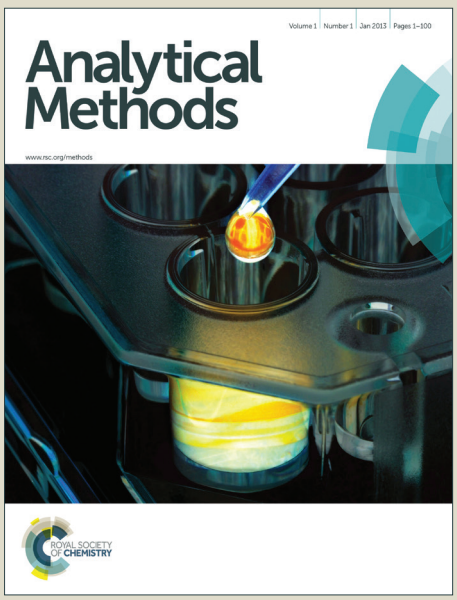
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Capillary electrophoresis of wide range DNA fragments in mixing solution of hydroxyethyl cellulose

Zhenqing Li [a, *], Chenchen Liu [a], Yoshinori Yamaguchi [b, a], Yi Ni [a], Qingxiang You [c], Xiaoming Dou [a, d]

[a] Engineering Research Center of Optical Instrument and System, Ministry of Education, Shanghai Key Lab of Modern Optical System, University of Shanghai for Science and Technology, No. 516 JunGong Road, Shanghai 200093, China

[b] Photonics Advanced Research Center, Graduate School of Engineering, Osaka University, Yamadaoka Suita-city, Osaka 565-0871, Japan

[c] Computer and Information Engineering, Changzhou Institute of Technology, No.299 Tong Jiang Road, Changzhou 213002, China

[d] Institute for Biomedical Engineering, Consolidated Research Institute for Advanced Science and Medical Care (ASMeW), Waseda University, 513 Wasedatsurumaki-cho, Shinjuku-ku, Tokyo 162-0041, Japan

Correspondence: zhenqingli@163.com

Phone: +86-21-55276023; **Fax:** +86-21-55276023

Abbreviations: HEC, hydroxyethyl cellulose; **kbp**, kilo base pairs; ***Mw***, Molecular weight; **CE**, capillary electrophoresis; **TBE**, Tris-borate-EDTA

Keywords: DNA separation/ HEC/ capillary electrophoresis

Abstract:

We carried out the capillary electrophoresis of 0.1-10.0 kilo base pairs DNA fragments in mixed hydroxyethyl cellulose (HEC) polymer. The mixed HEC polymer was prepared with different molecular weight (*Mw*) (90 k, 250 k, 720 k and 1300 k). The effects of important parameters, including the ratio of the mixture composition and the concentration of the mixing polymer, were investigated on the separation performance. Results show that it cannot only shorten the migration time of DNA without great deterioration in resolution, but also can decrease the viscosity of the polymer, and thus make it easy to fill into the capillary. Finally, we separated the φ×174-*Hirc* II and λ-*Eco*T14 I DNA digest with high resolution in the mixed HEC solution within 18 min.

Introduction

Because of its short separation time, high efficiency, low detection limits, and reduced usage of samples and consumables, capillary electrophoresis (CE) has become a universal technique for the separation and identification of DNA fragments[1-4]. Traditionally, cross-linked gels (e.g., polyacrylamide or agarose) were used as gel matrices in capillary electrophoresis because of their known utility in slab gels for the separation of proteins and DNA. However, due to the instability over time, irreproducibility in the polymerization processes, and the fragile nature of the medium, cross-linked gels are not suitable for large-scale DNA separation[5]. Thus, entangled and uncross-linked water soluble polymers are deemed to provide advantages over cross-linked gel, such as easy flushed into the capillaries, more capillary utilization times and greater speed. These polymers mainly include poly(ethyleneoxide) (PEO)[6], polyvinylpyrrolidone (PVP)[7], poly-N,N-dimethylacrylamide(PDMA)[8], and hydroxyethyl cellulose (HEC)[9, 10].

The migration mechanisms of DNA in CE were elaborated in Ref [[11-14]]. In this work, we mainly discuss the DNA separation by CE performed in entangled polymer solution over threshold centration c^*. In entangled polymer sieving matrix, the polymer chains overlap and form networks with dynamic pores. When DNA fragments migrate through, the polymer chains will proceed with a constraint release by changing their interacting partners. At the same time, the DNA molecules will undergo reptation[15], and then they are resolved by length. It is reported that DNA fragments of radii much larger than the mean pore size of the sieving matrix will disrupt the polymer-polymer entanglements and locally destroy the polymer network[16-18]. So, large DNA molecules are most efficiently separated in relatively dilute solutions of high molecular weight polymers, while small DNA fragments are better resolved in concentrated solutions of homogenous polymer with lower molecular weight[19-21]. In order to resolve the DNA fragments within a wide size range, researchers employed mixtures of polymers with different molecule weight (*Mw*), and even copolymers of different monomers, as the sieving matrix[22-26].

The hydrophilic HEC polymer can form highly entangled networks in aqueous solutions and its stiffness is suitable for sieving DNA fragments[27]. The c^* of HEC can be calculated by the empirical formula[28] in Eq. 1. And the mean pore size[29] ζ of the sieving matrix can be evaluated by Eq. 2.

$$c^* = 3.63[M_n/M_o]^{-1.2}+1.18\times10^{-4} \quad (1)$$

$$\zeta \approx 1.43R_g(c/c^{*(1+a)/3a}) \quad (2)$$

where M_n is the number average molecular weight, M_o is the average monomer molecular weight of HEC, R_g is the radius of gyration of the polymer and c is the concentration of the polymer. The exponent a varies between 0.5 and 0.8 for different polymers. It can be deduced from Eq. 1 that the larger the HEC polymer is, the lower c^* it will possess. And from Eq. 2, the mean pore size of an entangled polymer solution does not depend on the polymer length but mainly on its concentration and on the nature of the polymer[14]. Therefore, if we add some higher *Mw* HEC molecules into a lower *Mw* HEC solution, whose c is relative dilute (slightly above its c^*), the long polymer chains will strengthen the structure of the sieving network. This might help to produce a better degree of entanglement. Moreover, this kind of mixing solution possess a more ideal viscosity, which is between those of the two single HEC solutions[27]. Alexander P. Bünz and his coworkers reported the separation of DNA restriction fragments in dilute (non-entangled) HEC mixture solutions[30], A.R. Isenberg, B.R. McCord etc. employed a mixture of *Mn* (number-average molecular weight) 40,000 and *Mn* 140,000~160,000 HEC and separate DNA fragments of range 150~1,000 base pairs (bp) with baseline[31]. However, so far there is no detailed report on the DNA analysis in entangled mixed HEC solution.

In this paper, we have separated the DNA fragments ranging in size from 0.1 to 10.0 kilo base pairs (kbp) in mixing solutions of HEC by CE, and investigated the influence of concentration of HEC mixture and the ratio of mixture composition, on the separation performance of DNA. Such a study may provide new insight on the fast separation of DNA by CE.

Experimental

Chemicals

0.1 kbp, 1.0 kbp DNA ladder, φ×174-*Hirc* II digest and λ-*Eco*T14I digest were purchased from Takara (Shiga, Japan). SYBR Green I was got from Invitrogen (Carlsbad, CA, USA). HEC with *Mw* of 1300 k, 720 k, 250 k and 90 k HEC were from Sigma (St Louis, MO, USA). 10× Tris-borate-EDTA (TBE) buffer was from Bio-Rad (Hercules, CA, USA). HEC polymer solution containing 1× SYBR Green I was prepared by dissolving in the 0.5× TBE buffer. DNA samples were dissolved in 0.5× TBE buffer and mixed to make each DNA ladder concentration 16ug/ml. Prepared DNA samples were kept frozen at –20 °C before use.

Apparatus

The CE system used in this study has been described in detail elsewhere[32, 33]. Briefly, it consisted of a microscope with epi-illumination (IX71, Olympus, Tokyo, Japan) and a high-voltage power supply (MODEL 610E, TREK, Medina, NY, USA). The power supply was controlled by the locally programmed LabVIEW software (National Instrument). A mercury lamp produced the excitation light of a wavelength of 460-495 nm, which was the wavelength of the excitation maximum of the conjugate of SYBR Green I and the nucleic acid, by the optical filter (U-MWIB-3, Olympus, Tokyo, Japan). The fluorescence emission was collected by a 100× objective (PlanApo/IR, Olympus). The fluorescence signal was detected by use of a photo multiplier tube (R928, Hamamatsu Photonics, Hamamatsu, Japan), and the signal was digitized by National Instrument PCI-6024E (Austin, TX, USA). Fused-silica capillaries (PolymicroTehchnologies, Phoenix, AZ, USA) with ID/OD=75/365μm were covalently coated with polyacrylamide[34, 35]. DNA samples were electro kinetically injected at 100 V/cm for 2.0 sec. The entire detection system was enclosed in a black box.

Results and discussion

Separation of DNA fragments in HEC with different *Mw*

In order to review the role of polymer *Mw* in the DNA separation performance of CE, we first resolved DNA fragments (0.1-10.0 kbp) in mixed HEC solutions at 100

V/cm of electric field. The mixed solutions were prepared from equal amount of HEC with different *Mw* (1300 k, 720 k, 250 k, and 90 k) in 0.5× TBE buffer. Fig.1A-C shows a typical trend of DNA separation results change with the molecular weight of mixed HEC solutions. It shows that DNA fragments from 0.1 to 7.0 kbp were well resolved in three mixed HEC solutions, except that the separation time is different, implicating that two solutions of the same type of polymer and concentration but different *Mw* may have the same "pore size" if they are entangled. Fig.1D depicts the effect of mixed polymer on the migration times of DNA. It shows that in terms of speed, the migration time corresponding 0.1 kbp DNA fragment was nearly the same in each different mixing solutions, but the other DNA fragments (0.2-10.0 kbp) move faster when 1300 k HEC mixed with the lower *Mw* ones. Furthermore, the slope of the short DNA fragments (0.1-1.0 kbp) was decreased with the decrease of the *Mw* of HEC added in the background electrolyte, but the slope of the longer DNA fragments was very stable, emphasizing that the successful separation of short DNA fragment was mainly attributed to the low *Mw* HEC, while the separation of longer DNA fragments was dependent on the high *Mw* ones. Moreover, we found that DNA separation process could be finished within 11.0 min, while the resolution for the larger DNA fragments (> 1.5 kbp) was not deteriorated, thus we chose mixed HEC solution (250 k/1300 k) as our research object in the following sections.

Effect of ratio of HEC with different *Mw* on separation performance

Through Fig.1, we have found that the composition of mixed polymer will influence the migration time of DNA, and thus it is necessary to research the effect of the ratio of the mixed polymer composition on the separation performance. The separation performance was evaluated by the migration times of DNA and the resolution between adjacent peaks in the electropherogram. The resolution (R) [36] is calculated as the following equation:

$$R=\Delta t/1/2(W_1+W_2) \quad (3)$$

Where Δt is the difference between two adjacent peaks and W_1 and W_2 are the peak widths measured at the baseline.

The mixed HEC polymers were prepared with different ratios: 0/10, 2/8, 4/6, 6/4, 8/2, and 10/0. Then we have separated the DNA samples in various mixed HEC polymers by CE at 100 V/cm. Fig.2 demonstrates an example of the separation of DNA in 0.4% HEC (250 k/1300 k) solution with ratios of 10/0, 6/4 and 2/8. Corresponding to Fig.2, Fig.3 displays its separation performance in the mixed HEC solution with ratios from 0/10 to 10/0. It shows that even though the ratio of the composition of the mixing solution changes, the trend of DNA fragments move with remains the same (Fig.3A). Moreover, because the viscosity is positively related to the *Mw* of the polymer[14], the migration time of DNA fragment increases in solutions with excessive higher *Mw* of HEC (1300 k). Another interesting phenomenon is that, the resolution (Fig.3B) between the short adjacent DNA fragments (0.1-1.0 kbp) degrades with the increase volume of 250 k HEC in the mixing solution, while the resolution of the larger DNA fragments (1.0-10.0 kbp) seems very stable. Furthermore, it is worth noting that the resolution of longer DNA fragments (>1.0 kbp) was nearly the worst when they were resolved in single-*Mw* HEC solutions of 250 k. This was mostly because the pore size of the matrices is too small to have a sieving power for those longer DNA fragments.

Effect of mixed polymer concentration on separation performance

In Fig.3, we find that if there is too much lower *Mw* of HEC in the mixed polymer, the sieving solution offers poor resolution for the DNA fragments, and when the ratio of higher *Mw* of HEC is high, the migration of DNA will be prolonged because of the high viscosity of the polymer matrices. Furthermore, it is obvious that it will be harder to fill the capillary with polymer solution with a higher viscosity. Therefore, we choose the mixed HEC polymer comprised of different *Mw* with volume ratio 1:1 as the separation buffer. Fig.4 depicts an example of the DNA separation performance in mixed polymer (250 k and 1300 k) with concentration ranging from 0.4% to 1.2% at 100 V/cm of electric field. The plot is also derived from the electropherogram similar to Fig.1. We find that when the concentration of the mixing solution is lower than 0.4%, DNA fragments larger than 1.5 kbp almost migrate together (data not shown). When the concentration of the mixing HEC solution is above 1.2%, the situation

contributions more to the increasing of the DNA fragments migration time rather than the resolution, especially for the larger ones (> 5.0 kbp). Fig.4A shows that the migration time of DNA increases with the growth of mixed polymer concentration because of the increase of the viscosity of the polymer. Data in Fig.4B demonstrate that with the increase of mixed polymer concentration, the resolution of the short DNA fragments (< 1.0 kbp) improves. When the concentration is higher than 0.8%, it seems that there is no great improvement for the resolution of longer DNA fragments (> 0.3 kbp). Furthermore, 1.2% mixed HEC solution offers the most ideal resolution for a wide range, but it is at the cost of longer separation time of DNA because of its high viscosity.

Separation of φ×174-*Hirc* II digest and λ-*Eco*T14 I DNA digest in mixed HEC polymer

Based on the results obtained above, we separated φ×174-*Hirc* II and λ-*Eco*T14 I DNA digest in mixing solution of 0.8% HEC (250 k and 1300 k) by CE. The electric field strength is 100 V/cm, and the ratio of two mixing HEC solutions with different *Mw* is 1:1. The DNA digest mainly contains 24 DNA fragments, and the sizes of the gene fragments were 74, 79, 162, 210, 291, 297, 335, 341, 345, 392, 421, 495, 612, 770, 925, 1057, 1489, 1882, 2690, 3472, 4254, 6223, 7743, and 19329 bp. As shown in Fig.5, DNA samples were successfully resolved in a wide range within 18 min.

Concluding remarks

This paper systematically studied the separation of DNA fragments (0.1-10.0 kbp) in mixed polymer with different *Mw* of HEC by CE. We have mainly investigated the factors (i.e. the ratio of the mixed polymer composition and the concentration of the mixing solution) on the separation performance. Results show that the mixed HEC entangled solution can provide a comparative DNA separation performance at a lower viscosity. And mixed HEC polymer (250 k and 1300 k) at 0.8% with ratio 1:1 offers a high resolution for DNA ranging from 74 to 19329 bp within 18 min.

Acknowledgements

We gratefully acknowledge financial support from China Scholarship Council and Consolidated Research Institute for Advanced Science and Medical Care (ASMeW) of Waseda University (Japan). The work was also supported by the National Natural Science Foundation of China (No. 21205078), Research Fund for the Doctoral Program of Higher Education of China (No.20123120110002), and Cultivating of Teacher's Innovation Ability Program in University of Shanghai for Science and Technology (No. GDCX-Y-1205).

The authors have declared no conflict of interest.

References

1. J. v. d. Greef, P. Stroobant and R. v. d. Heijden, *Current opinion in chemical biology*, 2004, **8**, 559-565.
2. S. R. Bean and G. L. Lookhart, *Electrophoresis*, 1998, **19**, 3190-3198.
3. K. Inatomi, S. Izuo, S. Lee, H. Ohji and S. Shiono, *Microelectronic engineering*, 2003, **70**, 13-18.
4. L. Song and M. Maestre, *J. Biomol. Struct. Dyn.*, 1991, **9**, 525-536.
5. H.-T. Chang and E. S. Yeung, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1995, **669**, 113-123.
6. H. Chang and E. Yeung, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1995, **669**, 113-123.
7. Q. Gao and E. Yeung, *Analytical Chemistry*, 1998, **70**, 1382-1388.
8. C. Heller, *Electrophoresis*, 1998, **19**, 3114-3127.
9. Z. Li, X. Dou, Y. Ni and Y. Yamaguchi, *Anal. Bioanal. Chem.*, 2011, **401**, 1665-1671.
10. Z. Li, X. Dou, Y. Ni, K. Sumitomo and Y. Yamaguchi, *J. Sep. Sci.*, 2010, **33**, 2811-2817.
11. H. J. Jung and Y. C. Bae, *Journal of chromatography. A*, 2002, **967**, 279-287.
12. G. W. Slater, M. Kenward, L. C. McCormick and M. G. Gauthier, *Current opinion in biotechnology*, 2003, **14**, 58-64.
13. N. C. Stellwagen and E. Stellwagen, *Journal of chromatography. A*, 2009, **1216**, 1917-1929.
14. C. Heller, *Electrophoresis*, 2001, **22**, 629-643.
15. J. L. Viovy and T. Duke, *Electrophoresis*, 1993, **14**, 322-329.
16. M. N. Albarghouthi, B. A. Buchholz, E. A. Doherty, F. M. Bogdan, H. Zhou and A. E. Barron, *Electrophoresis*, 2001, **22**, 737-747.
17. H. Cottet and P. Gareil, *Electrophoresis*, 2002, **23**, 2788-2793.
18. W. M. Sunada and H. W. Blanch, *Electrophoresis*, 1997, **18**, 2243-2254.
19. B. Chu and D. Liang, *Journal of Chromatography A*, 2002, **966**, 1-13.
20. Y. Baba, N. Ishimaru, K. Samata and M. Tsuhako, *Journal of chromatography. A*, 1993, **653**, 329-335.
21. C. W. Kan and A. E. Barron, *Electrophoresis*, 2003, **24**, 55-62.
22. V. Barbier, B. A. Buchholz, A. E. Barron and J.-L. Viovy, *Electrophoresis*, 2002, **23**, 1441-1449.
23. M. Huang, C. Huang and H. Chang, *Electrophoresis*, 2003, **24**, 2896-2902.
24. H. S. Hwang, G. W. Shin, H. J. Park, C. Y. Ryu and G. Y. Jung, *Electrophoresis*, 2013.
25. L. Song, D. Liang, Z. Chen, D. Fang and B. Chu, *Journal of chromatography. A*, 2001, **915**, 231-239.
26. C. P. Fredlake, D. G. Hert, T. P. Niedringhaus, J. S. Lin and A. E. Barron, *Electrophoresis*, 2012, **33**, 1411-1420.
27. B. A. Siles, D. E. Anderson, N. S. Buchanan and M. F. Warder, *Electrophoresis*, 1997, **18**, 1980-1989.
28. A. E. Barron, H. W. Blanch and D. S. Soane, *Electrophoresis*, 1994, **15**, 597-615.
29. D. Broseta, L. Leibler, A. Lapp and C. Strazielle, *EPL (Europhysics Letters)*, 1986, **2**, 733.
30. A. P. Bünz, A. E. Barron, J. M. Prausnitz and H. W. Blanch, *Industrial & engineering chemistry research*, 1996, **35**, 2900-2908.

31. A. R. Isenberg, B. R. McCord, B. W. Koons, B. Budowle and R. O. Allen, *Electrophoresis*, 1996, **17**, 1505-1511.
32. Z. Li, X. Dou, Y. Ni, Q. Chen, S. Cheng and Y. Yamaguchi, *Journal of Chromatography A*, 2012, **122**, 274-279.
33. Z. Li, X. Dou, Y. Ni, K. Sumitomo and Y. Yamaguchi, *Electrophoresis*, 2010, **31**, 3531-3536.
34. S. Hjertén, *J. Chromatogr. A.*, 1985, **347**, 191-198.
35. D. Schmalzing, C. A. Piggee, F. Foret, E. Carrilho and B. L. Karger, *J. Chromatogr. A.*, 1993, **652**, 149-159.
36. K. R. Mitchelson and J. Cheng, Capillary Electrophoresis of Nucleic Acids: Introduction to the capillary electrophoresis of nucleic acids, Springer, 2004

Figure Captions

Fig.1 Electrophoretic separation of DNA in 0.4% mixed solutions of (A) HEC (1300 k + 90 k); (B) HEC (1300 k + 720 k); (C) 1300 K HEC. (D) Migration times of DNA versus the DNA size corresponding to Fig.1A-C. Electrophoretic conditions: the ratio of two HEC polymers is 1:1; sample loadings 100 V/cm (2.0 sec), electric field strength 100 V/cm. the sample was diluted in 0.5× TBE buffer. The total length (l_t) and the effective length (l_e) of the capillary are 14 cm and 8.0 cm, respectively.

Fig.2 Electrophoretic separation of DNA in 0.4% mixed solutions of HEC (250 k/1300 k) with different ratio: (A) 10/0 (B) 6/4 ; (C) 2/8. The other electrophoretic conditions are the same as in Fig.1.

Fig.3 The effect of the ratio of HEC mixture on the separation performance of DNA by CE. The other electrophoretic conditions are the same as in Fig.1.

Fig.4 The effect of concentration of HEC mixture on the separation performance of DNA by CE. The other electrophoretic conditions are the same as in Fig.1.

Fig.5 Separation of φ×174-*Hirc* II and λ-*Eco*T14 I DNA digest in mixing solution of 0.8% HEC (250 k and 1300 k) by CE. The other electrophoretic conditions are the same as in Fig.1.

Fig.1

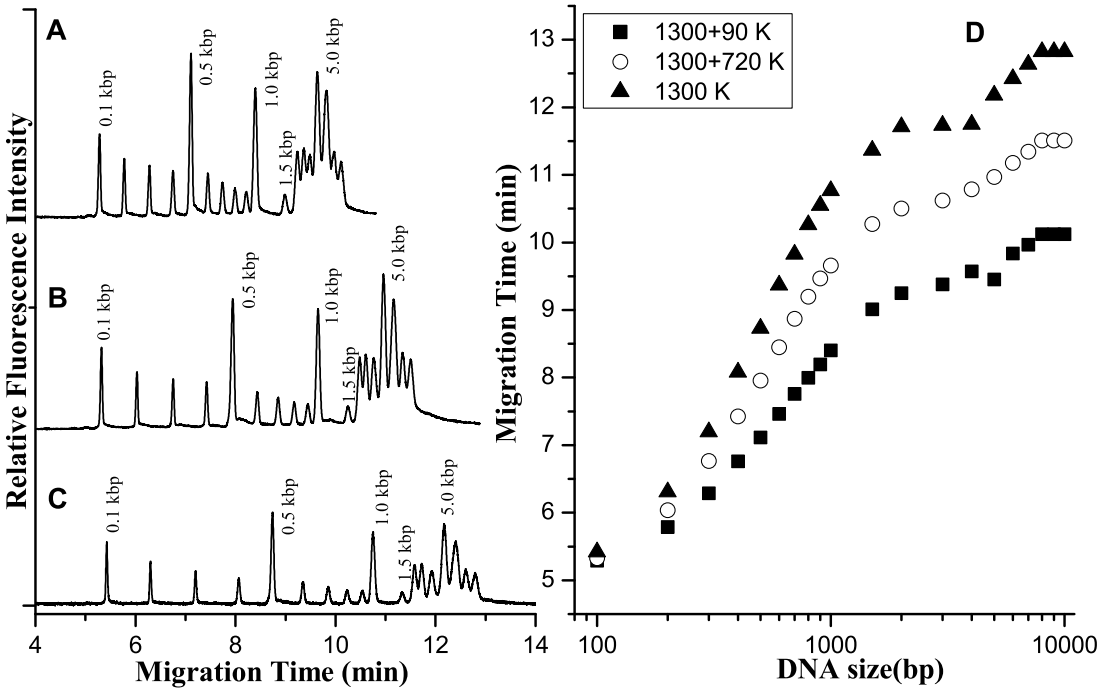


Fig.1 Electrophoretic separation of DNA in 0.4% mixed solutions of (A) HEC (1300 k + 90 k); (B) HEC (1300 k + 720 k); (C) 1300 K HEC. (D) Migration times of DNA versus the DNA size corresponding to Fig.1A-C. Electrophoretic conditions: the ratio of two HEC polymers is 1:1; sample loadings 100 V/cm (2.0 sec), electric field strength 100 V/cm. the sample was diluted in 0.5× TBE buffer. The total length (l_t) and the effective length (l_e) of the capillary are 14 cm and 8.0 cm, respectively.

Fig.2

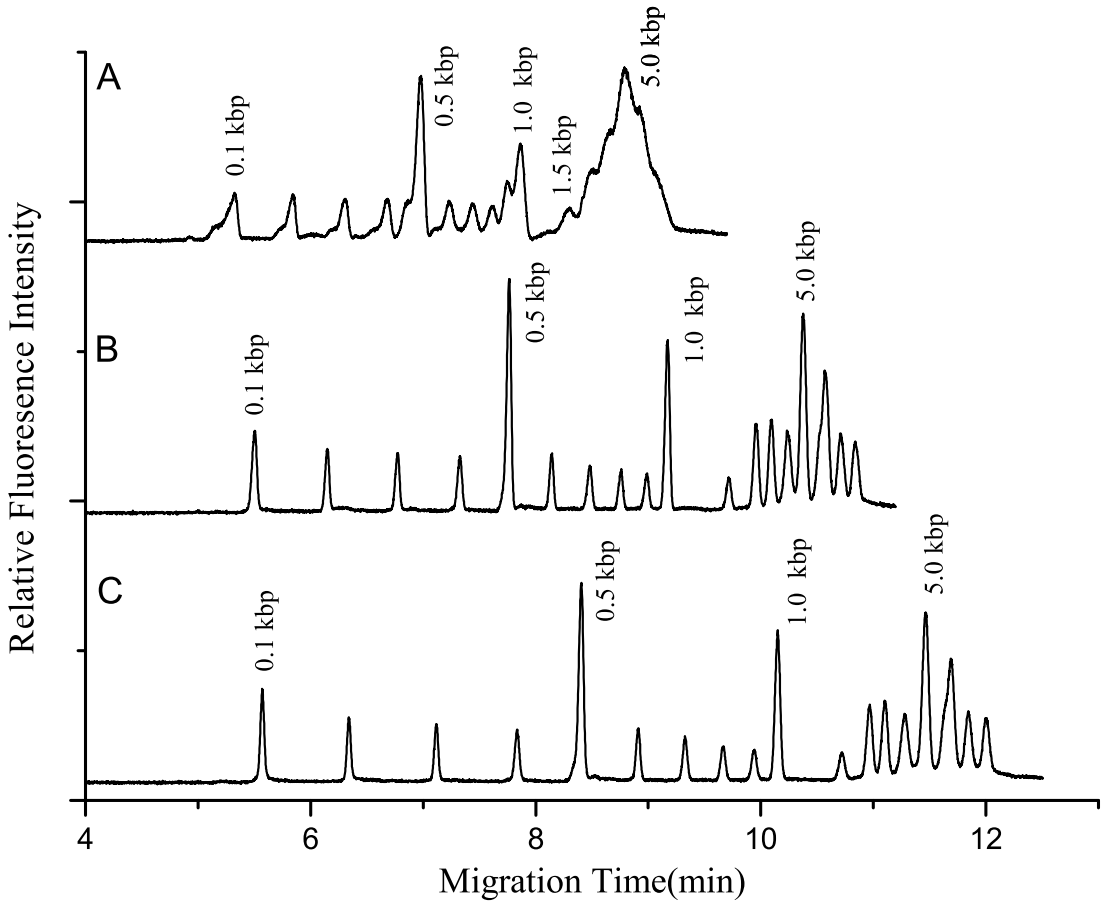


Fig.2 Electrophoretic separation of DNA in 0.4% mixed solutions of HEC (250 k/1300 k) with different ratio: (A) 10/0 (B) 6/4 ; (C) 2/8. The other electrophoretic conditions are the same as in Fig.1.

Fig.3

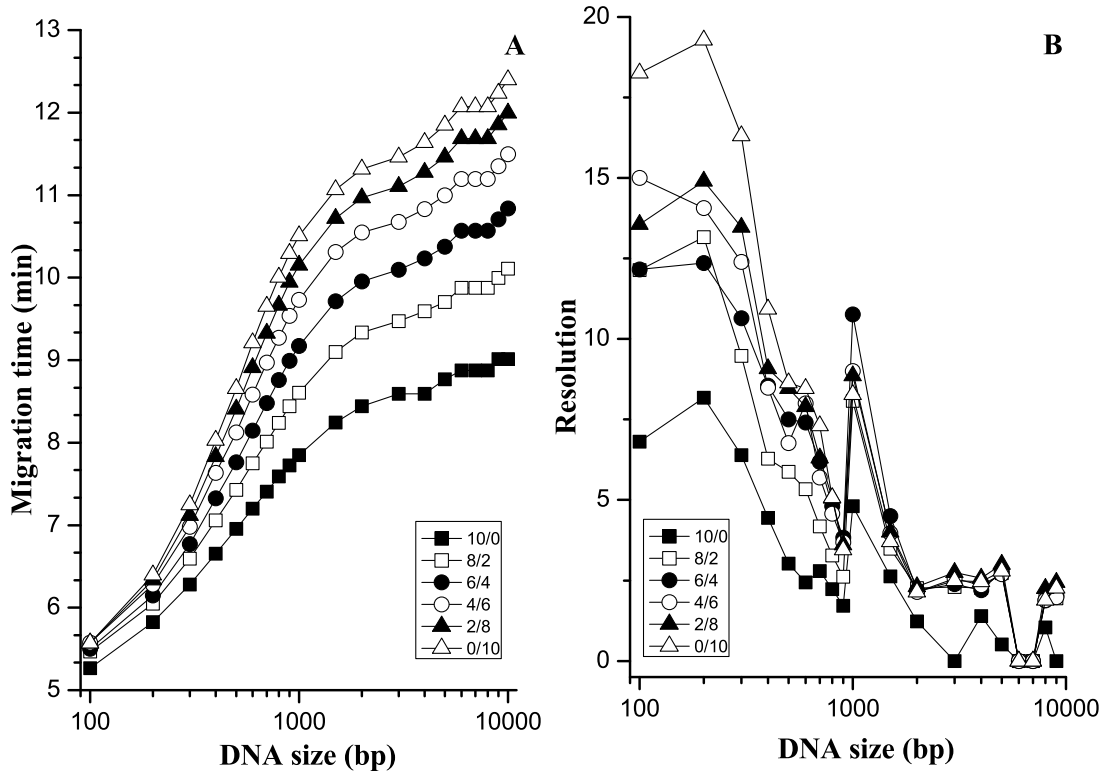


Fig.3 The effect of the ratio of HEC mixture on the separation performance of DNA by CE. The other electrophoretic conditions are the same as in Fig.1.

Fig.4

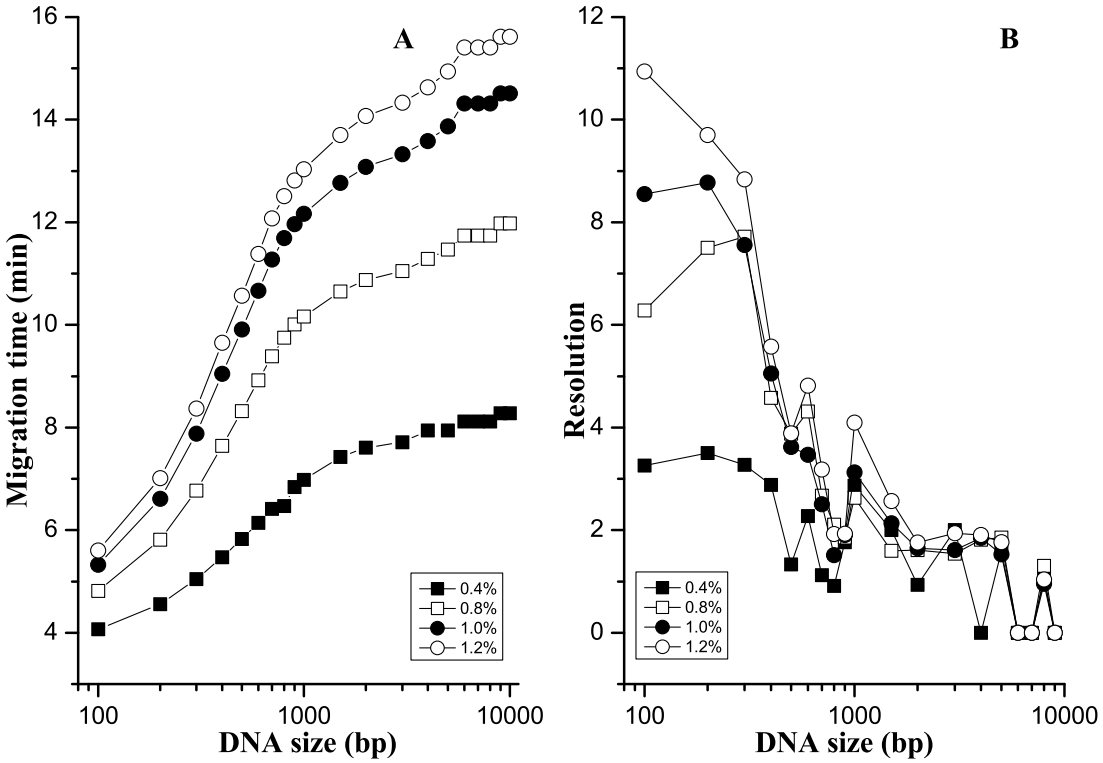


Fig.4 The effect of concentration of HEC mixture on the separation performance of DNA by CE. The other electrophoretic conditions are the same as in Fig.1.

Fig.5

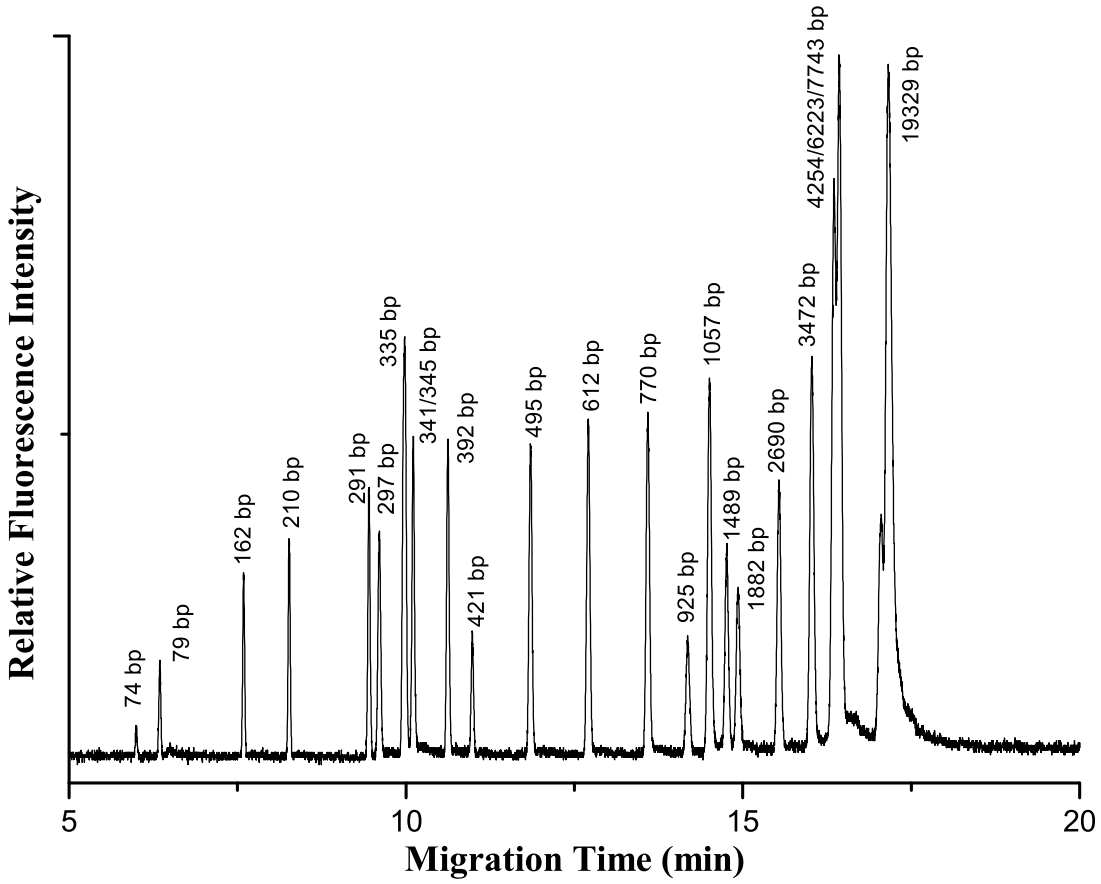


Fig.5 Separation of φ×174-*Hirc* II and λ-*Eco*T14 I DNA digest in mixing solution of 0.8% HEC (250 k and 1300 k) by CE. The other electrophoretic conditions are the same as in Fig.1.