

Article

Optimization of Polymer Sieving Matrix Composition for DNA Fragment Separation in a Laboratory-Built Multicapillary Electrophoresis System

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Abstract

Capillary electrophoresis (CE) is widely used for DNA fragment analysis, but its separation performance depends strongly on the composition of the polymer sieving matrix, especially in multicapillary systems where matrix operability and reproducibility are critical. Herein, a laboratory-built multicapillary electrophoresis system was used to optimize the sieving matrix for high-throughput DNA fragment separation. The system integrated twelve fused-silica capillaries, high-voltage electrokinetic injection, fluorescence detection, and digital electropherogram acquisition. Using a 100 bp DNA ladder as the model sample, the effects of poly(ethylene oxide) (PEO), Tween 20, and glycerol on electropherogram quality were systematically investigated. PEO concentration was the dominant factor controlling the dynamic sieving network: 0.1% PEO provided insufficient separation, whereas 0.8–1.0% PEO produced clearly resolved DNA peaks. Tween 20 improved peak regularity and electropherogram quality under the tested conditions, with 0.05% providing sufficient improvement without prolonging migration time. Glycerol affected peak distribution by increasing apparent migration resistance in the polymer matrix; however, excessive glycerol slowed DNA migration and markedly extended the separation window. Considering separation quality, matrix operability, and analytical efficiency, 1.0% PEO, 0.05% Tween 20, 2.5% glycerol, 1× SYBR Gold, and 0.5× TBE were selected as the optimized sieving matrix. Under the optimized matrix, inter-capillary migration-time alignment improved the consistency of parallel capillary outputs, with corrected migration-time RSD values generally below 0.5%. The optimized formulation provides a practical basis for high-throughput CE-based DNA fragment analysis.

Keywords: capillary electrophoresis; multicapillary electrophoresis; DNA fragment separation; polymer sieving matrix; PEO; Tween 20; glycerol; fluorescence detection; high-throughput analysis



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1. Introduction

DNA fragment separation is a routine analytical task in molecular biology, PCR product verification, nucleic acid quality control, and fragment-size assessment [1–3].

Conventional slab-gel electrophoresis is widely used because of its simplicity and low cost, but it is limited by relatively long analysis time, manual operation, low automation level, and limited reproducibility [4]. These limitations become more pronounced when multiple samples must be analyzed in parallel. Therefore, analytical platforms that provide high separation efficiency, low sample consumption, fluorescence detection, and improved throughput are desirable for routine DNA fragment analysis.

Capillary gel electrophoresis (CE) is an effective alternative to slab-gel electrophoresis for DNA separation [5,6]. The small inner diameter of fused-silica capillaries provides efficient heat dissipation, allowing high electric fields to be applied while maintaining narrow peak widths. CE also consumes only small amounts of sample and reagent, and it is compatible with electrokinetic injection, fluorescence detection, and automated data acquisition [7,8]. In particular, multicapillary electrophoresis systems can improve sample throughput by performing parallel separations in multiple capillaries [9]. Such systems are useful for DNA ladder analysis, PCR product screening, and nucleic acid quality control workflows where repeated or batch analysis is required [10,11].

Polymer-based replaceable sieving matrices are essential for DNA fragment separation in CE [12,13]. In free solution, DNA fragments of different lengths possess nearly identical charge-to-mass ratios, making size-dependent migration unattainable. Entangled polymer networks overcome this limitation by imposing a molecular-sieving effect that retards longer fragments more strongly than shorter ones. Among water-soluble polymers, poly(ethylene oxide) (PEO) is widely adopted due to its favorable solubility and replaceability [14]. However, its separation performance is critically concentration-dependent. An insufficiently low PEO concentration yields a network that is too dilute, resulting in compressed migration windows and poor resolution of adjacent fragments. Conversely, an excessively high concentration elevates viscosity, complicates capillary filling, prolongs migration times, and reduces practical operability, which is particularly detrimental in multicapillary systems where matrix handling must be reproducible across parallel channels [15]. Therefore, PEO concentration must be carefully balanced rather than simply maximized, aiming to achieve both adequate resolution and practical handling feasibility.

In addition to the polymer component, additives can also influence electropherogram quality. Tween 20, a nonionic surfactant, can reduce nonspecific interactions between DNA fragments, matrix components, and the capillary wall [16]. This wall-passivating effect may improve peak symmetry, reduce peak distortion, and stabilize migration behavior [17]. Glycerol can modify the viscosity of the sieving matrix and influence DNA migration and peak distribution [18]. Moderate glycerol content may improve peak definition, whereas excessive glycerol can markedly extend migration time and reduce analytical throughput. Thus, optimization of the sieving matrix requires a balance among separation efficiency, peak quality, migration time, and matrix operability.

In this study, a laboratory-built multicapillary electrophoresis system was used to optimize polymer sieving matrix composition for DNA fragment separation. The system integrated twelve parallel fused-silica capillaries, high-voltage electrokinetic injection, fluorescence detection, and digital electropherogram acquisition. Using a 100 bp DNA ladder as the model sample, the effects of PEO concentration, Tween 20 concentration, and glycerol content on electropherogram quality were systematically investigated. Representative electropherograms were plotted directly from raw fluorescence data to compare migration-time distribution, peak clarity, peak spacing, and analysis time. The objective was to identify a practical sieving matrix formulation suitable for high-throughput DNA fragment separation in a laboratory-built multicapillary CE system.

2. Materials and Methods

2.1. Reagents and DNA Standards

All reagents used in this study were of analytical grade or higher. Ultrapure water with a resistivity of 18.2 M Ω ·cm was used for reagent preparation and capillary rinsing. Tris-borate-EDTA (TBE) (Solarbio, Beijing, China) buffer was used as the background electrolyte and was diluted to 0.5 $\times$ working concentration for CE separation. PEO (5000k, Aladdin, Shanghai, China) was used as the polymer sieving component. Tween 20 (Aladdin, Shanghai, China) was used as a nonionic surfactant to reduce nonspecific adsorption on the capillary wall. Glycerol (Aladdin, Shanghai, China) was used as a viscosity modifier. SYBR Gold was used as the fluorescent dye for DNA detection. A commercial 100 bp DNA ladder (Solarbio, Beijing, China) was used as the model DNA sample for matrix optimization. The ladder contained DNA fragments with nominal sizes of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, and 1500 bp. The 500 bp fragment had a higher nominal concentration than the other fragments and therefore produced a relatively stronger fluorescence peak. Before CE analysis, the DNA ladder was diluted with ultrapure water according to the experimental requirements. The same DNA ladder was used throughout the single-factor experiments to ensure that differences in electropherogram quality mainly resulted from changes in matrix composition rather than sample variation. A 100 bp plus DNA ladder containing fragments from 100 to 5000 bp was used for inter-capillary migration-time alignment and consistency evaluation.

2.2. Laboratory-Built Multicapillary Electrophoresis System

DNA fragment separation was performed using a laboratory-built multicapillary electrophoresis system (Figure 1A), and data in Figure 1B is a typical electropherogram obtained in our system. The system consisted of a capillary array, a high-voltage power supply, an automatic electrokinetic injection module, a CMOS camera, and a digital data acquisition system. Twelve fused-silica capillaries (Yongnian Ruifeng Chromatography Devices Co., Ltd., Handan, China) were arranged in parallel for multicapillary operation. Each capillary had an inner diameter of 75 μ m, an effective separation length of 22 cm, and a total length of 40 cm. The high-voltage power supply provided a stable direct current electric field for sample injection and electrophoretic separation, with an output voltage range of 0–8 kV. DNA samples were introduced into the capillaries by electrokinetic injection. SYBR Gold-labeled DNA fragments were detected using a fluorescence detection module. A compact laser diode (LD, OXLASERS Flagship Store, Shanghai, China) with a center wavelength of 450 nm was used as the excitation source for DNA stained with SYBR Gold, SYBR Green I, or GelGreen. The emission peak wavelengths were 520 nm for SYBR Green I and GelGreen, and 540 nm for SYBR Gold, with an optical filter bandwidth of 20 nm.

2.3. Capillary Electrophoresis Procedure

Before each experiment, the capillaries were rinsed with ultrapure water and running buffer to remove residual contaminants and stabilize the capillary wall condition. The polymer sieving matrix was freshly prepared before use. PEO was dissolved thoroughly in 0.5 $\times$ TBE buffer, and the required amounts of Tween 20, glycerol, and SYBR Gold were added according to the experimental design. The matrix solution was mixed thoroughly to ensure homogeneity. Because polymer concentration and glycerol content can substantially affect solution viscosity, sufficient mixing time was used to obtain a uniform matrix. DNA samples were centrifuged briefly before injection to remove possible insoluble particles. Electrokinetic injection was performed at 4000 V for 3.5 s. After injection, electrophoretic separation was conducted under a constant electric field of 100 V/cm. The detector expo-

sure time was set to 20,000 μ s. Unless otherwise specified, the acquisition time was 50 min. For conditions with high glycerol content or extended migration time, signal acquisition was continued long enough to observe the major fragment peaks. Fluorescence signals were recorded digitally as electropherograms for subsequent separation performance evaluation and inter-capillary migration-time analysis.

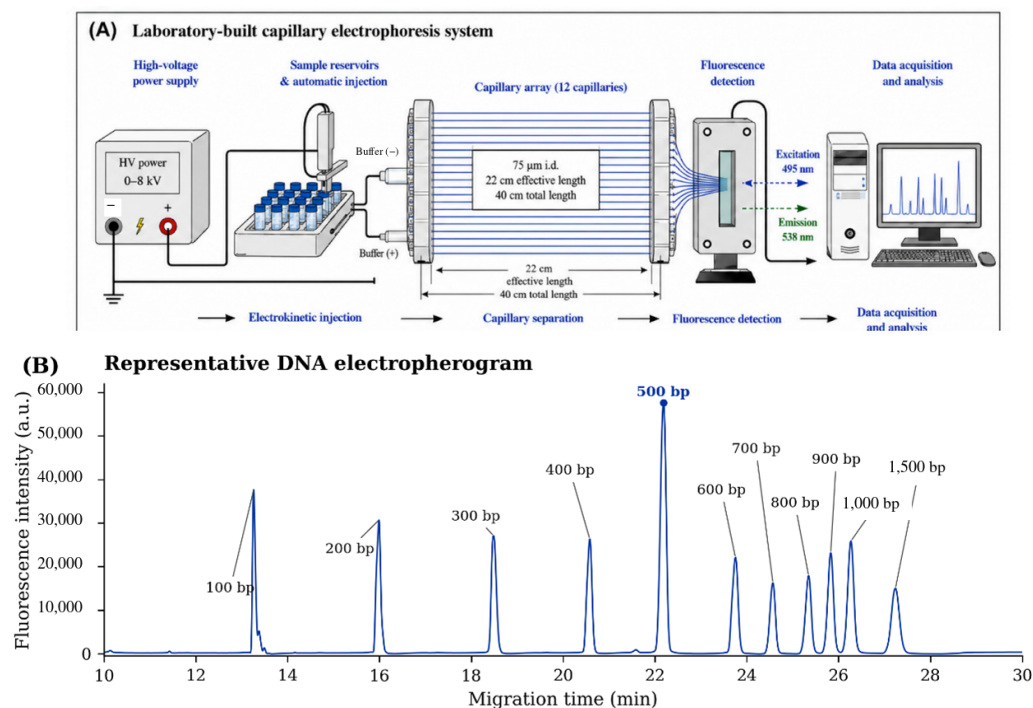


Figure 1. Laboratory-built multicapillary electrophoresis system and representative DNA ladder electropherogram. **(A)** Schematic illustration of the laboratory-built multicapillary electrophoresis system. **(B)** Representative electropherogram for DNA fragment analysis.

2.4. Single-Factor Experimental Design

The influence of sieving matrix composition on DNA fragment separation was evaluated using a single-factor experimental design. For each set of experiments, only one matrix component was varied, whereas the DNA ladder sample, buffer composition, fluorescent dye concentration, capillary format, injection condition, separation voltage, and detection parameters were kept constant. This design allowed the individual effects of polymer concentration, surfactant content, and viscosity modifier content to be compared under the same multicapillary CE operating conditions.

PEO concentration was first optimized because it determines the density of the dynamic polymer sieving network. The tested PEO concentrations were 0.1%, 0.5%, 0.8%, 1.0%, and 1.5%, with Tween 20, glycerol, SYBR Gold, and TBE fixed at 0.05%, 2.5%, 1 $\times$, and 0.5 $\times$, respectively. Tween 20 was then evaluated as a wall-passivating additive at concentrations of 0%, 0.05%, 0.1%, 0.2%, and 0.5%, while PEO, glycerol, SYBR Gold, and TBE were maintained at 1.0%, 2.5%, 1 $\times$, and 0.5 $\times$, respectively. Finally, glycerol was tested as a viscosity modifier at 0%, 2.5%, 5%, and 10%, with PEO, Tween 20, SYBR Gold, and TBE fixed at 1.0%, 0.05%, 1 $\times$, and 0.5 $\times$, respectively. The 1.5% PEO condition was included in the initial experimental design, but no effective electropherogram was obtained under the present operating conditions, most likely because of excessive matrix viscosity. For glycerol optimization, the 7.5% condition was not included in the final comparative analysis because the available data was incomplete. Therefore, the final glycerol comparison focused on 0%, 2.5%, 5%, and 10%.

2.5. Evaluation of Separation Performance

Separation performance was evaluated using both representative electropherogram profiles and calculated separation parameters. For the main figure presentation, raw fluorescence traces were used to preserve the original peak shape, baseline characteristics, and migration behavior under different matrix conditions. Each trace was normalized only for display and vertically offset to allow direct visual comparison among different PEO, Tween 20, and glycerol conditions. No smoothing or baseline correction was applied to the representative electropherograms shown in the composition-comparison figures. The visual evaluation focused on features that directly affect practical DNA fragment analysis, including migration-time distribution, number of distinguishable peaks, peak sharpness, peak spacing, peak symmetry, baseline stability, and total analysis time. A matrix condition was considered favorable when it produced clearly separated DNA peaks within an acceptable migration-time range and remained suitable for multicapillary operation.

In addition to visual comparison, the resolution between adjacent fragments was calculated as $(R_s = 2(t_2 - t_1)/(w_1 + w_2))$, where (t_1) and (t_2) are the migration times of the two adjacent peaks, and (w_1) and (w_2) are their corresponding peak widths at the baseline. These (R_s) values were used as supplementary quantitative evidence after peak assignments had been verified according to the known fragment order of the DNA ladder. The final evaluation emphasized agreement between raw electropherogram profiles and verified separation parameters. The optimized matrix formulation was selected by considering separation quality, adjacent-fragment resolution, migration-time window, and practical handling of the polymer solution together. Conditions that produced high apparent resolution but required excessively long migration times or showed poor matrix operability were not considered optimal for high-throughput multicapillary CE operation.

2.6. Inter-Capillary Migration-Time Alignment

To evaluate the consistency of parallel capillaries under the optimized sieving matrix, migration-time alignment was performed using the 100 bp plus DNA ladder as the reference sample. Because small differences in capillary inner-wall condition, effective separation length, injection amount, and local electric-field distribution may cause migration-time shifts among capillaries, direct comparison of raw electropherograms can lead to peak-position mismatch. One capillary with a complete and stable electropherogram was selected as the reference channel. The major DNA peaks in the other capillaries were assigned to the corresponding DNA fragments based on the lower and upper internal markers and the expected fragment sizes of the DNA ladder. A time-axis correction was then applied to reduce the deviation between each capillary and the reference channel. The correction was based on peak-position alignment rather than intensity normalization; therefore, the original peak shape and fluorescence intensity profile were preserved. After alignment, the corrected migration times of the assigned DNA fragments were used to evaluate inter-capillary consistency. For each DNA fragment, the mean migration time, standard deviation, and relative standard deviation (RSD) were calculated across parallel capillaries.

3. Results and Discussion

3.1. Effect of PEO Concentration on DNA Fragment Separation

PEO was first optimized because it provides the primary dynamic sieving network for DNA fragment separation. Thus, we separated the DNA ladders (100–1500 bp) in $0.5 \times$ TBE (pH 8.0) containing PEO (5000k), 2.5% glycerol, and $1 \times$ SYBR Gold. The concentration of PEO was varied from 0.1% to 1.0% (Figure 2). A 1.5% PEO matrix was also tested, but no effective electropherogram was obtained under the present operating conditions, most likely because excessive viscosity hindered stable capillary operation. As shown

in Figure 2, 0.1% PEO produced a highly compressed migration profile, with most DNA peaks clustered in the early migration region. Although several peaks were detectable, adjacent fragments were poorly separated, indicating that the polymer network was too dilute to provide sufficient size-dependent retardation. Increasing the PEO concentration to 0.5% broadened the migration-time distribution and made more peaks distinguishable, but partial peak crowding remained, especially among short fragments. Further increasing the PEO concentration to 0.8% substantially improved the electropherogram profile. DNA peaks were distributed over a wider time range, and adjacent fragments became more clearly separated. At 1.0% PEO, the electropherogram showed the most balanced separation behavior among the effective conditions, with clearly distributed major peaks and improved fragment discrimination. Although the migration time was longer than that at lower PEO concentrations, the gain in peak spacing and separation quality was more evident. The calculated resolution values further supported this trend. For the 100/200, 200/300, and 300/400 bp peak pairs, the resolution values were 0.287, 0.177, and 0.440 at 0.1% PEO, respectively, confirming insufficient separation of adjacent short fragments. These values increased to 1.588, 1.173, and 0.963 at 0.5% PEO and further to 2.491, 1.960, and 1.557 at 0.8% PEO. At 1.0% PEO, the corresponding resolution values reached 4.161, 2.596, and 2.135, demonstrating that increasing PEO concentration enhanced the size-dependent sieving effect.

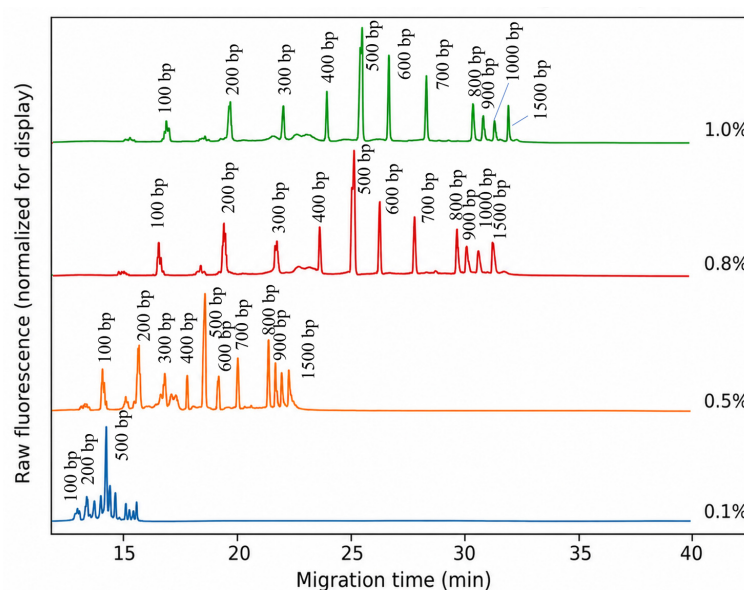


Figure 2. Representative electropherograms under different PEO concentrations. Capillary electrophoresis conditions: total length of the capillary (40 cm), effective length of the capillary (22 cm), the electric field strength (100 V/cm). The background electrolyte was $0.5\times$ TBE (pH 8.0) containing PEO (5000k), 2.5% glycerol, 0.05% Tween 20 and $1\times$ SYBR Gold. The concentration of PEO was varied from 0.1% to 1.0%.

These results indicate that PEO concentration must balance separation efficiency and matrix operability. Increasing PEO concentration improved adjacent-fragment resolution, which was attributed to the enhanced size-dependent sieving effect of the polymer matrix. Because polymer-chain entanglement was not directly characterized in this study, no direct conclusion was made regarding the degree of polymer-chain entanglement or effective pore size. However, excessive polymer content compromised practical operation, as shown by the failure of the 1.5% PEO condition. Considering peak distribution, adjacent-fragment resolution, migration time, and handling feasibility, 1.0% PEO was selected as the optimized polymer concentration for subsequent additive optimization.

3.2. Effect of Tween 20 Concentration on Electropherogram Quality

Tween 20 was evaluated as a nonionic surfactant for improving electropherogram quality in the PEO-based sieving matrix. Thus, we separated the DNA sample in $0.5\times$ TBE (pH 8.0) containing 1.0% PEO (5000k), 2.5% glycerol and $1\times$ SYBR Gold. The concentration of Tween 20 was varied from 0% to 0.5%. Tween 20 was varied from 0% to 0.5%. Unlike PEO, which primarily regulates the sieving network density, Tween 20 was evaluated as an additive for improving electropherogram quality, possibly by reducing wall-related interactions in the capillary. As shown in Figure 3, DNA fragments were detectable under all Tween 20 concentrations, indicating that the 1.0% PEO matrix provided sufficient baseline sieving ability. Without Tween 20, multiple peaks were observed, but the peak pattern was less well defined, with less uniform peak spacing and more variable peak shapes among adjacent fragments. After adding Tween 20, especially at 0.05% and 0.1%, the electropherograms showed clearer peak patterns and more stable migration behavior. Therefore, Tween 20 was evaluated only according to the observed electropherogram quality, migration-time distribution, and adjacent-fragment resolution under the tested conditions. The resolution values were consistent with the electropherogram observations. For the 100/200, 200/300, and 300/400 bp peak pairs, the values were 2.850, 2.105, and 1.658 without Tween 20. At 0.05% Tween 20, the corresponding values were 2.482, 1.977, and 1.653, indicating that adjacent-fragment separation was maintained while the peak profiles became more regular. Increasing Tween 20 to 0.1%, 0.2%, and 0.5% further increased the resolution values to 3.828/2.298/1.960, 4.099/2.546/2.139, and 4.461/2.643/2.234, respectively. However, the highest Tween 20 concentration was not selected as optimal. Although 0.5% Tween 20 gave the highest resolution values among the tested conditions, it also broadened the migration-time window, which would reduce analytical efficiency in multicapillary operation. Therefore, Tween 20 optimization required a balance between improved separation and practical analysis time. Considering peak regularity, maintained adjacent-fragment resolution, and shorter migration time, 0.05% Tween 20 was selected as the optimized surfactant concentration for the final sieving matrix.

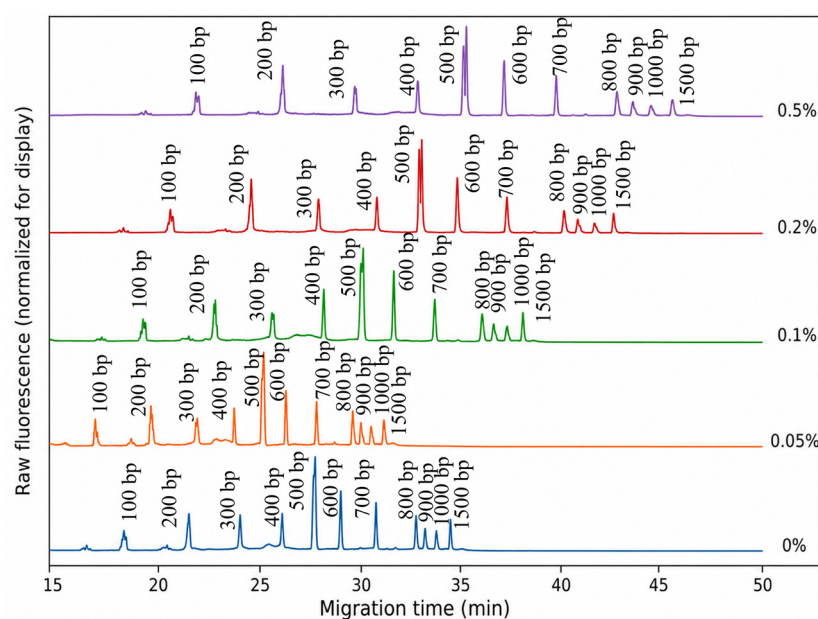


Figure 3. Representative electropherograms under different Tween 20 concentrations. Capillary electrophoresis conditions: total length of the capillary (40 cm), effective length of the capillary (22 cm), the electric field strength (100 V/cm). The background electrolyte was $0.5\times$ TBE (pH 8.0) containing 1.0% PEO (5000k), 2.5% glycerol and $1\times$ SYBR Gold. The concentration of Tween 20 was varied from 0% to 0.5%.

3.3. Effect of Glycerol Content on Migration Behavior and Separation Quality

Glycerol was investigated as a viscosity modifier in the PEO/Tween 20 sieving matrix. In this experiment, PEO, Tween 20, SYBR Gold, and TBE were fixed at 1.0%, 0.05%, 1×, and 0.5×, respectively, while the glycerol content was varied from 0% to 10%. Because the 7.5% glycerol dataset was incomplete, the final comparison focused on 0%, 2.5%, 5%, and 10% glycerol. As shown in Figure 4, glycerol content strongly influenced the migration behavior of DNA fragments. Without glycerol, the electropherogram showed detectable DNA peaks, but several fragments migrated within a relatively narrow time range. This indicated that the matrix provided limited viscosity-mediated modulation of fragment migration [19]. After adding 2.5% glycerol, the peak distribution became clearer, and the electropherogram showed improved separation among adjacent fragments while maintaining a moderate migration-time window. This condition improved peak definition without substantially prolonging the analysis time. When the glycerol content was increased to 5%, the migration-time distribution was further broadened, and the DNA peaks remained distinguishable. The calculated resolution values also increased for most adjacent peak pairs. For the 100/200, 200/300, and 300/400 bp peak pairs, the resolution values were 2.586, 1.895, and 1.579 without glycerol, and 2.531, 1.949, and 1.508 at 2.5% glycerol, respectively. At 5% glycerol, these values increased to 3.518, 2.589, and 2.122, indicating enhanced adjacent-fragment separation. However, compared with 2.5% glycerol, 5% glycerol produced a longer migration-time window, suggesting a trade-off between resolution and analysis time. At 10% glycerol, the calculated apparent resolution values were higher, with resolution values of 5.29, 4.14, and 5.29 for the same peak pairs. The major DNA peaks shifted to a much later migration region, markedly extending the separation window and reducing practical throughput. In practical multicapillary CE operation, excessively long migration time reduces analytical throughput and is therefore not desirable, even when peak spacing appears improved. These results indicate that glycerol improves separation mainly by increasing matrix viscosity and modulating DNA migration resistance. However, the benefit of higher viscosity must be balanced against analysis time. Considering electropherogram quality, adjacent-fragment separation, migration-time window, and practical throughput, 2.5% glycerol was selected as the optimized content for the final sieving matrix.

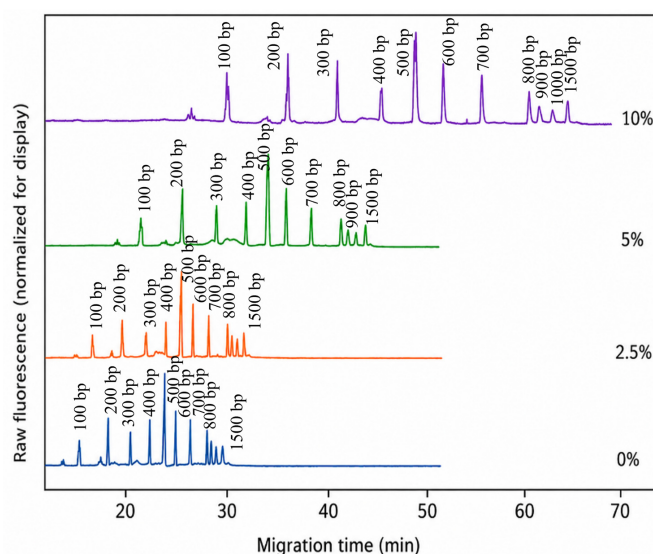


Figure 4. Representative electropherograms under different glycerol contents. Capillary electrophoresis conditions: total length of the capillary (40 cm), effective length of the capillary (22 cm), the electric field strength (100 V/cm). The background electrolyte was 0.5× TBE (pH 8.0) containing 1.0% PEO (5000k), 0.05% Tween 20, and 1× SYBR Gold. The concentration of glycerol was varied from 0% to 10%.

3.4. Inter-Capillary Migration-Time Consistency Under the Optimized Matrix

Using the optimal electrophoretic conditions determined above, we resolved DNA ladders of 100–3000 bp in a running buffer composed of $0.5\times$ TBE (pH 8.0), 1.0% PEO (5000k), 0.05% Tween 20, 2.5% glycerol, and $1\times$ SYBR Gold (Figure 5). Ten replicate runs were performed to assess reproducibility. Inter-capillary migration-time consistency was evaluated under the optimized sieving matrix to verify whether the laboratory-built multicapillary system could provide comparable separation results across parallel capillaries. Before alignment, visible migration-time shifts were observed in the original electropherograms, although the overall peak patterns were similar. These shifts were more evident in the middle and late migration regions and could affect DNA fragment assignment if the raw electropherograms were compared directly. To improve inter-capillary comparability, one capillary with a complete and stable electropherogram was selected as the reference channel. The major DNA peaks in the other capillaries were matched to the corresponding reference peaks according to their elution order and expected fragment sizes, followed by a time-axis transformation. As shown in Figure 5, the aligned electropherograms showed closely matched peak positions across parallel capillaries after correction. The correction mainly adjusted the migration-time axis while preserving the original electropherogram profiles. The corrected migration-time statistics further confirmed the improvement in inter-capillary consistency. For DNA fragments from 100 to 5000 bp, the RSD values of corrected migration times were generally below 0.5%. For example, the RSD values were 0.29% for 200 bp, 0.43% for 300 bp, 0.46% for 400 bp, 0.42% for 500 bp, 0.16% for 1500 bp, and 0.22% for 3000 bp (Table 1). These results demonstrate that the optimized sieving matrix, combined with migration-time alignment, enabled reproducible DNA fragment separation across the multicapillary array.

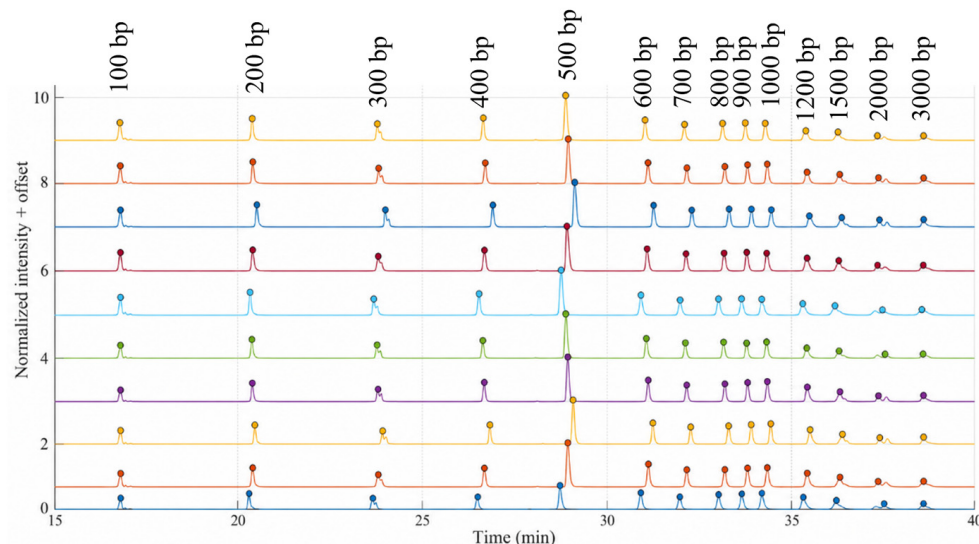


Figure 5. Aligned electropherograms and transformed peak positions under the optimized sieving matrix. The electropherograms were aligned using one capillary as the reference channel. Colored circles indicate transformed peak positions assigned according to the DNA ladder fragment order. Capillary electrophoresis conditions: total length of the capillary (40 cm), effective length of the capillary (22 cm), the electric field strength (100 V/cm). The background electrolyte was $0.5\times$ TBE (pH 8.0) containing 1.0% PEO (5000k), 0.05% Tween 20, 2.5% glycerol and $1\times$ SYBR Gold.

Table 1. Detailed information about the migration time of each DNA fragment.

DNA Size (bp)	Average Time (min)	SD (min)	RSD (%)
100	16.7806	0.0000	0.00
200	20.3649	0.0599	0.29
300	23.7717	0.1033	0.43
400	26.6495	0.1239	0.46
500	28.8914	0.1221	0.42
600	31.0621	0.1142	0.37
700	32.1111	0.1033	0.32
800	33.1376	0.0920	0.28
900	33.7560	0.0843	0.25
1000	34.2959	0.0808	0.24
1500	35.3903	0.0577	0.16
2000	36.2682	0.0555	0.15
3000	37.3823	0.0838	0.22
5000	38.5567	0.0000	0.00

SD: standard deviation, RSD: relative standard deviation.

3.5. Optimized Matrix Composition and Practical Considerations

Based on the single-factor optimization experiments, the final sieving matrix was determined to contain 1.0% PEO, 0.05% Tween 20, 2.5% glycerol, $1\times$ SYBR Gold, and $0.5\times$ TBE. This formulation was selected according to the overall balance among adjacent-fragment separation, peak regularity, migration-time window, and matrix operability rather than according to the highest resolution value alone. The optimized composition reflected the complementary functions of the three matrix components. PEO provided the primary dynamic sieving network for size-dependent DNA migration, Tween 20 improved electropherogram regularity by reducing possible wall-related interactions, and glycerol adjusted matrix viscosity and peak distribution. Although higher polymer or glycerol contents could increase apparent peak spacing, they also increased matrix viscosity, prolonged migration time, and reduced practical suitability for multicapillary operation [20]. Therefore, the selected formulation represented a practical compromise between separation efficiency and analysis throughput. Together with inter-capillary migration-time alignment, this matrix improved electropherogram quality and the comparability of parallel capillary outputs, making it suitable for repeated high-throughput DNA fragment analysis in the laboratory-built multicapillary electrophoresis system.

4. Conclusions

In this study, the polymer sieving matrix for DNA fragment separation was optimized using a laboratory-built twelve-capillary electrophoresis system. Systematic single-factor experiments revealed that PEO concentration primarily governed sieving network density and adjacent-fragment resolution, while Tween 20 improved peak regularity and electropherogram quality under the tested conditions. Glycerol modulated matrix viscosity and peak distribution, although excessive glycerol prolonged the migration-time window. The balanced formulation—1.0% PEO, 0.05% Tween 20, 2.5% glycerol, $1\times$ SYBR Gold, and $0.5\times$ TBE—enabled effective DNA fragment separation while maintaining practical operability for multicapillary operation. Under this optimized matrix, inter-capillary migration-time alignment reduced RSD values to below 0.5% for fragments ranging from 100 to 5000 bp, demonstrating reproducible parallel outputs. This work provides a ready-to-use matrix

formulation and an alignment strategy for high-throughput CE-based DNA analysis in routine laboratory settings.

Author Contributions: Conceptualization, Supervision, Project administration and Funding acquisition, Z.L. and Y.Y.; Methodology and Data curation, B.Y. and W.L.; Validation and Investigation, Formal analysis, Investigation, Methodology, X.W., W.B., C.M., S.Y., Y.J., P.W. and B.Y.; Writing—original draft and Writing—review and editing, B.Y., P.W., Z.L. and Y.Y. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study is available from the corresponding author upon reasonable request.

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