Prediction of DNA Separation by Capillary Electrophoresis with Polymer Additives

Chun Ye Liu[1,*], Jian Zhang[2], Xu Xu[3], and **Jierong Chen[4]**

[1]School of Pharmacy, Xi'an Medical University, Xi'an, China 710021, [2]Xi'an Research Institute of Hi-Tech. Hongqing Town, Xi'an, China, 710025, [3]Laboratory of Analytical Chemistry, State Key Laboratory of Organometallic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China 200032, and [4]School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, China 710049

Abstract

The paper is focused on the powerful prediction ability of the quantitative DNA sieving model in DNA separations by capillary electrophoresis, which was proposed by us previously. First, the DNA resolution can be predicted by the theory. The model predicts that the most difficult and easiest separation will be 184bp/192bp and 234bp/267bp respectively, which is consist with experimental results. Furthermore, the average relative differences of predicted and experimental resolution values (R_S) for ssDNA 184b/192b or dsDNA 184bp/192bp were all smaller than 2.8% if the diffuse parameter D considered was 8×10^{-5} cm^2/s. Secondly, the optimum polymer concentrations for DNA separation were also calculated by the model, and the results show that polymer concentration should be as high as possible in DNA separation. Thirdly, the sieving ability of polymer will be predicted by the model. Polymer with smaller k, a polymer parameter calculated by the model, is prior to use as DNA sieving media.

Introduction

Capillary electrophoresis (CE) has been proven to be superior to classical slab-gel electrophoresis for the separation of DNA due to the following characteristics: higher efficiency, faster speed, better sensitivity, and easier for automation (1–6). The separation of DNA in CE is often achieved in the mold of capillary gel electrophoresis (CGE) where a polymeric material, such as polyacrylamide gel, is formed to provide a sieving matrix for DNA separation. Alternatively, different polymeric materials can be added into the buffer to form pseudo gel sieving matrixes. The commonly used polymers for this purpose include linear polyacrylamide (7–9), polyethylene glycol with fluorocarbon tails (10), polyethylene oxide (11–12), polyvinylalcohol (13), and polyvinylpyrrolidone (14–15), etc. Each of those polymeric gels has demonstrated certain degree of success in the separation of DNA, and a general concept of sieving mechanism is widely accepted. However, the true mechanism for the interaction between DNA and those polymers has not been completely understood yet. Therefore, the selection of any specific polymer as well other experimental conditions such as the kind and concentration of the polymer is solely based on experience of the bench chemists and no theoretical basis is available for them to predict the outcome of a DNA separation when a specific polymer is chosen. It is desirable to better understand the mechanism of interaction between DNA and the polymers, which will provide certain theoretic basis for the selection of proper polymers or experimental conditions in meeting the specific separation requirements.

It is expected that the concentration of polymers for DNA separations varies broadly as each of them has very different solubility in the buffer (16–18). Therefore, different theoretical models may be needed to address the unique characteristics at different concentrations of polymer additives. Barron (19–20), Hubert (21), and Sunada (22) have proposed a "transient entanglement coupling" model to explain mechanism of DNA separation in unentangled polymer solutions. This is a simple model based on the collision of the polymer and the DNA molecules during the separation process. Y. Jin et al. have proposed another DNA-polymer collision model which gives out the mathematical relationship between DNA mobility and some experimental conditions (23). The models discussed previously have described the DNA separation mechanism in dilute polymer. However, differing from models for dilute polymer solutions, the current theoretical models describing the interaction mechanism of DNA separation in entanglement polymer solutions only described the process qualitatively. For example, both Ogston (24) and Slater and Noolandi (25) proposed the repetition model, which assumes that DNA separation in entangled solutions was similar to that in gels, and where the "pores" or "tube" formed in the gel was the basis for achieving the separation.

Previously, we proposed a different and quantitative model for CE separation of DNA in entangled polymer solutions (26). In this model, DNA molecules move through the transient pores formed in the polymeric solutions and collide with blobs of polymer molecules encountered during their migration. Based on that model, a quantitative mathematical equation was derived and was proven to be related to the average retardation time (t_c) and the total collision number (Nc) of DNA-blob collision. Therefore, the mobility of DNA fragments can be calculated as a

*Author to whom correspondence should be addressed: email zzjj1230@163.com.

function of the experimental conditions, like the size of DNA molecules, the concentration of the polymers as well as the electric field strength. For example, it had been shown that the average retardation time (t_c) in a linear polyacrylamide and hydroxyethyl cellulose containing solution was about (2~3) × 10^{-6} s. On the basis of the theoretical fundamentals established there, it was intended to further demonstrate the feasibility of using that model and make predictions for new DNA separations. By predicting the resolution and other related conditions, it can be seen that the resources can be used more effectively. Further, it is even possible to understand that sometime the optimum polymer concentrations may even obtainable in practice. Therefore, a wise decision can be made without wasting much time. It is also possible to use this model for selecting excellent sieving materials.

Brief description of the model

It is assumed that the collision of DNA with the "blobs" of polymers will reduce its mobility in an electrophoretic system. For example, when the concentration of polymer is above its entanglement threshold, transient "pores" will be formed in the solution and DNA molecules must crawl through these "pores" in order to pass through. The size of those pores is represented by ξ_c. When multiple polymer chains are crossed together, they would form large clusters with each of the individual polymer chain can be viewed as a succession of independent subunits, called "blobs", which has an average size, as represented by , much larger than the aforementioned pores (27). The linear circumvolving DNA fragments crawl through these "pores" and collide with the "blobs" during the electrophoresis process (28). It was further assumed that only a fraction of the DNA and a part of polymer (i.e., blobs) would collide into each other. Thus, the effect of each collision on DNA mobility should be similar because of similar characteristics for each part of the molecule. Therefore, the time of each collision can be statistically viewed the same as the average retardation time, t_c, of DNA-"blob" collision. During the electrophoretic separation process, DNA collides with "blobs" many times and the total migration time of DNA is thus prolonged. Of course, the longer it takes for the DNA fragment to collide with the polymer and the larger the number of collisions the longer the overall DNA migration time. Therefore, DNA fragments with different sizes can be separated based on the total number of DNA-"blob" collisions (N_c). The total number of collisions is able influenced by the concentration of polymers and the size of the DNA fragments. Thus, whole delayed time of DNA migration caused by collisions is directly linked to the product of t_c and N_c.

The DNA mobility in polymer solutions is given by Eq. 1. The detailed derivation of this equation can be found in reference (26).

$$\frac{1}{\mu} - \frac{1}{\mu_0} = \frac{EN_A\pi L_P t_c C_P^{\frac{1+4a}{3a}}}{M_p k} \times \left[f_E (5.66 \times 10^{-14} M_{DNA})^{1/2} + kC_P^{-\frac{1+a}{3a}} \right]^2 \quad \text{Eq. 1}$$

where M_{DNA} is the molecular size of the DNA (in base pair, or bp), E is the electric field strength in V/cm, N_A is the Avogadro's number, M_p and L_p is the molecular weight and molecular length of polymer (cm), respectively, t_c is the average retardation time of collision (s), C_P is the polymer concentration (g/mL). $k = 1.43(K / 6.2N_A)^{1/3}(K/1.5)^{-(1+a)/3a}$, a, and K are characteristic constants for a given polymer-solvent system in Mark-Houwink equation (29), K is a ratio constant (mL/g), a is an experiential parameter about the molecular shape, and f_E is an inverse function of E:

$$f_E = -0.37 \ln E + 2.59$$

Materials and Methods

Instrumentation

All experiments were performed with the BioFocus 3000 capillary electrophoresis system (Bio-Rad, Hercules, CA) in the reversed polarity mode. The temperature was set at 25°C, and the UV absorbance was monitored at 260 nm. Instruments control and data collection were performed by the Bio-Rad station software. The fused silica capillary (36 cm in length, 31.2 cm from inlet to detection window, 50 µm i.d.) was purchased from Reafine Chromatograph Device Ltd. (Hebei, China). The capillary was coated according to Hjertén's method (30). Brief description of the method is as below. First, the capillary was activated by NaOH and HCl solution. Second, the capillary was treated by methacrylic acid 3-trimethoxysilylpropyl ester and heated for 1 h in 60°C. Thirdly, acrylamide solution was pumped into the capillary and reacted for 2 h. Before DNA sample injection the new coated capillary was first rinsed with deionized water for 5 min and then with phosphate buffer (pH 7.0) or TBE buffer (85 mmol/L Tris, 85 mmol/L boric acid, 2 mmol/L EDTA, which naturally reaches to a pH of 8.3) for 5 min. Next, the coated capillary was rinsed with linear polyacrylamide (LPA) or hydroxyethylcellulose (HEC) solution for 5 min. To prevent the sample from being contaminated, the inlet end of the capillary and the electrode were cleaned in deionized water before sample injection. The DNA sample was introduced into the capillary by eletromigration at 5 kV for 5 s.

Materials and reagents

pBR322/Hae III DNA marker was purchased from Sino-American Biotechnology Company (Shanghai, China), which consisted of 20 fragments in the range of 8~587bp. It was diluted to 0.05 µg/µL with deionized water before injection. HEC with different molecular weight (viscosity in 2% aqueous solution at 25°C are 200-300 cP, 800-1,500 cP, and 4,500-6,500 cP, respectively) were all obtained from TCI (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan). Acrylamide (AA), N,N,N',N'-tetramethylethylenediamine (TEMED) and amine persulfate (APS) were all electrophoretic grade and from Sino-American Biotechnology Co., (Shanghai, China). Tris(hydroxymethyl)aminomethane (Tris) was from Bio Life Science and Technology Co., Ltd. (Shanghai, China). Disodium, ethylenediaminetetraacetic acid (EDTA) and boric acid were purchased from Shanghai Lianshi Chemical Reagent Plant.

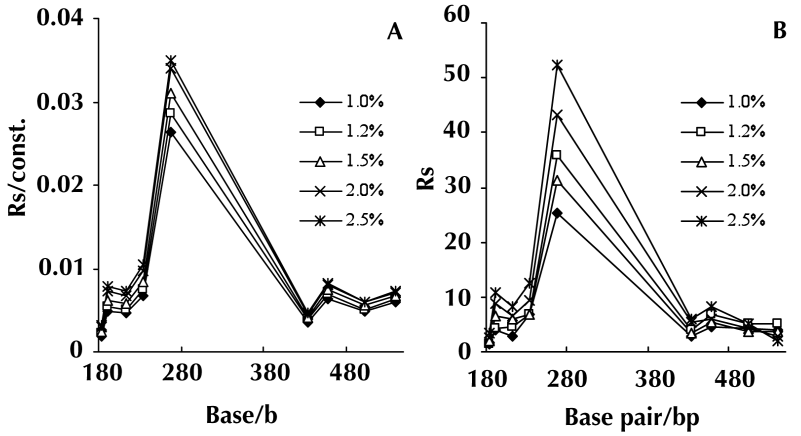

Figure 1. Changing regulation of R_s for different ssDNA. (A) Prediction results by the model and (B) results from experimental data. Data were from ssDNA separation in 800–1,500 cP HEC sieving medium. Conditions: applied voltage, 4 kV; UV detection at 260 nm; electrokinetic injection at 5 kV for 5 s. Constant voltage running mode with reversed polarity. Const. in R/const. of Y-axis in Figure 1A is $1/(4\sqrt{2})(l^2/D)^{1/2}$.

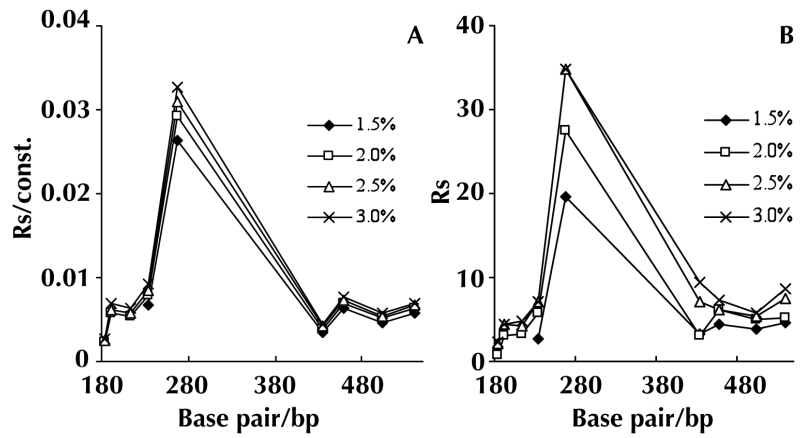

Figure 2. Changing regulation of R_s for different dsDNA. (A) prediction results by the models and (B) results from experimental data. Data were from dsDNA separation in LPA sieving medium. Separation conditions are same with Figure 1. Const. in R/const. of Y-axis in Figure 2A is. $1/(4\sqrt{2})(l^2/D)^{1/2}$.

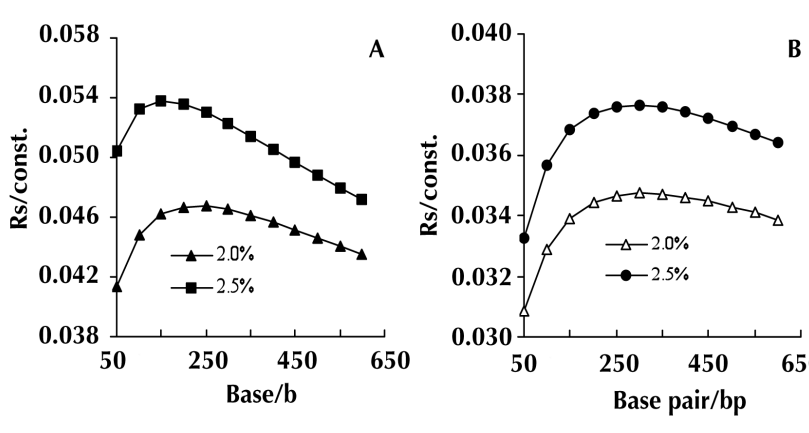

Figure 3. Changing trend of R_s for DNA that differed in one bp. (A) ssDNA separated in HEC media (800~1500 cP) and the predicted data by the model and (B) dsDNA separated in LPA sieving media and the predicted data by the model. Separation conditions are same with Figure 1. Const. in R/const. of Y-axis is $1/(4\sqrt{2})(l^2/D)^{1/2}$.

Method of ssDNA separation

The running or sample pretreatment method of ssDNA is different from that of dsDNA. For ssDNA running, the DNA sample was mixed with formamide (50:50, v/v), and shaken intensely for denaturation just before experiments. ssDNA is more like a random coil in separation than dsDNA, and more suitable to the R_{DNA} expression (the gyration radius of DNA) in our model. Therefore, the model is more suitable for ssDNA electrophoresis than dsDNA.

Prediction ability of the model for DNA in electrophoresis

Prediction for DNA resolutions

In reference 26, the average value t_c was used to calculate the migration time of DNA and compared it with experimental data. The results indicate that the calculated migration time was similar to that of the experimental data. Further more, those predicted mobility time can be used to estimate any DNA resolution, R_s. The diffuse parameter D is not a constant. But experimental results showed that, D is not fluctuant very much for DNA fragments in a little length range. Therefore, it was assumed that D is a constant for DNA in experiments. Of course, the assumption was not very reasonable, but the changing trend of R_s will not be affected seriously. R_s is estimated by Eq. 2 (31).

$$\mathrm{Rs} = \frac{1}{4\sqrt{2}}\left(\frac{l^2}{D}\right)^{1/2}\left(\frac{1}{t_{m2}}\right)^{1/2}\frac{\Delta t_m}{t_{m2}} \qquad \text{Eq. 2}$$

Where Δt_m is the difference of migration time between two adjacent peaks, s. t_{m2} is the migration time of slower DNA. Of course, the standard chromatographic method should be used to calculate the resolution of DNA. However, the area of chromatographic peak will not be clear before experiments doing. Instead, the prediction of DNA resolution based on the migration time of DNA can be calculated by Eq. 2. So, Figures 1A and 2A just showed the changing tendency of R_s for ssDNA and dsDNA respectively. Figures 1A and 2A showed that the most difficult and easiest separation will be 184bp/192bp and 234bp/267bp respectively, which is consist with experimental results that shown in Figures 1B and 2B. In Figures 1B and 2B, resolution were calculated by standard chromatographic method, [i.e., formula $R = (t_2 - t_1) / 1/2(w_1 + w_2)$] where t_1 and t_2 are migration time of DNA, w_1 and w_2 are peak area of DNA.

From Figures 1 and 2, it can be seen that the spread in R_s values is always larger than for the theory plots. Here, the R_s values in theory plots are not calculated out completely. There is a lack of value of D which is variational in experiments actually. In Figures 1 and 2, the biggest spreads are in R_s values of 267bp/434bp. Fortunately, R_s values of 267bp/434bp in Figure 1 and 2 did not have actual meanings, because they are not of two adjacent peaks. R_s values of two adjacent peaks in electrophoresis experiments are usually calculated out. Before doing experiments, it was decided to focus on which separations of two DNA fragments are the most difficult. Using the model, it was possible to find them out. Furthermore, if experimental statistical value of D of 8×10^{-5} cm^2/s was used to calculate R_s, the average relative differences of predicted and experimental R_s of ssDNA 184b/192b or dsDNA 184bp/192bp were all small than 2.8%, which is acceptable in analytical chemistry.

Using the theoretical migration time of DNA computed by the model, it was also possible estimate the changing trend of R_s for DNA differed in one bp (Figure 3) by Eq. 2. For example, by introduced migration time of 51 bp and 50 bp (computed by the model) to Eq. 2, their resolution can be computed out. The previously described estimations were conducted for DNA in the range of 50–600 bp in this section. In Figure 3, bigger R_s value obtained for DNA in the range of 150–250 bp, which indicate that DNA in the range of 150–250 bp will be separated easier. Separation of DNA longer than 250 bp or shorter than 150 bp was difficult which reflected by their small R_s displayed in Figure 3. The separation regulation about DNA described above is consist with our experience in DNA electrophoresis.

Prediction for the optimum polymer concentration

The experimental results showed that good resolution of DNA could be obtained when it was separated in HEC solutions with special concentration. The optimum concentration of HEC with lower molecular weight was bigger than that with larger molecular weight in DNA separation. For example, the optimum concentration of HEC with 200–300 cP is 2.0% in DNA sieving. While, for HEC with 4,500–6,500 cP, it is just 1.2%. In other words, the optimum concentrations were different for different polymer solutions in DNA separation. In practice, the optimum concentration was obtained by a large number of experiments. Here, the time and labor consuming method used in optimum concentration looking for can be partly replaced by our model. From Eq. 1, we can compute the optimum concentrations of LPA and HEC ($C_{optimum,LPA}$ and $C_{optimum,HEC}$) in separating DNA molecules of size M_{DNA}. The separation between DNA molecules of sizes M_{DNA} and M_{DNA+1} (in bp) is proportional to their relative mobility $\partial(\mu)/\partial M_{DNA}$. Thus, the optimal concentration to separate these molecules can be found by solving the equation $\partial^2(\mu)/\partial C_p \partial M_{DNA} = 0$. For LPA solutions, Eq. 1 was treated with second-order derivatives and changed to

$$2.93 \times 10^{-27} a^2 M_{DNA} C_P^{2.5} + 2.48 \times 10^{-20} k a^2 M_{DNA}^{1/2} C_P^{1.75} + 3.07 \times 10^{-14} a^2 k^2 C_P - 1.01 \times 10^{-7} a^2 k^3 M_{DNA}^{-1/2} C_P^{0.25} - 7.16 \times 10^{-14} a \mu_0^{-1} C_P^{-0.75} - 2.02 \times 10^{-7} \mu_0^{-1} a k M_{DNA}^{-1/2} = 0 \quad \text{Eq. 3}$$

where $t_c = 3.07 \times 10^{-8}$ min (computed in paper [26]), Lp ≈ 10 cm, $M_p \approx 5 \times 10^6$, $a = EN_A \pi t_c L_P / M_P k = 1.25 \times 10^{15}$, and for HEC polymer system, we transferred Eq. 1 to

$$2.88 \times 10^{-27} (a^*) 2 M_{DNA} C_P^{2.44} + 2.48 \times 10^{-20} k^* (a^*)^2 M_{DNA}^{1/2} C_P^{1.72} + 3.44 \times 10^{-14} (a^* k^*)^2 C_P - 8.90 \times 10^{-8} (a^*)^2 (k^*)^3 M_{DNA}^{-1/2} C_P^{0.28} - 7.03 \times 10^{-14} a^* \mu_0^{-1} C_P^{-0.72} - 2.02 \times 10^{-7} \mu_0^{-1} a^* k^* M_{DNA}^{-1/2} = 0 \quad \text{Eq. 4}$$

where $t_c^* = 3.92 \times 10^{-8}$ min [computed in paper (26)], $L_p^* \approx 10^{-4}$ cm, $M_p^* \approx 5 \times 10^6$, $a^* = EN_A \pi t_c^* L_P^* / M_P^* k^* = 2.25 \times 10^{15}$.

We solved Eq. 3 and 4 by drawing method and obtained the $C_{optimum,LPA}$ and $C_{optimum,HEC}$ for separating different DNA molecules. Using the solutions of Eq. 3 or 4, the relationship between the optimum concentration and DNA size was obtained by curve fitting method (presented by Eq. 5 and 6, the correlation coefficient were 0.9999 and 0.9998 respectively, see Figure 4).

$$C_{optimum,LPA} = 0.82 M_{DNA}^{-0.29} \quad \text{Eq. 5}$$

$$C_{optimum,HEC} = 0.70 M_{DNA}^{-0.30} \quad \text{Eq. 6}$$

From the predicted results, it is able to be seen that the optimum concentrations were too high to be used in CE actually. Obviously, other factors such as the polymer solubility, viscosity, and operating possibility must be taken account of in the optimum concentration choosing. However, the results told us that the polymer concentration should be as high as possible in DNA molecules separating by CE. The rule is suitable for all ssDNA and dsDNA. Of course, the behavior of dsDNA and ssDNA is different. However, dsDNA is flexible enough when it has larger length ($L_p > b$, L_p is the total curvilinear length of polymer, b is the Kuhn length of DNA), its radius of gyration, R_{DNA}, can be approximately expressed by the formula that was used in the model.

From Eq. 5 and 6, it can be seen that the larger the DNA fragments, the lower the optimum concentration of polymer asked in electrophoretic separation. To enable convenient operating and

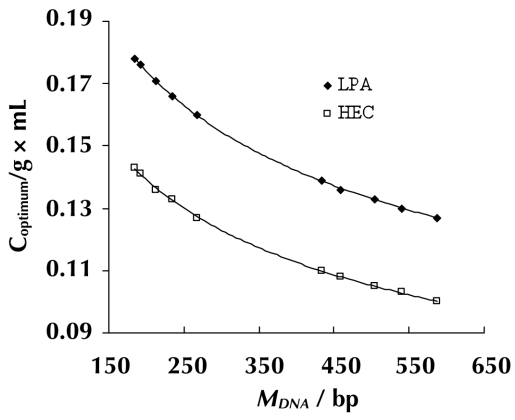

Figure 4. Curves of the optimum concentration of HEC and LPA solutions for separating DNA molecules (involving ssDNA and dsDNA) with different size, the exponential correlation coefficients were 0.9998 and 0.9999.separated in HEC media (800~1500 cP) and the predicted data by the model and (b) dsDNA separated in LPA sieving media and the predicted data by the model. Separation conditions are same with Figure 1. Const. in R/const. of Y-axis is $1/(4\sqrt{2})(l^2/D)^{1/2}$.

better resolution, the 2.0% polymer solution was determined to be more suitable for DNA separation in the experiments.

Prediction for the separation ability of polymer

Polymer has an important role in DNA separation by CE. However, the separation ability of polymers are different from each other. Generally, the sieving ability of any polymer is evaluated by doing a bench of experiments, then experimental results such as the resolution and the theoretical plate number of electrophoresis peaks will be used to select a more suitable polymer. Here, multiple experiments can be replaced by the model in selectivity of sieving materials. Depicted by our model, the characteristic constants of a given polymer-solvent system, such as a and K, are useful in polymer selecting. Eq. 1 shows that for a given polymer solution, the smaller the value of k, the better the resolution of DNA if other coefficients fixed. Where, values of k are calculated by a and K and listed in Table I. From Table I, we can see that the best suitable medium for DNA sieving should be methyl cellulose if we neglected other factors such as its hydrophilic and viscosity. Using the model, it was also possible to select excellent sieving materials by the values of k initially and save much time. In other words, the sieving ability of polymer can be predicted by the model.

Conclusion

In this paper, we focused on the prediction ability of the model proposed by ourselves previously (26). It is well known that good DNA resolution is our target in DNA sieving. By this model, DNA resolution can be predicted before doing experiments, which is useful for chemists in CE experiments.

From the model, the optimum concentrations for separating different DNA fragments were computed, which were too high to be performed in CE experiments. However, the results told us that more concentrated polymer solutions will be convenient to achieve better separation if other factors such as polymer solubility, viscosity, and operating possibility were neglected. In our experiments, the most suitable concentration was 2.0%. Polymer solution with this concentration will be convenient for performance. Furthermore, the satisfied resolution of DNA was ensured. The intense labor can be avoided by using the model in polymer selectivity. Described by the model, the sieving ability of someone polymer can be predicted just by the value of k calculated.

Table I. Molecular parameters of different polymers (30)

Polymer	$k\ \{1.43 \times [K/(6.2N_A)]^{1/3} \times (K/1.5)^{-(1+a)/3a}\}$
Polyacrylamide	7.21×10^{-8}
Hydroxylpropyl cellulose	7.21×10^{-8}
Polyethyleneoxide	5.71×10^{-8}
Hydroxyethyl cellulose	5.13×10^{-8}
Methyl cellulose	1.90×10^{-8}
Dextran	4.92×10^{-8}
Ethyl hydroxyethyl cellulose	9.01×10^{-8}

Temperature is another factor that will affect DNA separation except polymer concentration and electric field strength. Thus, further work should be conducted to modify the model and establish a dependence of DNA electrophoretic mobility on temperature. Furthermore, the model could extend to predict protein separation by CE. However, there is still much work needed to be done in future before the applied range of the model expanding.

Acknowledgement

The authors thank Professor James Bao of the Pharmacy School at Tianjin University (China) for his kind help and the support of the Innovation Foundation of Chinese Academy of Sciences (NO. KSCX1-06).

References

1. W.G. Kuhr and C.A. Monnig. Capillary electrophoresis. *Anal. Chem.* **64(12):** 389R–407R (1992).
2. Z.Q. Xu, T. Esumi, N. Ikuta, et al. High-sensitive analysis of DNA fragments by capillary gel electrophoresis using transient isotachophoresis preconcentration and fluorescence detection. *J. Chromatogr. A* **1216(7):** 3602–3605 (2009).
3. J.L. Viovy and T. Duck. DNA electrophoresis in polymer solutions: Ogston sieving, reptation and constraint release. *Electrophoresis* **14(4):** 322–329 (1993).
4. J. Z. Zhang, Y. Fang, and J.Y. Hou. Use of non-cross-linked polyacrylamide for four-color DNA sequencing by capillary electrophoresis separation of fragments up to 640 bases in length in two hours. *J. Anal. Chem.* **67(24):** 4589–4593 (1995).
5. H. Swerdlow and R. Gesteland. Capillary gel electrophoresis for rapid, high resolution DNA sequencing. *Nucleic Acids Res.* **18(6):** 1415–1419 (1990).
6. M.A. Quesada. Replaceable polymers in DNA sequencing by capillary electrophoresis. *J. Curr. Opin. Biotechnol.* **8(1):** 82–93 (1997).
7. K. Klepárník, Z. Malá, and P. Bocek. Fast separation of DNA sequencing fragments in highly alkaline solutions of linear polyacrylamide using electrophoresis in bare silica capillaries. *Electrophoresis* **22(4):** 783–788 (2001).
8. M.N. Albarghouthi, B.A. Buchholz, E.A.S. Doherty, et al. Impact of polymer hydrophobicity on the properties and performance of DNA sequencing matrices for capillary electrophoresis. *J. Electrophoresis* **22(4):** 737–747 (2001).
9. C.H. Wu, M.A. Quesada, D.K. Schneider, et al. Polyacrylamide solutions for DNA sequencing by capillary electrophoresis: Mesh sizes, separation and dispersion. *Electrophoresis* **17(6):** 1103–1109 (1996).
10. S. Menchen, B. Johnson, M.A. Winnik, et al. Flowable networks as DNA sequencing media in capillary columns. *Electrophoresis* **17(9):** 1451–1459 (1996).
11. E.S. Yeung and Y. Kim. DNA sequencing with pulsed-field capillary electrophoresis in poly(ethylene oxide) matrix. *Electrophoresis* **18(15):** 2901–2908 (1997).
12. R.S. Madabhushi, M. Vainer, V. Dolnik, et al. Versatile low-viscosity sieving matrices for nondenaturing DNA separations using capillary array electrophoresis. *Electrophoresis* **18(1):** 104–111 (1997).
13. M.H. Klemiβ, M. Gilges, and G. Schomburg. Capillary electrophoresis of DNA restriction fragments with solutions of entangled polymers. *Electrophoresis* **14(1):** 515–522 (1993).
14. Q.F. Gao and E.S. Yeung. A matrix for DNA separation: genotyping and sequencing using poly(vinylpyrrolidone) solution in uncoated capillaries. *Anal. Chem.* **70:** 1382–1388 (1998).

15. J.M. Song and E.S. Yeung. Optimization of DNA electrophoretic behavior in poly(vinyl pyrrolidone) sieving matrix for DNA sequencing. *Electrophoresis* **22(4):** 748–754 (2001).
16. S. Boulos, O. Cabrices, M. Blas, et al. Development of an entangled polymer solution for improved resolution in DNA typing by CE. *J. Electrophoresis* **29(23):** 4695–4703 (2008).
17. W.A. Maschke, H.T. Rasmussen, and D.M. Northrup. Size-Selective Capillary Electrophoresis (SSCE) Separation of DNA Fragments. *J. Liq. Chromatogr.* **15:** 1063–1080 (1992).
18. M. Chiari, M. Nesi, and P.G. Righetti. Movement of DNA fragments during capillary zone electrophoresis in liquid polyacrylamide. *J. Chromatogr. A* **652(1):** 31–39 (1993).
19. A.E. Barron, D.S. Soane, and H.W. Blanch. Capillary electrophoresis of DNA in uncross-linked polymer solutions. *J. Chromatogr. A* **652(1):** 3–16 (1993).
20. A.E. Barron, H.W. Blanch, and D.S. Soane. A transient entanglement coupling mechanism for DNA separation by capillary electrophoresis in ultradilute polymer solutions. *Electrophoresis* **15(1):** 597–615 (1994).
21. S.J. Hubert and G.W. Slater, Theory of capillary electrophoresis separation of DNA using ultradilute polymer solutions. *J. Macromolecules* **29(3):** 1006–1009 (1996).
22. W.M. Sunada, and H.W. Blanch. Microscopy of DNA in dilute polymer solutions. *J. Biotechnol. Prog.* **14(5):** 766–772 (1998).
23. Y. Jin, B.C. Lin, and Y.S. Fung. A collision model for DNA separation by capillary electrophoresis in dilute polymer solution. *Feresenius' J. Anal. Chem.* **370:8** 1015–1022 (2001).
24. A.G. Ogston. The spaces in a uniform random suspension of fibres. *J. Trans Faraday Soc.* **54:** 1754–1757 (1958).
25. G.W. Slater and J. Noolandi. The biased reptation model of DNA gel electrophoresis: mobility vs molecular size and gel concentration. *Biopolymers* **28(10):** 1781–1791 (1989).
26. C.Y. Liu, X. Xu, J.R. Chen, et al. Mathematical model for DNA separation by capillary electrophoresis in entangled polymer solutions. *J. Chromatogr. A* **1142(2):** 222–230 (2007).
27. L.C. McCormick, G.W. Slater, A.E. Karger, et al. Capillary electrophoretic separation of uncharged polymers using polyelectrolyte engines: Theoretical model. *J. Chromatogr. A* **924(1–2):** 43–52 (2001).
28. V. Dolnik, W.A. Gursk, and A. Padua. Sieving matrices in capillary electrophoresis: Inflection slope and double reciprocal plot. *J. Electrophoresis* **22(4):** 692–698 (2001).
29. M. Kurata, Y. Tsunashima, J. Brandup, and E.H. Immergut. Polymer Handbook. Wiley, New York, 1989, P.1, Chapter 7.
30. S. Hjertèn. High-performance electrophoresis: Elimination of electroendosmosis and solute adsorption. *J. Chromatogr. A* **347:** 191–198 (1985).
31. Y.Z. Deng and J.L. He. High performance capillary electrophoresis. Beijing: Science Press 1996: 34–35.

Manuscript received November 20, 2008;
revision received March 8, 2010.