%) and 1-bromohexane (99%) were purchased from Alfa Aesar (Haysham, UK). *N,N,N′,N″,N″*-Pentamethyldiethylenetriamine (PMDETA) was purchased from TCI (Tokyo, Japan). All other reagents and solvents used in the synthesis, including BBr_3, *N,N*-dimethylacrylamide, CuBr, triethylamine, CH_2Cl_2 and THF, were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Anhydrous and oxygen-free solvents were distilled over sodium or CaH_2. Other reagents and solvents were used without further purification. Tris(hydroxymethyl)aminomethane (Tris), borate and EDTA were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Φ174/HaeIII digested dsDNA was purchased from Sigma–Aldrich (St. Louis, USA). Water was purified using a Milli-Q (Millipore, Billerica, USA) pure water system.

2.2. Synthesis and characterization of polymers

The structures of the ATRP initiators that were designed for this study are shown in Scheme S1 (see Electronic Supplementary Material). These initiators contain multiple initiating sites as well as fluorene- or truxene-based fluorescent cores that enable detection of the polymers via UV–vis and fluorescence spectroscopy, which can potentially benefit the study of the separation mechanism.

The synthesis (Scheme S2, see Electronic Supplementary Material) of the linear initiator **L-II-ini** began with the reported dibromobifluorene compound **1** [27]. A methoxyphenyl group was introduced through a Suzuki coupling reaction to obtain **2**, and then **2** was demethylated using tribromoborane to yield the diphenol **3**. The subsequent esterification of **3** with 2-bromoisobutyryl bromide generated the initiator **L-II-ini**. The synthesis (Scheme S2) of the three-site and six-site initiators, **S-III-ini** and **S-VI-ini**, started from trimethoxy- or hexamethoxy-substituted truxene derivatives **4** and **7**, respectively [28]. A hexyl group was introduced to improve the solubility of **4**. Deprotection and esterification procedures similar to those described for **L-II-ini** were carried out to afford the desired three-site **S-III-ini** or six-site **S-VI-ini** initiators.

The structures of all three initiators were confirmed using ^1H and ^{13}C NMR, ESI–FTICR MS, and elemental analysis.

L-II-ini: ^1H NMR (300 MHz, $CDCl_3$): δ 7.78–7.82 (m, 4H), 7.70 (d, J = 8.4 Hz, 4H), 7.56–7.65 (m, 8H), 7.24 (d, J = 8.4 Hz, 4H), 2.10 (m, 20H), 1.01 (m, 24H), 0.75–0.78 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 152.0, 150.3, 140.8, 140.4, 140.08, 140.06, 139.3, 128.4, 126.4, 126.3, 121.7, 121.5, 121.3, 120.3, 55.6, 55.5, 40.6, 31.7, 30.9, 29.9, 24.0, 22.8, 14.2. ESI–FTICR MS: $[M•]^+$ Calc'd. for $C_{70}H_{84}Br_2O_4$:

1146.4731. Found: 1146.4743. Anal. Calc'd. for $C_{70}H_{84}Br_2O_4$: C, 73.16; H, 7.37. Found: C, 73.15; H, 7.33.

S-III-ini: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37–8.34 (d, J = 8.1 Hz, 3H), 7.24 (s, 3H), 7.22–7.19 (d, J = 8.1 Hz, 3H), 2.91 (m, 6H), 2.15 (s, 18H), 2.06 (m, 6H), 0.95–0.87 (m, 36H), 0.65–0.63 (t, 18H), 0.52 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 155.3, 149.8, 144.7, 137.8, 137.7, 125.1, 118.6, 114.8, 55.8, 55.5, 36.9, 31.4, 30.7, 29.4, 23.9, 22.2, 13.8. ESI–FTICR MS: $[M+K]^+$ Calc'd. for $C_{75}H_{105}Br_3O_6K$: 1377.5093. Found: 1377.5077.

S-VI-ini: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (s, 3H), 7.56 (s, 3H), 4.10 (s, 6H), 2.16 (s, 18H), 2.13 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 142.3, 141.8, 141.1, 140.1, 136.4, 136.1, 119.8, 115.7, 55.5, 36.1, 31.2, 31.1. ESI–FTICR MS: $[M+H]^+$ Calc'd. for $C_{51}H_{49}Br_6O_{12}$: 1326.8319. Found: 1326.8319. Anal. Calc'd. for $C_{51}H_{48}Br_6O_{12}$: C, 45.98; H, 3.63. Found: C, 46.14; H, 3.63.

As shown in Scheme 1, the ATRP polymerization of *N,N*-dimethylacrylamide was performed in bulk. CuBr and PMDETA ligand were used to prepare the catalyst complex. Polymerization was carried out at 120 °C for 48–96 h to afford PDMA with different topologies.

The molecular weight of the polymer was characterized by static light scattering in water, and the analysis was performed on a Brookhaven Instrument equipped with a BI-200SM goniometer and a BI-TurboCorr digital correlator. All rheological measurements were performed on a stress rheometer (MCR 301, Anton Paar) with cone-and-plate (25 mm diameter, 2° cone angle) geometry at 20 °C. The shear viscosity of the polymer solution in 1× TBE (100 mM Tris, 90 mM boric acid, 1.0 mM EDTA, pH 8.3) was measured as a function of the applied shear rate by static steady stress sweep tests. The shear rate was changed from 0.1 to 10 s^{-1}.

2.3. CE procedures

DNA fragment separation was performed using a P/ACE MDQ CE system (Beckman Coulter, Fullerton, CA, USA) with laser-induced fluorescence detection. A fused-silica capillary was purchased from Ruifeng Capillary (ID/OD = 75 μm/360 μm; Hebei, China). Capillary pretreatment was carried out as follows: flushing with 1 M HCl at 20 psi for 15 min, flushing with water at 20 psi for 10 min, and conditioning with 1× TBE at 20 psi for 30 min. Separation medium was loaded by flushing the capillary with the different polymer solutions containing commercial DNA dye GeneFinder™ (Biovision, China) in 1× TBE at 80 psi for 6 min. Before each electrophoresis run, the capillary was equilibrated under an electric field of 8 kV to stabilize the current. The DNA sample was electrokinetically injected into the capillary at an electric field of 4 kV for 3 s. Electrophoresis was performed under an electric field of 8 kV (31 cm in length) at 20 °C. Capillary refreshment was carried out by rinsing with 1× TBE at 80 psi for 6 min.

Detection of the DNA separation was performed using the laser-induced fluorescence detector (λ_{ex}: 488 nm, λ_{em}: 520 nm; Beckman Coulter, Fullerton, CA, USA).

3. Results and discussion

3.1. ATRP of DMAA

ATRP is one of the most important living/control polymerization methods. However, ATRP of the DMA monomer with linear amines or bipyridines as ligands in the catalyst complex is not a controlled process because the PDMA complexes copper and forms species with lower catalytic activity. Consequently, the molecular weight and polydispersity of resultant polymers cannot be precisely controlled [29]. Instead, we synthesized a series of polymers, and we chose three of these polymers with similar molecular weights and different topologies to evaluate their DNA separation abilities (Table 1).

Table 1
Molecular weights and topologies of the polymers.

Polymer	**L-II**	**S-III**	**S-VI**
Initiator	**L-II-ini**	**S-III-ini**	**S-VI-ini**
Arms	2	3	6
M_w[a]	980, 000	828, 000	916, 000

[a] Measured by static light scattering in water.

3.2. Viscosity of the different polymers

When a polymer is used as a separation medium, its solution viscosity is a very important factor for DNA analysis. High solution viscosity always results in low separation speed and causes problems for column refreshing. The viscosity of the polymer solution is related to the polymer molecular weight and its topology. Star-shaped polymers tend to have a lower viscosity than linear polymers with the same molecular weight. The viscosities of three polymers with similar molecular weights but different topologies were compared (Fig. 1). As expected, at the same concentration (10%), the linear polymer (**L-II**) has higher static viscosity than the star polymers. Furthermore, polymers with more arms tend to have lower viscosity. The static viscosity of the three-armed **S-III** polymer was about 0.116 Pa S, which is almost one-tenth that of **L-II**, and the six-armed **S-VI** polymer had a much lower viscosity value of 0.0229 Pa S.

3.3. Influence of polymer concentration on DNA sieving

The electrophoretic behavior of the DNA fragments was studied in buffer systems containing the **L-II**, **S-III**, and **S-VI** polymers, respectively. The sieving abilities of these polymers were obtained from Fig. 2. The 11 fragments of the ΦX174/HaeIII digest could be successively separated at an electric field of −8 kV with a 31 cm long capillary in 20 min (Fig. 2).

The sieving ability of the different polymers was closely related to their molecular weight, concentration and topology. In general, sieving ability increased with the concentration of the polymer solution. The migration time of the fragments also increased as the concentration of polymer solutions increased from 1% to 5% (Fig. 3). Longer fragments had a larger increase in migration time than shorter fragments, and all of the peaks were separated at a concentration of 5%. The separation process of the DNA fragments is based on the tangle interaction between the DNA strand and polymers. At a higher concentration, the tangle interaction became

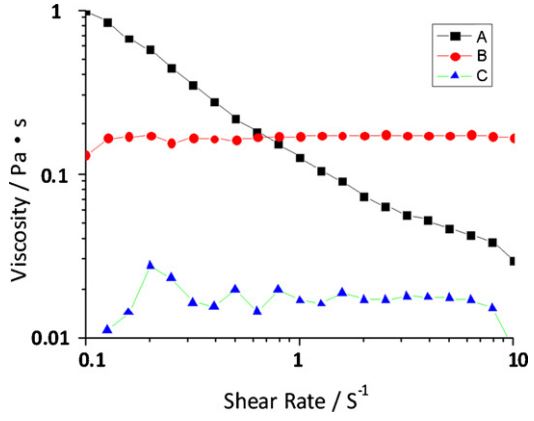

Fig. 1. Viscosities of different polymer solutions in 1× TBE at polymer concentrations of 10%. Polymer solutions A, B, and C were **L-II**, **S-III**, and **S-VI**, respectively.

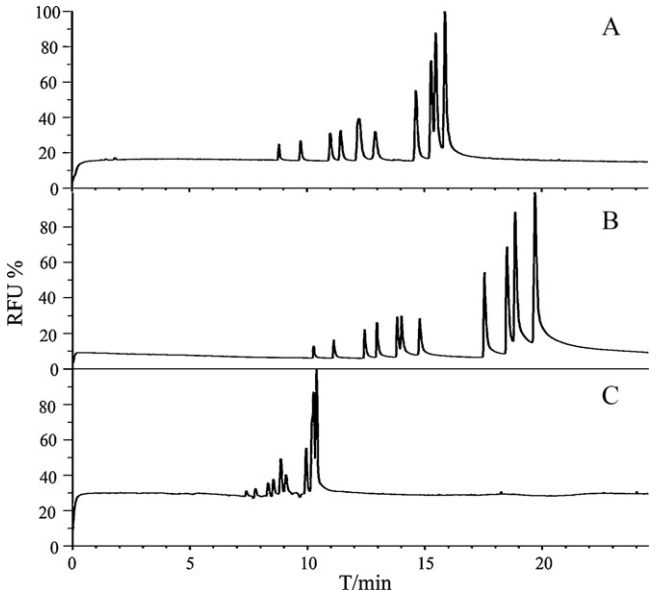

Fig. 2. Separation of dsDNA fragments in polymer media using CE. ΦX174/HaeIII digest (2.5 μg/ml), containing DNA fragments of 72, 118, 194, 234, 271, 281, 301, 603, 872, 1073, and 1353 bp, were separated in a bare capillary (21 cm effective length and 75 μm I.D.) filled with 5% polymer. Polymers A, B and C were **L-II**, **S-III** and **S-VI**, respectively. Electrokinetic injection at −4 kV for 3 s was used; the temperature was 20 °C, and the applied voltage was −8 kV. RFU: relative fluorescence units.

stronger, which resulted in stronger retaining of the fragments and longer migration time. The tangle interaction was also strengthened as the length of the DNA fragments increased, which resulted in increased migration time for longer fragments. As a result, DNA fragments had a better separation at a higher concentration.

3.4. Influence of polymer topology on DNA sieving

Polymer topology is a more important factor affecting the separation efficiency than polymer concentration. Although the molecular weights of the three polymers were similar, the DNA

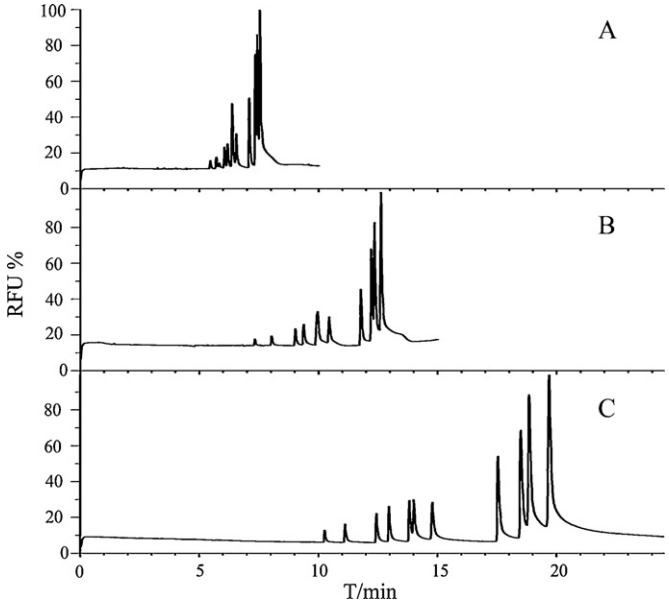

Fig. 3. Sieving ability analysis in different concentrations of **S-III** polymer solution. The concentrations of A, B and C were 1%, 3% and 5%, respectively. Analysis conditions: 21 cm effective length and 75 μm I.D. capillary; 1× TBE; −8 kv electronic field. Injection: 3 s × −4 kV.

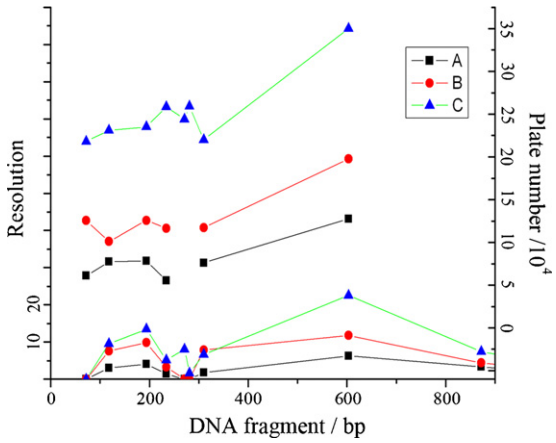

Fig. 4. Comparison of sieving abilities with different polymers. DNA samples were analyzed using a fused-silica capillary (21 cm effective length and 75 μm I.D.) filled with 5% polymer. Polymer solutions A, B and C were **L-II**, **S-III** and **S-VI**, respectively. Electrokinetic injection at −4 kV for 3 s was used; the temperature was 20 °C, and the applied voltage was −8 kV.

fragment separation performance was different for each of them (Figs. 4 and 5). The mobility of the DNA fragments was the highest in **S-VI** polymer solution because the interaction between the polymer and DNA fragments decreased as the arm length decreased. The lowest DNA fragment mobility was not observed with the **L-II** polymer, which has the longest arm length, but with the **S-III** polymer. **S-III** had the strongest interaction with the DNA fragments and achieved the best separation of 271-bp and 281-bp fragments (Fig. 2).

3.5. A strategy to reduce viscosity

As mentioned previously, the viscosity of the polymer solution significantly influences the separation efficiency. Stronger interaction between the polymer chains and DNA fragments is a prerequisite for achieving high separation efficiency. However, strong interactions between polymer chains also exist and lead to high viscosity of the polymer solution, and high viscosity leads to a low mass transfer rate and sample band broadening as well as difficulties in column refreshment. The use of a star-shaped polymer may be a way to address this dilemma. A comparison of the viscosity of the polymer solutions (Fig. 1) and the mobility of the DNA fragments in these polymers solutions (Fig. 5) showed

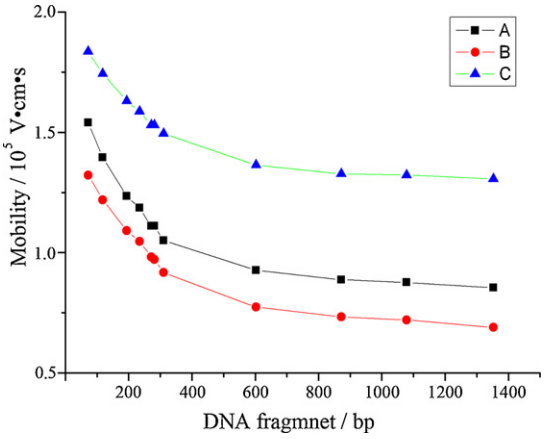

Fig. 5. Mobility of DNA fragments in different polymer solutions (5%). Polymer solutions A, B and C were **L-II**, **S-III**, and **S-VI**, respectively. Analysis conditions: 21 cm effective length and 75 μm I.D. capillary; 1× TBE; −8 kv electronic field. Injection: 3 s × −4 kV.

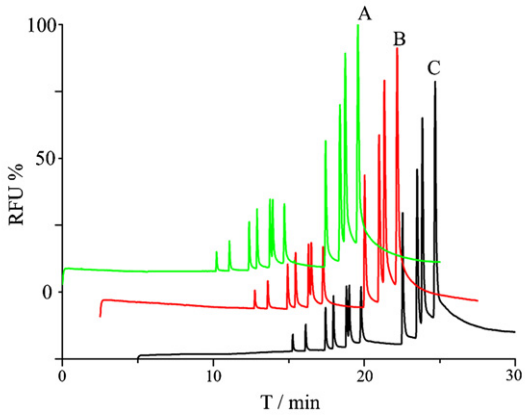

Fig. 6. Repeatability of the capillary for DNA separation. A, B and C present three sequential CE separations without refreshing the capillary. Separation matrix: 5% **S-III** 1× TBE; analysis conditions: 21 cm effective length and 75 μm I.D. capillary; 1× TBE; −8 kv electronic field. Injection: 3 s × −4 kV.

that DNA fragments had a lower mobility and a higher separation efficiency in the **S-III** polymer than in the **L-II** polymer even when the **S-III** polymer had a lower viscosity. This result was observed because the **S-III** polymer has one more arm than the **L-II** polymer, which provides additional opportunities to interact with the DNA fragments; the **S-III** polymer has shorter arms, which resulted in weaker interactions between polymer chains and lower viscosity. Thus, by controlling the topology of the polymers, the viscosity was reduced while the separation efficiency was maintained.

3.6. Adsorption of PDMA to capillary wall

In addition to high separation speed and strong sieving ability, the star-like PDMA polymer solutions exhibited good adsorption ability. The silica hydroxyl groups on the inner surface of the bare capillary will interact with DNA fragments if the hydroxyl groups are not protected before performing the separation. This interaction is unstable and results in low resolution and poor reproducibility of the CE separation. Different coating methods have been developed to eliminate these interactions. Although these modifications improved the separation efficiency, they have some disadvantages, including inhomogeneous modification, high cost, storage problems, and limited column life. Unlike other polymers reported for CE separation of DNA fragments, a coated capillary is not necessary for PDMA polymers. The PDMA can adsorb to the inner surface of the capillary, and the PDMA significantly reduces the interaction between samples and the capillary inner surface [12]. All of the polymers introduced here demonstrated this adsorption ability; thus, the coating process can be avoided. Adsorption of the PDMA polymers produces other benefits, including a homogeneous inner surface that results in good repeatability (Fig. S1, see Electronic Supplementary Material). In addition, the capillary maintained working status longer due to the reduced interaction between the capillary and DNA fragments. The results showed that refreshing the polymer solution for each analysis was unnecessary (Fig. 6).

4. Conclusion

Novel, star-shaped PDMA polymers were synthesized and employed as a matrix for DNA fragment analysis. These polymers showed improved sieving ability and separation time when compared to linear polymers. In addition, the ability of PDMA to adsorb to the inner surface of capillary obviates the need for a covalent coating process. The separation of DNA with star-shaped PDMA provides a novel strategy to optimize the CE matrix for DNA analysis. The matrix characteristics, including tangling ability, adsorption ability, and viscosity, are affected by the molecular weight and topology of these polymers. The results reported herein demonstrate that structural control of the synthetic polymers may permit the selection of different sieving matrices to satisfy different analysis requirements.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.03.027.

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