ELSEVIER

Journal of Chromatography A, 909 (2001) 271–278

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Mixed triblock copolymers used as DNA separation medium in capillary electrophoresis

Dehai Liang, Tianbo Liu, Liguo Song, Benjamin Chu*

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

Received 31 July 2000; received in revised form 31 October 2000; accepted 31 October 2000

Abstract

A polymer solution, formed by mixing two polyoxybutylene–polyoxyethylene–polyoxybutylene (BEB) triblock copolymers ($B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$), was tested as a new separation medium for double-stranded DNA separation in capillary electrophoresis. The mixture of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ has a viscosity-adjustable property and a dynamic coating ability, which makes the medium very easy to handle. The performance of the mixture on the DNA separation is greatly affected by the mass ratio of the two constituents. There is a minimum amount of concentration for $B_{10}E_{270}B_{10}$, below which the medium will lose its performance. The addition of $B_6E_{46}B_6$ increases both the selectivity and the separation efficiency. The optimal concentration, with 3% (w/v) $B_{10}E_{270}B_{10}$ and 5% (w/v) $B_6E_{46}B_6$, is determined with the consideration of both speed and resolution. A resolution of 1.3 was achieved on the separation of 123/124 base pairs in the pBR322/HaeIII digest within 20 min by using a 10 cm column of 75 μm I.D., demonstrating the potential use of mixtures of amphiphilic block copolymers as an effective DNA separation medium. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Polymer separation media; DNA

1. Introduction

Capillary electrophoresis (CE) has been demonstrated to be a powerful method for the analysis of charged biomolecules, such as DNAs and proteins. Polymer solutions, as an alternative to gels, are widely used at present in the high-performance CE for DNA separations. Among them, linear polyacrylamide (PAM), which has achieved more than 1000 base read length in DNA sequencing analysis [1,2], showed good potential. However, its high viscosity and the need for column treatment have prompted an effort to find more promising separation media that may circumvent these shortcomings but with even better resolution.

When PAM, cellulose and its derivatives [3–6] are used as separation media, it is necessary to coat the capillary inner wall in order to suppress electroosmosis and analyte adsorption. In DNA analysis, bonding a polymer chemically to the capillary surface by reaction of part of the silanol groups on the silica surface with a reagent is often used, such as the Hjertén method [7]. The chemical bonding approach usually includes several steps and is time and effort consuming. Dynamic coating only needs little or no pretreatment of the column and has been acknowledged and appreciated as one of the desirable properties for the separation medium. The more

*Corresponding author. Tel.: +1-631-6327-928; fax: +1-631-632-6518.

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

0021-9673/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(00)01101-8

popular approach is to use polymers, such as poly-(vinylpyrrolidone) (PVP) [8], poly(ethyleneoxide) (PEO) [9,10] and polydimethylacrylamide [11] with dynamic coating ability as the separation medium.

The automation of CE for DNA analysis requires the separation medium with low viscosity, for easy filling and replenishment of the separation medium. Unfortunately, most of the separation media used at present have high viscosity at working concentrations, except for PVP whose performance is worse than that of PAM or PEO [8]. Recent study showed that polymers with high molecular mass at low concentration have longer reader length and better resolution when mixed with corresponding low-molecular-mass polymers [1]. However, the high viscosity problem remains. Polymer solutions with viscosity-adjustable properties can circumvent this shortcoming and become very easy to handle.

Some polymers, such as poly(*N*-isopropylacrlyamide) (PNIPAM) [12], may possess both the dynamic coating ability and the viscosity-adjustable property, but they cannot be used as a DNA separation medium because of the low sieving ability. On the other hand, copolymers that can combine the properties of two or more homopolymers may have the potential to be an effective DNA separation medium. Two kinds of such copolymers have been developed and tested in our laboratory, with one being the triblock copolymer $E_{99}P_{69}E_{99}$ [where P=poly(propyloxide) and E=poly-(ethyleneoxide)] [13,14] and the other one being the PNIPAM–g-PEO [12,15]. $E_{99}P_{69}E_{99}$ has a very convenient temperature dependent viscosity-adjustable property. At low temperature (about 4.0°C), 25% (w/v) $E_{99}P_{69}E_{99}$ is in a solution state with very low viscosity. The same polymer solution becomes gel-like at room temperatures. The mesh size of the matrix formed by $E_{99}P_{69}E_{99}$ is very small mainly due to the presence of more hydrophobic cores, which results in longer migration time at comparable applied electric field strength. On the contrary, it has a good separation for the small size oligonucleotides and can be used with very short columns (e.g., 8 mm). Furthermore, with lower overall dielectric constant because of higher polymer concentration, higher applied electric field strength becomes acceptable with less Joule heating effect. PNIPAM–g-PEO showed a high sieving ability: one base pair (123 and 124) resolution was achieved with the pBR322/HaeIII digest being separated within 12.5 min at a polymer concentration of 8% (w/v). It can also be used in a high-speed separation of ΦX174/HaeIII digest, with a run time within 24 s [15]. However, the viscosity-adjustable property is not so obvious because of the densely grafted PEO.

Based on our knowledge about the triblock copolymers, a new separation medium, consisting of a mixture of a long-chain copolymer $B_{10}E_{270}B_{10}$ (where B=polyoxybutylene) and a shorter chain copolymer $B_6E_{46}B_6$, was tested at different mass ratios. The copolymer mixture has a dynamic coating ability and a viscosity-adjustable property; but it also has a higher mesh size when compared with that of $E_{99}P_{69}E_{99}$. Studies on the structure of the copolymer mixtures and its relationship with the DNA separation can be found elsewhere [16]. In brief, pure $B_{10}E_{270}B_{10}$ is not a very efficient separation medium and $B_6E_{46}B_6$ is even worse. But a copolymer mixture within a certain range of mass ratio can produce a polymer network of appropriate mesh size so that the copolymer mixture becomes an effective separation medium. This mixture forms mixed micelles in aqueous solution at low polymer concentrations and open network gel-like structures connected by hydrophobic clusters at higher concentrations. Small angle X-ray scattering (SAXS) measurements indicate no ordered structure in these mixtures, different from $E_{99}P_{69}E_{99}$ where very ordered micellar packing could be observed.

In this paper, the effect on the separation of pBR322/HaeIII digest at different mass ratio by changing either $B_{10}E_{270}B_{10}$ or $B_6E_{46}B_6$ has been systemically studied and the role in the DNA separation that the components played has also been discussed. An optimal mass ratio is found with the consideration of both resolution and speed and the separation of other types of DNAs has been tested.

2. Experimental

2.1. Material

The $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ triblock copolymer samples were obtained as a gift from Dow (Freeport,

TX, USA) and used without further purification. Tris, boric acid, EDTA and ethidium bromide (EtBr) were purchased from Sigma (St. Louis, MO, USA). All chemicals were electrophoresis grade and no further purification was performed. The mixtures of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ were prepared by mixing them with 1×TBE buffer [89 m*M* tris(hydroxymethyl)aminomethane, 89 m*M* boric acid and 2 m*M* EDTA in deionized water] to the desired concentration with different copolymer mass ratio. The mixtures were stored in the refrigerator at 4°C for a few days before use. pBR322/HaeIII digest and pBR322/MspI digest were purchased from Sigma and New England Biolabs (Beverly, MA, USA), respectively, and were diluted to 10 μg/ml by deionized water.

2.2. CE

Separation was performed using a laboratory-built CE system with laser-induced fluorescence detection. The detailed description has been described elsewhere [17]. A 13 cm fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with I.D.s of 75–100 μm was flushed with 1 *M* HCl for 10 min. A detection window of 1.5 mm width was opened at the predetermined length from the cathodic end by stripping the polyimide coating off the capillary with a razor blade. Both cathode and anode reservoirs (1.6 ml volume) were filled with 1×TBE buffer and 3 μg/ml ethidium bromide. The copolymer solution was injected into the capillary tubing by using a 50-μl syringe. The capillary tubing was then assembled onto the holder and a pre-run at a constant electric field strength of 200 V/cm was used to introduce the fluorescent dye into the separation medium and to stabilize the current. The DNA sample was electrokinetically injected into the capillary. The voltage was generated by using a PCI-MIO-16E-4 multifunction I/O board (National Instruments, Austin, TX, USA) together with a high-voltage power supply FC30P04 (Glassman High Voltage, Whitehouse Station, NJ, USA). The fluorescence was detected by using a Hamamatsu R928 photomultiplier tube (Rockaway, NJ, USA) and data were acquired by the same I/O board and processed by a Pentium personal computer.

2.3. Viscosity measurement

A digital viscometer LVTDCP (Brookfield Engineering Labs., Stoughton, MA, USA) was used to determine the viscosity of the polymer mixture at different temperatures. The viscometer was calibrated by the viscosity standards (Brookfield Engineering Labs.) at 975 cP.

3. Results and discussion

3.1. Separation performance

The $B_{10}E_{270}B_{10}$ molecules have much longer chains than those of $B_6E_{46}B_6$ and tend to form flower-like micellar structures with dangling E chains. On the other hand, the $B_6E_{46}B_6$ chains are shorter and could form mixed micelles with $B_{10}E_{270}B_{10}$. To find out the effect of mixed micelles as a separation medium and also the optimal mass ratio for the copolymer mixture, two series of polymer mixtures were prepared with one case keeping the concentration of $B_{10}E_{270}B_{10}$ constant and the other keeping $B_6E_{46}B_6$ constant. Although $B_6E_{46}B_6$ and $B_{10}E_{270}B_{10}$ can form homogeneous solutions over a wide range, we set the total mass concentration to less than 10% (w/v) based on the consideration of low viscosity for the copolymer mixture and the appropriate mesh size.

Fig. 1a–d shows the electropherogram of pBR322/HaeIII digest by using the triblock copolymer mixtures having a different mass concentration of $B_6E_{46}B_6$ (from 3 to 6%, w/v) but a constant mass concentration of $B_{10}E_{270}B_{10}$ (3%, w/v). All the other conditions, such as column dimension and size, injection time and voltage, running electric field strength, were kept constant. With increasing mass ratio of $B_6E_{46}B_6$, the migration time became longer while the resolution became better. With 3% (w/v) $B_6E_{46}B_6$ added as shown in Fig. 1a, the separation of pBR322/HaeIII digest was completed within 11 min with poor resolution for DNA fragments greater than 400 base pairs (bp) where only three peaks were obtained instead of five. However, all the five fragments were separated by increasing the $B_6E_{46}B_6$ concentration to 4% (w/v) as shown in Fig. 1b, where the two fragments of 184 bp and 192 bp were

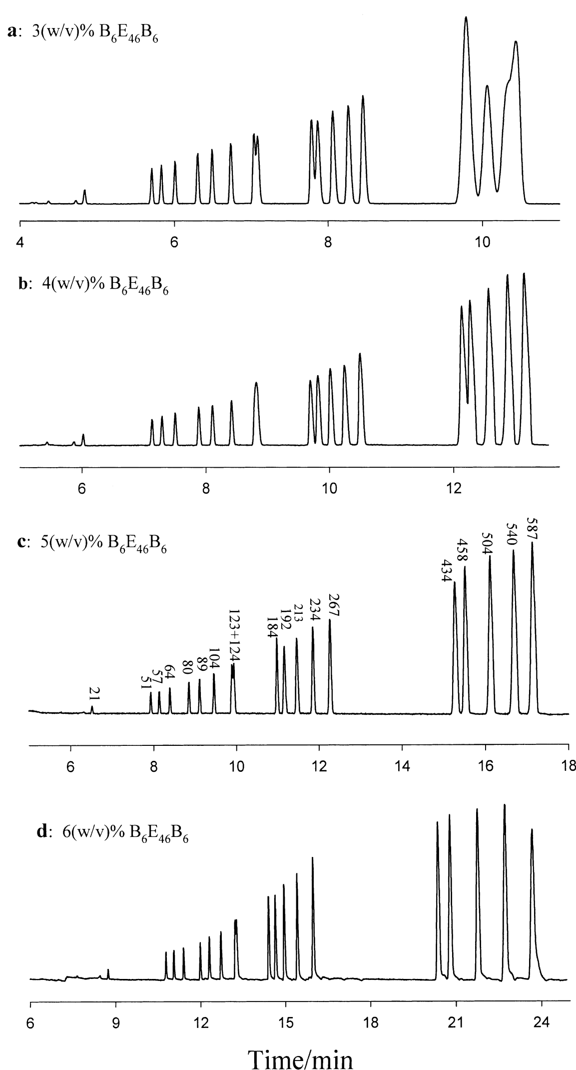

Fig. 1. Electropherogram of pBR322/HaeIII digest. Experimental conditions: 10 cm effective length×100 μm I.D. capillary, 1× TBE; 3 μg/ml EtBr, running electric field strength: 150 V/cm; electrokinetic injection at 300 V/cm for 1 s; room temperature; separation medium: mixture of 3% (w/v) $B_{10}E_{270}B_{10}$ and (a) 3% (w/v), (b) 4% (w/v), (c) 5% (w/v), (d) 6% (w/v) $B_6E_{46}B_6$. 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.

also baseline separated (instead of half in Fig. 1a). Further improvements on the separation of the large fragment (>400 bp) were achieved by increasing the $B_6E_{46}B_6$ concentration to 5% (w/v). When 6% (w/v) $B_6E_{46}B_6$ was added, the resolution was even better but with even longer run time.

To clearly understand the effect of $B_6E_{46}B_6$ on the separation, we fitted the electropherogram data by using the Peakfit 4 (SPSS, San Rafael, CA, USA) software. The selectivity (S) and the efficiency (N or plate number) were calculated according to the following equations [18]:

$$S = 2(t_1 - t_2)/(t_1 + t_2) \tag{1}$$

with t_1 and t_2 being the migration time of two fragments and $t_1 > t_2$.

$$N = 5.54(t/w_{1/2})^2 \tag{2}$$

with t and $w_{1/2}$ being the migration time and full peak width at half maximum height, respectively.

From Eq. (1), we can see the selectivity used to characterize how far two fragments can be separated within a certain column, i.e., how good the sieving ability the medium has. As S is directly related to the size difference of two fragments ($N_1 - N_2$), $S/(N_1 - N_2)$ is used here for a proper comparison of different fragment pairs. From Eq. (2), the efficiency implies the peak broadening effect since the peak width is involved. If the DNA bands become broad in medium 1 within a certain time period while they are broadened less in medium 2 within the same time period, medium 2 has a higher efficiency than medium 1.

The 123 bp and 124 bp fragments showed poor resolution in this and the next electropherogram sets, they are not included in the peak fitting data. All the other peak fittings have $r^2 > 0.99$. Fig. 2a shows the change in selectivity of the DNA fragments at different concentrations of $B_6E_{46}B_6$. The selectivity remained almost the same for the smaller size DNA fragments (<300 bp) with concentrations of $B_6E_{46}B_6$ ranging from 3 to 6% (w/v). For the DNA fragments larger than 300 bp, the selectivity increased with increasing concentration of $B_6E_{46}B_6$, i.e., with more $B_6E_{46}B_6$ added into the 3% (w/v) $B_{10}E_{270}B_{10}$, the medium increased its sieving ability for the larger DNA fragments. Fig. 2b shows the change in efficiency at different $B_6E_{46}B_6$ concentrations. The higher the concentration of $B_6E_{46}B_6$,

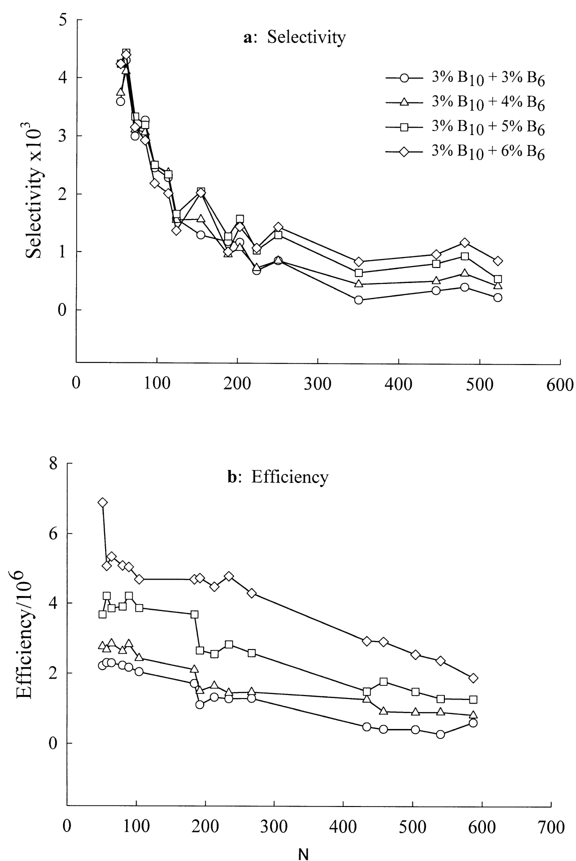

Fig. 2. Plot of (a) selectivity and (b) efficiency versus DNA size (N). All the data points were calculated from the electropherogram in Fig. 1.

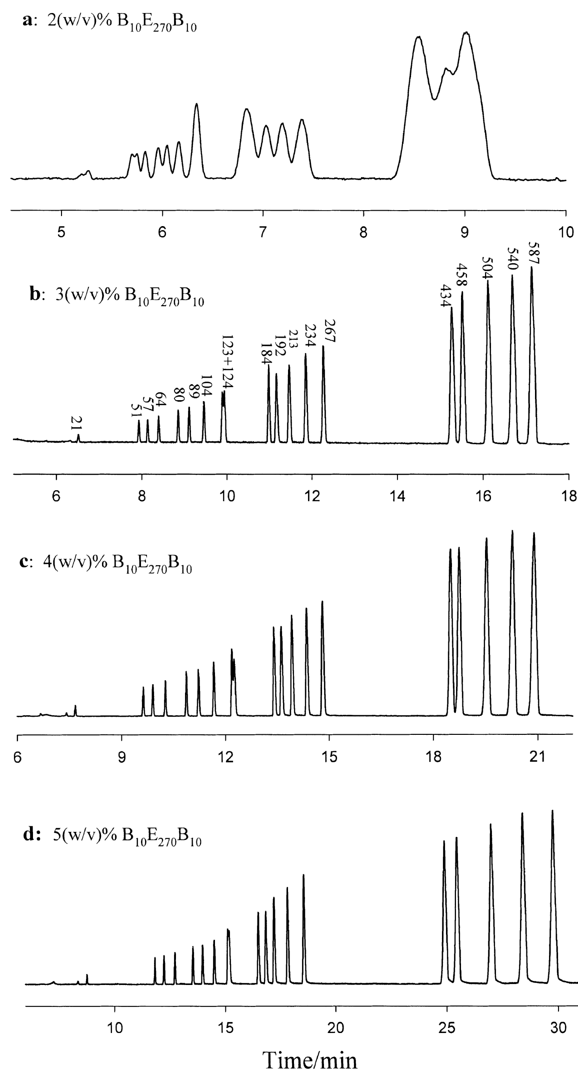

Fig. 3. Electropherogram of pBR322/HaeIII digest at constant $B_6E_{46}B_6$ concentration of 5% (w/v) and various concentrations of $B_{10}E_{270}B_{10}$ at (a) 2% (w/v), (b) 3% (w/v), (c) 4% (w/v), (d) 5% (w/v) $B_6E_{46}B_6$. Other conditions as in Fig. 1.

the higher the efficiency, i.e., the addition of the $B_6E_{46}B_6$ decreased the band broadening effect.

Fig. 3a–d shows the electropherogram set of pBR322/HaeIII digest by using the mixtures having different concentrations of $B_{10}E_{270}B_{10}$ but constant concentration of $B_6E_{46}B_6$ at 5% (w/v). As shown in Fig. 3a, the resolution was really poor with 2% (w/v) $B_{10}E_{270}B_{10}$ in the mixture. Some of the DNA fragments could not be separated at all. Great improvement was achieved with 3% (w/v) $B_{10}E_{270}B_{10}$ being added there, all the fragments were baseline separated except for the 123 bp and 124 bp pair, as shown in Fig. 3b. However, further increase in the $B_{10}E_{270}B_{10}$ content did not lead to a better improvement, as shown in Fig. 3c and d. Another conclusion from Fig. 3a–d is that with higher concentrations of $B_{10}E_{270}B_{10}$, the migration time became longer. Fig. 4a shows the change in selectivity at different concentrations of $B_{10}E_{270}B_{10}$. By using 2% (w/v) $B_{10}E_{270}B_{10}$, only electropherogram data below 300 bp were fitted because of the poor separation. From 2% (w/v) $B_{10}E_{270}B_{10}$ to 3% (w/v) $B_{10}E_{270}B_{10}$, there was a substantial increase in the selectivity, especially for the small size DNA fragments, whereas from 3% (w/v) $B_{10}E_{270}B_{10}$ to

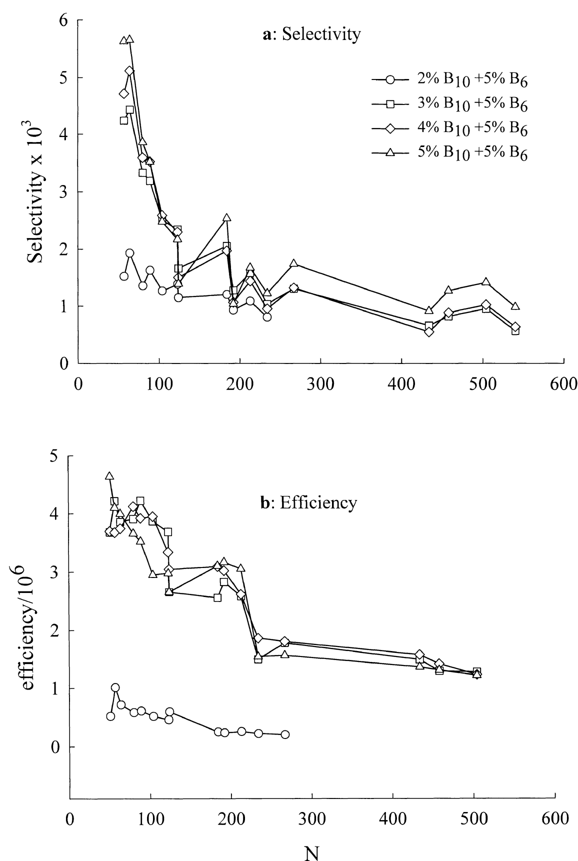

Fig. 4. Plot of (a) selectivity and (b) efficiency versus DNA size (*N*). All the data points were calculated from the electropherogram in Fig. 3.

5% (w/v) $B_{10}E_{270}B_{10}$, there was almost no difference in selectivity except that the 5% (w/v) $B_{10}E_{270}B_{10}$ had a higher selectivity than the other two for large DNA fragments. The change in efficiency showed more obvious trends as seen in Fig. 4b, where the efficiency of the DNA fragments obtained by using the mixtures whose $B_{10}E_{270}B_{10}$ concentration was from 3 to 5% (w/v) was almost the same but much higher than that of 2% (w/v) $B_{10}E_{270}B_{10}$.

Neither $B_{10}E_{270}B_{10}$ nor $B_6E_{46}B_6$ was a good medium for DNA separation, but the mixtures in different mass ratios had a different performance as shown in Figs. 1–4. The mass ratio change in $B_{10}E_{270}B_{10}$ or $B_6E_{46}B_6$ has a different effect on the DNA separation, suggesting that they play a different role in the formation of the medium network. In the presence of $B_6E_{46}B_6$, there is a minimum amount of $B_{10}E_{270}B_{10}$ about 2–3% (w/v) required to achieve a successful network. Below that amount, the polymer matrix is not strong enough to be an effective separation medium even by adding more $B_6E_{46}B_6$, as can be seen from the separation in Fig. 1a by using 3% (w/v) $B_{10}E_{270}B_{10}$ with 3% (w/v) $B_6E_{46}B_6$ and in Fig. 3a by using 2% (w/v) $B_{10}E_{270}B_{10}$ with 5% (w/v) $B_6E_{46}B_6$. Even though the medium in Fig. 3a has the total amount of polymer (7%, w/v) more than that in Fig. 1a (6%, w/v), its resolution on DNA separation is worse. Once the minimum amount of $B_{10}E_{270}B_{10}$ is reached, the essential scaffolding for the matrix is established. The further addition of $B_{10}E_{270}B_{10}$ will not improve much on the sieving ability of the medium. The network formed by $B_{10}E_{270}B_{10}$ itself is coarse and crude due to its long chains [16]. The addition of $B_6E_{46}B_6$ makes the network less crude and more uniform since $B_6E_{46}B_6$ has short chains and it modified the network like an homogenizer. The more $B_6E_{46}B_6$ added, the more uniform the network is. The dispersion effect will be alleviated when the DNA bands migrate through the medium. The increase in the amount of $B_{10}E_{270}B_{10}$ or $B_6E_{46}B_6$ slows down the speed due to the decrease in the medium pore size.

The mixture of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ is different from those mixtures of the same polymer type with different molecular mass both in the structure and in the purpose. The high-molecular-mass polymer at lower concentrations showed better resolution for large DNA fragments and longer read length but at some loss on the resolution of small DNA fragments. The addition of the low-molecular-mass polymer into the matrix could maintain the separation of small fragments without much increase in the viscosity. Since they are same polymer type, there is little change in the matrix structure when mixed except for the overall average pore size.

The polymers used in the mixtures with different molecular mass are usually good DNA separation media, such as PAM [2,17] and PEO [10]. The mixture of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ has a structure and pore size different from those formed by themselves [16]. Our purpose in mixing $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ together is to find a suitable DNA sepa-

ration medium that has a high sieving ability and is easy to handle. Amphiphilic block copolymers with different types and architectures are commercially available and have some applications in drug delivery and other pharmaceutical systems [18]. They are not toxic and their viscosity adjustable property and self-coating ability are two useful features if they can be used as an effective separation media for DNA separation. Unfortunately, most of them showed low sieving ability when used by themselves, except $E_{99}P_{69}E_{99}$. The mixture of two or more of them with a certain ratio could drastically improve the performance in DNA separation as discussed above. The study on the $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ also enlightens us to explore the mixtures formed by other amphiphilic block copolymers as separation media as further improvements are clearly within our group.

3.2. Optimal conditions

Based on the discussion above and by considering the resolution, speed and mixture viscosity at the same time, 3% (w/v) $B_{10}E_{270}B_{10}$ with 5% (w/v) $B_6E_{46}B_6$ was chosen as the optimal concentration for the selected pair. Fig. 5 shows the change of viscosity versus temperature. At 2°C, the viscosity was below 500 cP, making it very easy to inject the separation medium into the capillary. The viscosity increased with increasing temperature. At ambient temperature, the viscosity was about 25 000 cP, 50-times higher than that at 2°C.

By decreasing the column I.D. to 75 μm and the injection amount of DNA to 50 V/cm for 3 s, all the DNA fragments in the pBR322/HaeIII digest were clearly separated as shown in Fig. 6, except for the first two fragments (8 bp and 11 bp), which were hardly observed. The resolution (R_s) was calculated according to the following equation:

$$R_s = 2(t_1 - t_2)/(w_1 + w_2) \quad (3)$$

with w_1 and w_2 being the full width of DNA fragments 1 and 2, respectively, and t_1 and t_2 being the migration time of DNA fragments 1 and 2, respectively, and $t_1 > t_2$. The calculated data showed that the 123 bp and 124 bp fragments were successfully separated with a resolution of 1.3.

The sieving ability of the copolymer mixture of 3% (w/v) $B_{10}E_{270}B_{10}$ with 5% (w/v) $B_6E_{46}B_6$ was also demonstrated by the separation of pBR322/MspI in Fig. 7. In this sample, there are four pairs of fragments with the same lengths: 26, 34, 147 and 160 bp. It is noted that even though the separation

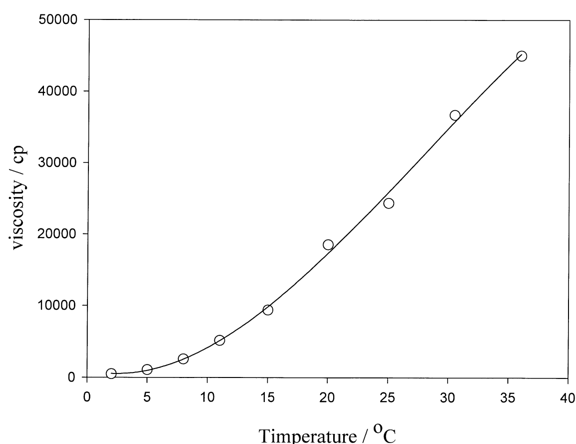

Fig. 5. Temperature dependence of the viscosity of 3% (w/v) $B_{10}E_{270}B_{10}$ with 5% (w/v) $B_6E_{46}B_6$ in 1×TBE buffer.

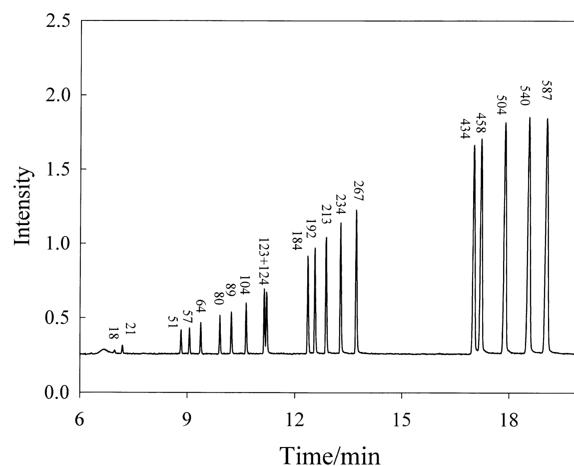

Fig. 6. Electropherogram of pBR322/HaeIII digest obtained by using 3% (w/v) $B_{10}E_{270}B_{10}$ with 5% (w/v) $B_6E_{46}B_6$ as a separation medium. Experimental conditions: 10 cm effective length×75 μm I.D. capillary, 1×TBE; 3 μg/ml EtBr, running electric field strength: 150 V/cm; electrokinetic injection at 50 V/cm for 3 s; room temperature.

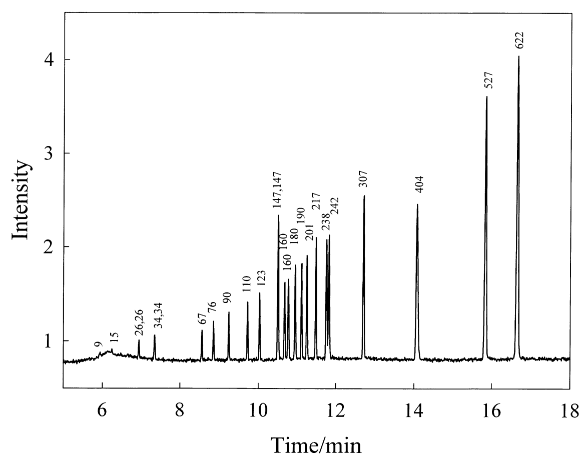

Fig. 7. Electropherogram of pBR322/MspI digest obtained by using 3% (w/v) $B_{10}E_{270}B_{10}$ with 5% (w/v) $B_6E_{46}B_6$ as a separation medium. Peak identification from left to right in bp are: 9, 15, 26 (26), 34 (34), 67, 76, 90, 110, 123, 147 (147), 160, 160, 180, 190, 201, 217, 238, 242, 307, 404, 27, 622. Other conditions as in Fig. 6.

was completed within only 17 min, the two 160 bp fragments were still successfully baseline separated.

4. Conclusion

The copolymer mixture of $B_{10}E_{270}B_{10}$ and $B_6E_{46}B_6$ over a range of mass ratios has been demonstrated as a potentially useful separation medium for double-stranded DNA analysis. It has a dynamic coating ability and its viscosity-adjustable property makes it very easy to handle. The sieving ability of the mixture can be controlled by varying the mass ratio of the two components. There is a minimum amount of $B_{10}E_{270}B_{10}$ required for successful separation. The addition of $B_6E_{46}B_6$ increases both the selectivity and the efficiency. At optimal conditions, a resolution of 1.3 on the separation of 123/124 bp was achieved by using the pBR322/HaeIII digest as a reference with a 10 cm effective length column of 75 μm I.D. at 150 V/cm running electric field strength. The two fragments of 160 bp in pBR322/MspI digest were baseline separated under the same conditions.

Acknowledgements

B.C. gratefully acknowledges the support of this research by the National Human Genome Research Institute (5R01HG0138606).

References

[1] O. Salas-Solano, E. Carrilho, L. Kotler, A.W. Miller, W. Geotzinger, Z. Sosic, B.L. Karger, Anal. Chem. 70 (1998) 3996.
[2] H. Zhou, A.W. Miller, Z. Sosic, B. Buchholz, A.E. Barron, L. Kotler, B.L. Karger, Anal. Chem. 72 (2000) 1045.
[3] M. Strege, A. Lagu, Anal. Chem. 63 (1991) 1233.
[4] P.D. Grossman, D.S. Soane, Biopolymers 31 (1991) 1221.
[5] A.E. Barron, D.S. Soane, H.W. Blanch, J. Chromatogr. A 652 (1993) 3.
[6] O. Muller, M. Minarik, F. Foret, Electrophoresis 19 (1998) 1436.
[7] S. Hjertén, J. Chromatogr. 347 (1985) 189.
[8] Q. Gao, E.S. Yeung, Anal. Chem. 70 (1998) 1382.
[9] N. Iki, E.S. Yeung, J. Chromatogr. A 731 (1996) 273.
[10] Y. Kim, E.S. Yeung, J. Chromatogr. A 781 (1997) 315.
[11] M.A. Quesada, H.S. Dhadwal, D. Fisk, F.W. Studier, Electrophoresis 19 (1998) 1415.
[12] D. Liang, S. Zhou, L. Song, V.S. Zaitsev, B. Chu, Macromolecules 32 (1999) 6236.
[13] C. Wu, T. Liu, B. Chu, Electrophoresis 19 (1998) 231.
[14] D. Liang, B. Chu, Electrophoresis 19 (1998) 2447.
[15] D. Liang, L. Song, S. Zhou, V.S. Zaitsev, B. Chu, Electrophoresis 19 (1999) 2856.
[16] T. Liu, D. Liang, L. Song, V.M. Nace, B. Chu, Electrophoresis, in press.
[17] C. Wu, M.A. Quesada, D.K. Schneider, R. Farinato, F.W. Studier, B. Chu, Electrophoresis 17 (1996) 1103.
[18] J.C. Gilbert, J. Hadgraft, A. Bye, L.G. Brookes, Int. J. Pharm. 32 (1986) 223.