Academia Open

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

Academia Open

By Universitas Muhammadiyah Sidoarjo

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

Table Of Contents

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

Originality Statement

The author[s] declare that this article is their own work and to the best of their knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the published of any other published materials, except where due acknowledgement is made in the article. Any contribution made to the research by others, with whom author[s] have work, is explicitly acknowledged in the article.

Conflict of Interest Statement

The author[s] declare that this article was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright Statement

Academia Open

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

EDITORIAL TEAM

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

Article information

Check this article update (crossmark)

Check this article impact (*)

Save this article to Mendeley

(*) Time for indexing process is various, depends on indexing database platform

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

Biophysical Characterization of DNA Fragment Separation by Agarose Gel Electrophoresis: Quantitative Analysis of Electrophoretic Mobility : Karakterisasi Biofisik Pemisahan Fragmen DNA dengan Elektroforesis Gel Agarosa: Analisis Kuantitatif Mobilitas Elektroforesis

Zeina Salman, zsalman@uowasit.edu.iq (*)

Department of Physics, College of Science, University of Wasit, Al-Kut, Iraq, Iraq

(*) Corresponding author

Abstract

General Background: Agarose gel electrophoresis is a fundamental technique for separating DNA fragments based on size and charge in molecular biology and biophysics. **Specific Background:** Quantitative characterization of DNA migration behavior provides deeper understanding of electrophoretic mobility and molecular interactions within gel matrices. **Knowledge Gap:** Despite established theoretical models, precise quantitative relationships between DNA fragment size, topology, and migration dynamics under controlled conditions remain insufficiently detailed. **Aims:** This study aims to analyze the relationship between DNA fragment size and migration velocity, determine electrophoretic mobility values, and evaluate the role of DNA topology using image-based quantification. **Results:** The findings demonstrate an inverse exponential relationship between fragment size and migration velocity, with smaller fragments migrating faster than larger ones. Electrophoretic mobility values ranged from 0.25×10^{-4} to 0.829×10^{-4} $cm^2/V\cdot s$, while structural differences between circular single-stranded DNA and linear double-stranded DNA resulted in distinct migration behaviors and frictional resistance. **Novelty:** The study provides quantitative validation of electrophoretic principles using digital image analysis and highlights the role of molecular conformation in migration patterns. **Implications:** These results offer reference data for DNA fragment analysis and support improved interpretation of electrophoretic experiments in molecular biology applications.

Keywords: Gel Electrophoresis, DNA Migration, Electrophoretic Mobility, Agarose Gel, Image Analysis

Key Findings Highlights

1. Smaller fragments exhibit faster movement under constant electric field conditions
2. Molecular conformation produces distinct separation behavior despite equal mass
3. Digital measurement enables precise evaluation of migration parameters

Published date: 2026-03-24

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

1. Introduction

Gel electrophoresis is among the most existing and most commonly used technologies in the fields of molecular biology, biochemistry, and genetics [1,2]. Since Oliver Smithies invented it in 1955, this method has become a staple of the separation, identification, and purification of nucleic acids and proteins by their inherent physical properties [3,4]. Essentially, the principle of electrophoresis is based on the movement of charged molecules through a porous medium under a static electric field, which provides a separation process whose major characteristic is based on the size of the molecule and charge [1,5].

Electrophoresis Agarose gel is the technique of choice when analyzing DNA fragments due to its simplicity, usefulness, and ability to resolve strands of a few dozen pairs of bases to several million [2,6]. Agarose gel is an extract of seaweed polysaccharides that turns into a three-dimensional mesh when dissolved in buffer and cooled down; and thus, creates a network of pores in which the DNA moves [7]. The size of the pore can be controlled by varying the concentration of agarose such that a smaller concentration yields bigger pores that permit larger fragments of DNA to be separated [6,8].

The principles that determine the movement of the DNA through the gel are controlled by a few physical and chemical principles [9,10]. The DNA has a consistent negative charge on the backbone of sugar-phosphate; and hence, there is one negative charge per nucleotide [1]. As a result, DNA molecules move towards the positive electrode (anode) when placed in an electric field. However, the migration rate does not increase uniformly with all the fragment sizes. The smaller the fragment is, the more freely it passes the gel pores than the larger the fragment. Thus, a pattern of size-dependent separation spectrum is obtained [11,12]. This sifting effect is the basic mechanism that lies behind determining the DNA fragment size. Theoretical basis that describes this behavior balances the electrostatic driving force with the resistance caused by a frictional force exerted on the gel matrix [9,13]. In small DNA fragments, the migration can be observed according to the Ogston sieving model, where molecules pass through the pores that are smaller than the effective radius of their size [14]. Conversely, the larger molecules of the DNA molecule are repeated, which means that the polymer takes up a long form and passes through the matrix end-first [15,16]. An understanding of such mechanisms is vital in ensuring optimization of the separation parameters and also in interpreting electrophoretic outcomes correctly.

Biophysically, gel electrophoresis is a noteworthy interpretation of basic physical laws - electrostatic force, frictional resistance, and molecular hydrodynamics - in terms of biological macromolecule characterization. Gel electrophoresis represents an example of the use of quantitative physical measurements in gaining insight into biomolecular structure and behavior as an interface between physics and molecular biology.

Agarose gel visualization of DNA has been mainly based on intercalation with dyes. Ethidium bromide has been mostly used despite its safety concerns [17,18]. Recently, there have been some safe alternatives, such as SYBR dyes and GelRed, which also provide similar sensitivity but less mutagenicity [19,20]. Quantitative gel analysis has enhanced by the introduction of digital imaging systems and advanced image-analysis software, especially ImageJ, which has made a possible accurate measurement of distance and band intensities when migrating through gel lanes [21,22].

Gel electrophoresis is no longer a novel field of study today. Some of the uses include clinical diagnostics, forensics, and biotechnological production [23,24]. In clinical labs, it helps in the diagnosis of genetic diseases, detection of pathogens, and therapeutic examining regimens. DNA profiling and evidence analysis is a method used by forensic investigators [25]. More recent advances in automation and miniaturization have only served to increase the method throughput and accessibility [22,26].

This paper applies biophysical characterization of DNA fragment movement in 1% agarose gel electrophoresis using quantitative image analysis to define basic relationships among molecular attributes and electrophoretic behavior. The objectives of the present work are as follows: (1) to define how the size of DNA fragments and their migration velocity depend on each other; (2) to estimate the values of electrophoretic mobility of DNA fragments of 250 to 10000 bp size; (3) to analyze the migration of DNA based on its topology by comparison of circular ssDNA and linear dsDNA; and (4) to demonstrate that digital image analysis is appropriate to achieve good precision of measurements concerning the migration of DNA fragments. The results provide an addition to the basic knowledge of the work of electrophoretic and provide practical reference data on the application of a variety of molecular biology studies.

2. Materials and Methods

2.1 Materials and Reagents

All experiments were conducted in the University of Arkansas Biophysics Laboratory. The reagents and DNA samples were purchased at New England Biolabs (NEB). They included a 1 kbp (DNA) ladder marker (NEB #N3232) that acted as the molecular-weight marker, a 2.7 kbp double-stranded DNA (dsDNA) fragment, a 750 bp dsDNA fragment, a 4.4 kbp circular dsDNA fragment from PBR322 that was of plasmid φX174 digested with restriction enzyme BstNI, a 5.4 kbp circular ssDNA fragment of bacteriophage φX174 (φX174I), and a 5.4 kbp linear dsDNA from bacteriophage φX174 (φX174 II). Other tools were molecular-biology grade agarose powder, 10 x TrisBoric acid EDTA (TBE) buffer concentrate, 6x DNA loading dye, ethidium bromide (EtBr) at 10mg/ml solution, and TE buffer (10mm TrisHCl, 1mm EDTA, pH 8.0).

2.2 Equipment

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

The equipment that was utilized included horizontal gel electrophoresis apparatus with 8.4 cm electrode separation distance, power supply capable of constant voltage output (Bio-Rad PowerPac), gel casting tray with appropriate sample combs, UV transilluminator for DNA visualization (312 nm wavelength), digital camera for gel documentation, microwave oven for agarose melting, micropipettes (2-20 µL range) and sterile tips, and protective safety equipment (UV-blocking goggles, gloves).

2.3 Agarose Gel Preparation

The standard protocols were followed in preparation of a 1% (w/v) agarose gel. Using 5 mL of 10x TBE stock buffer, 1x TBE running buffer was first prepared by placing 50 mL of 1x TBE stock buffer in 45 mL of deionized water to get a final volume of 50 mL. In the case of gel casting, the agarose powder weighing 0.5 g was put in a 50 mL beaker containing 1x TBE buffer that was heated in a heat-flask. This solution was warmed in a microwave oven between 70 and 80 seconds and swirled at a few intervals until all the agarose dissolved resulting in a clear and uniform solution.

To avoid thermal destruction of ethidium bromide, the solution of molten agarose was allowed to cool to about 60°C. 5 µL of ethidium bromide stock solution (10 mg/mL) was added carefully in order to attain a final ethidium bromide concentration of 0.5 µg/mL at this temperature. The fluorescent dye was swirled in the solution gently to evenly spread out the dye. The gel casting tray was fitted in the electrophoresis chamber with rubber gaskets adequately covered to avoid any leaks. The comb sample was loaded into the proper slot to have wells to load the sample.

Agarose solution with ethidium bromide was placed in the gel tray carefully to avoid creation of air bubbles under the comb. The gel was left to dry at room temperature in 30-60 minutes until fully solidified as indicated by the gel being transparent and solid. After the polymerization, the comb was gently lifted up to expose sample wells that were intact.

2.4 DNA Sample Preparation

The final volume of the DNA samples was 12 µL, which had 10 µL DNA in TE buffer and 2 µL loading dye. There was approximately 50 ng of DNA in the final volume. Six lanes were prepared for gel electrophoresis analysis. Each lane contained approximately 50 ng of DNA in a final volume of 10 µL. First lane served as the molecular weight reference and was loaded with 1 kbp DNA ladder, which was prepared by mixing 5 µL of stock ladder solution with 5 µL of TE buffer. Second lane contained 2.7 kbp dsDNA, which was prepared using 2.5 µL of stock DNA diluted in 7.5 µL of TE buffer. Third lane was loaded with 750 bp dsDNA, consisting of 2 µL stock DNA and 8 µL TE buffer. Fourth lane contained 4.4 kbp PBR322 DNA digested with BstNI restriction enzyme, which was prepared from 2 µL stock DNA and 8 µL TE buffer. Fifth lane was loaded with 5.4 kbp circular ssDNA from bacteriophage φX174 form I, which was prepared using 2 µL stock DNA in 8 µL TE buffer. Finally, the sixth lane contained 5.4 kbp linear dsDNA from bacteriophage φX174 form II, which was prepared with 4 µL stock DNA and 6 µL TE buffer. After preparing each DNA dilution, 2 µL of 6× loading dye (containing glycerol and tracking dyes) was added to each sample and thoroughly mixed by pipetting. The loading dye plays two crucial roles. First role is increasing sample density for proper settling into wells, and second role is providing visible tracking dyes to monitor electrophoresis progress.

2.5 Electrophoresis Procedure

The gel tray was oriented in a 90° counterclockwise rotation after the gel was solidified, and placed on the electrophoresis chamber in a way that the wells were facing the negative (cathode) electrode. Fresh 1x TBE running buffer was made by mixing 30 mL of 10x TBE stock with 270mL deionized water [27]. The buffer was then added to each electrode chamber in the gel box until the gel surface was entirely covered with buffer such that there was adequate electrical conductivity. Micropipettes were used to carefully load DNA samples (12 µL each) into the wells without puncturing the gel or making any bubble. The power supply of the electrophoresis was adjusted to constant voltage mode at 90 V with the current limit of 80 mA. Electric field strength (E) was found out as 10.7 V/cm where the distance between the two electrodes was 8.4 cm [1, 9].

The electrophoresis was performed at room temperature (around 22°C) for 80 minutes. Visualization of the movement of tracking dyes was performed periodically during the run: GoTaq yellow dye (approximately 10 bp of DNA), Orange G (approximately 50 bp), Bromophenol Blue (approximately 300 bp), and Xylene Cyanol FF (approximately 4 kbp) [4]. The run was stopped when the bromophenol blue dye was within two- thirds of the gel length to ensure that enough separation was achieved without the smaller pieces being washed off of the gel.

2.6 DNA Visualization and Image Acquisition

After electrophoresis, the DNA bands were observed under UV transilluminator with a wavelength of 312nm [17]. Ethidium bromide when intercalated in the DNA gave out orange-red fluorescence on UV excitation [17. 18]. To ensure the best visualization of bands within the entire concentration range to detect DNA free bands, the gel images were obtained with a few numbers of exposures with a digital camera. ImageJ software (National Institutes of Health, USA) was used in quantitative analysis of distance of band migration. The gel picture was scaled to 8-bit grayscale and the distances to the base of the well to the center of the band (d) were measured using millimeter scales. The measurements were made three times, so as to guarantee accuracy [17].

2.7 Quantitative Image Analysis

Analysis of DNA fragment migration was done quantitatively through ImageJ software (version 1.54f; National Institutes of

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

Health, Bethesda, MD, USA) [21]. The gel images in the UV light were transferred and imported in the original TIFF format and converted into 8-bit grayscale (256 gray levels) to maximize band detection capabilities. Calibration of space was done by known physical separation of electrodes of the gel apparatus (8.4 cm), and calibration factor was checked by comparing with physical measurements. Migration distances (d) were calculated as the linear movement of the bottom of the sample loading well to the centroid of the maximal pixel density in each DNA band, calculated using ImageJ Floyd-Steiner plot profile. A reference baseline was set at the bottom of the loading wells and kept constant throughout all the gel lanes to ensure measurement consistency and reproducibility as well as positioning of bands determined by measuring the peak of the greatest intensity at the migration axis. All readings were obtained in centimeters, to three decimal points and applied as triplicate readings in independent analysis, the mean number of values obtained was recorded and standard deviation estimated to determine the precision of the measurement (coefficient of variation < 2%). Bands where there was smearing, or inter-replicate fluctuation more than 5% were marked to be examined again.

2.8 Calculations and Data Analysis

Using the below equation, migration velocities (v) were calculated [1, 9]:

$$v = d / t$$

where d is the migration distance in cm, and t is the electrophoresis time that is 4800 seconds.

Electrophoretic mobility (μ) was governed using the following relationship [1, 9, 10]:

$$\mu = v / E$$

where E is the electric field strength that is 10.7 V/cm in this experiment as described in section 2.5 earlier.

The ratio of frictional coefficient (f) to net charge per base (q^*) was determined using [9, 10]:

$$f / q^* = N / \mu$$

N being the number of phosphate groups contained and is the same as the number of base pairs in the case of a dsDNA and twice the number of bases in the case of a ssDNA.

3. Results

3.1 Gel Image and Band Visualization

The separation of electrophoresis DNA samples in 1% agarose gel resulted in well-defined bands with obvious size dependency. In this study, there were six lanes as illustrated in Fig 1a. Lane 1 had the 1 kbp DNA marker ladder, which presented 13 distinct bands ranging between 250 bp and 10,000 bp. Lane 2 had a sharp band at 2.7 kbp showing the 250 to 10,000 bp fragment of the extracted DNA, whereas Lane 3 had a well-resolved band at 750 bp of a 5000 to 250 bp fragment of the extracted DNA. Lane 4 had the PBR322-BstNI restriction digest, which had 4 visible fragments of the sequences 1857, 1058, 929, and 383 bp as primary and indistinct secondary band respectively. Lane 5 places 5.4 kbp circular ssDNA φX174I with a primary band and an indistinct secondary band retained at the loading well. Additionally, φX174II as 5.4 kbp linear ds DNA appeared as a diffuse smeared spot above the 10,000 bp length marker in Lane 6.

Ethidium bromide-DNA complexes were visualized under UV light (312 nm) on a dark background with bright orange-red fluorescence that was used to visualize and further quantify the complexes by ImageJ software [17,21]. There were sharp and well-resolved DNA fragments of 60 to 1000bp size, which could be used to make accurate distance measurements. The φX174I bigger size samples exhibited band broadening, whereas φX174II linear dsDNA form presented strong smears which is indicative of heterogeneity of the sample or degraded samples.

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

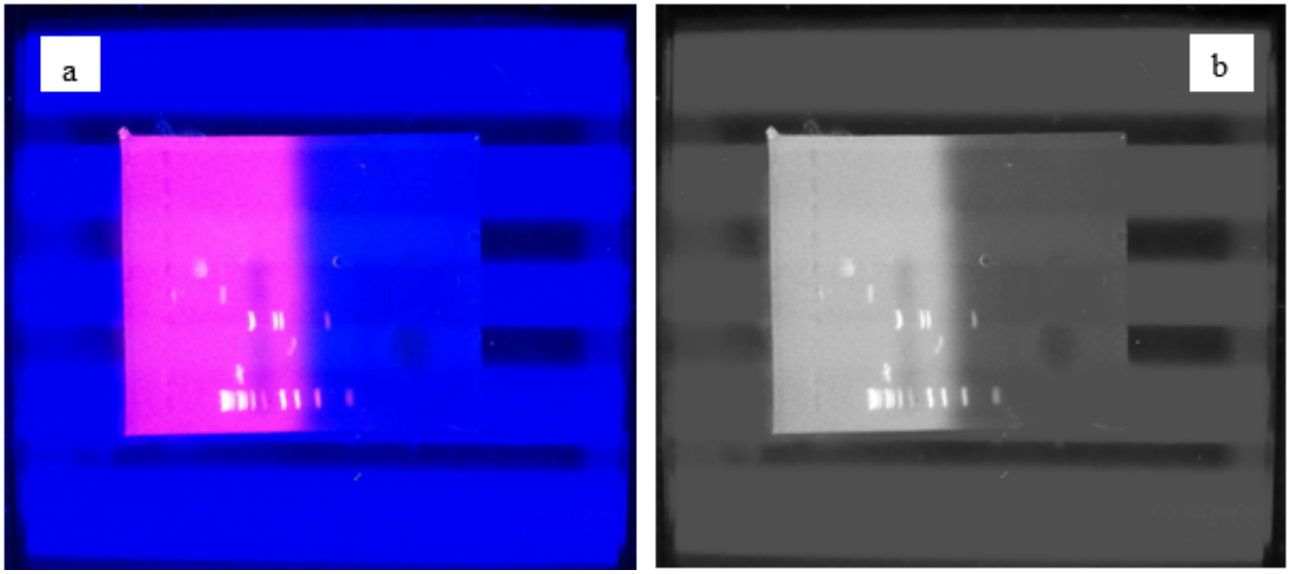

Figure 1. Figure 1. Electrophoresis of agarose gel DNA samples. Lane 1: 1 kbp DNA marker (13 fragments between 250-10,000 bp); Lane 2: 2.7 kbp fragment of dsDNA; Lane 3: 750 bp fragment of dsDNA; Lane 4: 4.4 kbp PBR322-BstNI digest (4 fragments) of dsDNA; Lane 5: 5.4 kbp ssDNA fragment of φX174I; Lane 6: 5.4 kbp Linear dsDNA fragment of φX174II Gel: 1% agarose in 1x TBE buffer; Electrophoresis: 90V, 80 minutes; Staining: Ethidium bromide; Visualization: UV transillumination, 312 nm. Figure 1a is an Agarose gel electrophoresis of DNA bands in the presence of ethidium bromide. The image in figure 1b is the processed image with ImageJ that is utilized in the quantitative distance measurements.

3.2 Migration Distance and Velocity Analysis for 1 kbp DNA Ladder

The quantitative measurement of the 1 kbp DNA ladder showed a definite negative correlation between fragment size and the distance of its migration. Table 1 provides complete information on all 13 ladder bands, with the length of migration varying between 1.289 cm (10,000 bp fragment) and 4.256 cm (250 bp fragment). Migration velocities of the biggest fragment were 2.69x10-4 cm/sec and the smallest fragment was $8.87x10^{-4}$ cm/sec, showing that smaller DNA fragments move about 3.3-fold faster than bigger ones with the same electrophoretic conditions. Similar trends of electrophoretic mobility were observed, μ increasing between $0.25x10^{-4}$ cm^2/V.s with the 10,000 bp fragment and $0.829x10^{-4}$ cm^2/V.s with the 250 bp fragment. This increase in mobility of smaller fragments by approximately 3.3 times is an indication of the lower frictional resistance at the time of migration through the gel matrix of agarose [9,12].

Fragment Size (bp)	Distance d (cm)	Velocity v (×10⁻⁴ cm/sec)	Mobility μ (×10⁻⁴ cm²/V·s)	f/q* (×10⁷ V·s/cm²)	v vs Size Trend
10000	1.289	2.69	0.25	40	Slowest
8000	1.385	2.89	0.27	29.6	↓
6000	1.44	3	0.28	21.4	↓
5000	1.445	3.01	0.281	17.8	↓
4000	1.509	3.144	0.294	13.6	↓
3000	1.683	3.506	0.328	9.15	↓
2500	1.807	3.765	0.352	7.1	↓
2000	1.972	4.11	0.384	5.21	↓
1500	2.238	4.663	0.43	3.49	↓
1000	2.697	5.62	0.525	1.9	↓
750	3.013	6.28	0.587	1.28	↓
500	3.499	7.29	0.68	0.735	↓
250	4.256	8.87	0.829	0.302	Fastest

Table 1. **Table 1. Migration parameters for 1 kbp DNA ladder fragments**

It was found that the frictional coefficient of analysis to charge ratio (*f*/*q**) increased numbers between $40.0x10^7$ V.s/ cm^2 of 10,000 bp and $0.302x10^7$ V.s/ cm^2 with 250bps, which means that larger DNA molecules are proportionately affected by higher frictional force in migration. The exponential decrease in the velocity when increasing the size of the fragmentations agree with theory calculations of DNA in migration porous gel matrices [12,14,15].

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

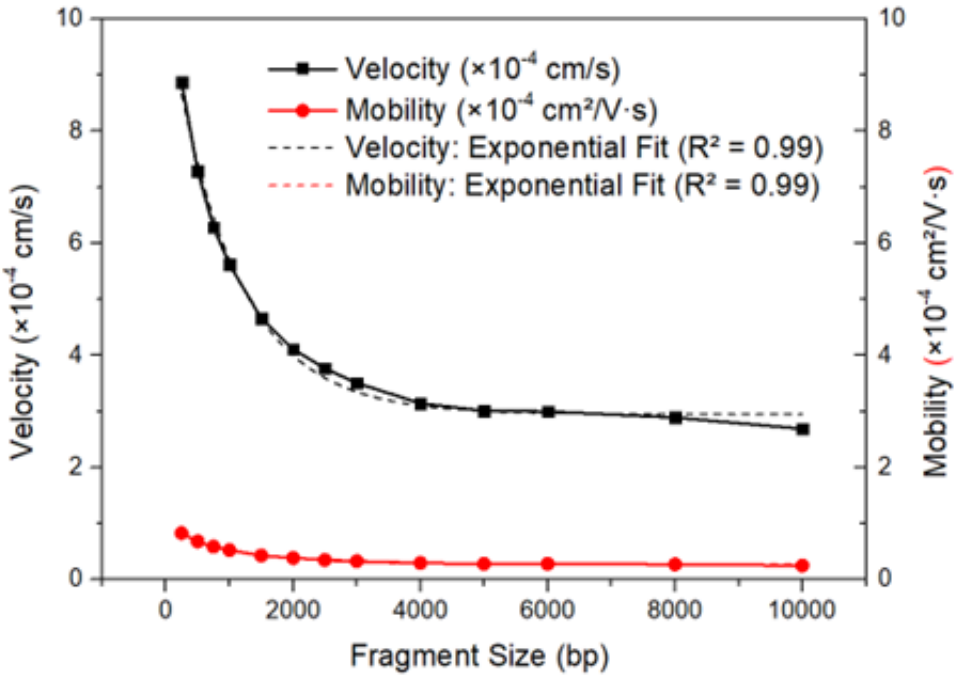

Figure 2. Figure 2: Correlation of DNA fragment size and migration characteristics. Electrophoretic mobility (red circles, right y-axis) and migration velocity (black squares, left y-axis) of DNA ladder fragments of 1 kbp (250-10,000 bp) as a function of DNA fragment size. The two parameters are both inversely exponentially with respect to the fragment size (dashed lines, R^2 = 0.99). The data is the means of repeated measurments when the standard deviations are below 2%.

3.3 Analysis of Known DNA Samples

A 2.7 kbp fragment of the dsDNA moved to the range between 2500 to 3000 bp ladder bands, which validates its molecular weight. Quantitative analysis provided a distance of migration of 1.651 cm and a velocity of $3.44x10^{-4}$ cm/sec and a mobility in the electrophoresis of $0.32x10^{-4}$ cm^2/V.s. Fractional coefficient to charge was determined as $16.875x10^7$ V.s/cm^2 using 5400 phosphate groups in the doubled strand structure. Our gel separation system was confirmed to be right through this correspondence with the marker on the ladder.

The 750 bp segment of the dsDNA migrated perfectly with the 750 bp band on the corresponding liquid ladder, indicating a high level of system reproducibility. The fragment moved 2.912 cm along the gel at a velocity of $6.067x10^{-4}$ cm/sec with a mobility of $0.567x\ 10^{-4}$ cm^2/ V.s. The f/q^* value of $2.646x10^7$ V.s/cm^2 calculated was much lower than the frictional resistance of larger fragments.

PBR322 plasmid that was subjected to restriction digestion using BstNI enzyme was successful in producing four different fragments that had been determined effectively through electrophoresis. The 1857 bp fragment moved between the 1500 and the 2000 bp positions. The two mid-range fragments of 1058 bp and 929 bp were located on either side of the marker of the 1000 bp gel with the first fragment having slightly larger size and the later fragment having slightly smaller size, indicating that this gel was capable of determining fragments with their differences of only 129 bp. The shortest piece that was observed at 383 bp moved quickly to the lower gel position between the 250 and 500 bp markers. In this case, the theoretical fifth fragment of 121 bp was not observed, which could be due to insufficient concentration of the DNA, or not recognizing the fragment because it migrated beyond the visible gel edge.

3.4 Structural Conformation Effects: Circular vs. Linear DNA

The bacteriophage φX174I circular ssDNA exhibited specific behaviors in the electrophoresis which was categorized into 1.280 cm along which it moved. The corresponding velocity was $2.667x10^{-4}$ cm/sec and the electrophoretic mobility was $0.249x10^{-4}$ cm^2/V.s. Since N in this ssDNA molecule is 5400, the ratio of frictional coefficient to charge (f/q^*) was computed as $21.69x10^7$ V. s/cm^2. The main band was transferred to the range of 5000 bp-6000 bp reference band markers on the DNA ladder, which was in accordance with the anticipated molecular weight of the band. Interestingly, there was an indistinct second faint band at the well position indicating that there was a supercoiled or aggregated version of DNA forms, which could not penetrate the gel-matrix. This effect is usually exhibited by circular DNA molecules that can take on many conformations states as a result of the level of supercoiling and conditions of the solution at the time of electrophoresis [11].

The linear structure of the bacteriophage φX174II showed a significantly different electrophoretic pattern compared to its circular single-stranded counterpart. This particular sample moved a distance of 0.832 cm out of the loading well which was equivalent to a velocity of $1.733x10^{-4}$ cm/sec and an electrophoretic mobility significantly less than $0.162x10^{-4}$ cm^2/V.s. When

Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13932

N was10800 for this particular dsDNA molecule, the calculated ratio of frictional coefficient-to-charge ranged at $66.67x10^7$ V .s/cm^2 which was the greatest resistance to migration in all samples. Instead of giving out a band, this sample emerged as a smeared area located over the 10,000 bp mark at the top of a ladder. The smearing effect must have been due to degradation of the samples with time causing a partial denaturation or separation of strands or development of heterogeneous conformational states of the molecules. A sharp contrast of the two φX174 forms was also quite instructive in that distinctly, although both forms had the same molecular weights of 5.4 kbp, the linear dsDNA moved at a much slower rate in comparison with the circular ssDNA. This disparity highlights the dramatic evidence on the importance of DNA topology and secondary structure on electrophoretic motility. The ratio *f/q**of the linear dsDNA was quantitatively 3.1 times higher than that of the circular ssDNA ($66.67x10^7$ vs. $21.69x10^7$ V.s/cm^2), indicating the significantly greater actual resistance that the rigid, rod-like double helical structure met on its way through the gel matrix in comparison to the smaller frictional resistance to the structure of the circular single-stranded structure [11].

4. Discussion

This paper shows an exponential dependence between the DNA fragment size and the migration velocity of DNA fragment in 1% agarose gel electrophoresis, which match the proven theory of electrophoresis [1,9,12,28]. Velocity increased to $8.87x10^{-4}$ cm/sec on 250 bp fragments to $2.69x10^{-4}$ cm/sec on 10,000 bp fragments, which is about 70% slower than the 250 bp fragments. The fact that this separation is size-dependent is revealing two different theoretical action mechanisms that are dependent on the size of the fragment [14,15,16]. Smaller fragments of DNA, which are less than or equal to 2000 bp, can pass through the gel according to the Ogston sieving model with minimal distortion in passing through the pore of the gel. In this model, the velocity of a fragment is determined by the ratio of molecular radius to pore size [14]. The larger fragments, which are greater than 2000 bp, use reptation forming a lengthy snake-like structure and pass all the way through the gel matrix as the end-first run targeting [15,16]. In our study, it was observed that some forms of transition between these mechanisms took place around 2000-3000 bp and this is indicated by some minor shifts in the slope of the velocity-size relationship.

The values of electrophoretic mobility ($0.25\text{-}0.829x10^{-4}$ cm^2/V.s) are within the ranges of expected values in 1% agarose gels [9,10,12]. Mobility increased by about 3.3-fold between the largest (10,000 bp) and the smallest (250 bp) DNA fragments, which indicated the reduced frictional resistance of smaller DNA molecules. The f/q, ratio increased exponentially from $0.302x10^7$ and $40.0x10^7$ V.s/cm^2. This result shows that the resistance due to friction increases in greater rate compared to charge as the size of the fragment increases. This non-linear behavior is due to the fact that the more DNA gets attached to the agarose net, the more energy is required to make it through the gel pores [13,16].

The circular ssDNA and linear dsDNA φX174 forms are compared and have been found that strong effects of the DNA topology on the electrophoretic characteristics are noticed [11]. They separated completely differently in spite of the same molecular weights (5.4 kbp) although the linear dsDNA presented with *f/q** ratio that was 3.1-fold greater ($66.67x10^7$ vs. $21.69x10^7$ V.s/cm^2) as well as deep smearing of their bands. The circular ssDNA, with its short and flexible diameter and intramolecular base-pairing, passes through the pores of the gel more easily than the rigid and rod-like double-helical form dsDNA because the surface experiences fewer frictions [11]. The reported smearing could be explained by the degradation of the sample or non-homogeneous conformations. This is in fact significantly highlights the DNA sample quality importance in the electrophoretic analysis [8,12].

A good agreement between fragment sizes and their migration positions compared with a 1 kbp size marker confirms ImageJ quantitative analysis results and our experimental procedure [4, 21]. The sample at 2.7 kbp was positioned between 2500-3000 bp markers, the sample at 750 bp co-migrated with the marker at 750 bp, and the PBR322-BstNI fragments were positioned exactly with the expected size. The one deleted 121 bp fragment shows how the standard agarose gels are weak with very small fragments as they either move too fast or are too diffusive to be visualized [6,8]. Such fragments would be better resolved on higher percentage agarose, such as 2-3%, or polyacrylamide gel [8].

ImageJ digital image analysis is a vast improvement of visual estimation, allowing both the precise calculation of distance and repetitive results [21,22]. The recent gel documentation and additional high-end software has revolutionized electrophoresis into more of a quantitative than qualitative procedure [22]. Constant voltage mode (90V) ensured that the same amount of electric field was applied consistently [4], whereas application of 1x TBE buffer maintained constant pH and ionic strength required in the reproducible migration [27]. Newer solutions, such as SYBR Green and GelRed, are sensitive with reduced potentiation of mutagenicity though ethidium bromide is still valuable despite the safety concerns [19,20].

The quantitative relationships in this study have practical usages in molecular biology, such as verification of PCR products, restriction site analysis, quality control of DNA products, purification of a fragment to be cloned, and diagnosis of genetic diseases [2,23,24]. Our reference data may be used to estimate the unknown fragment sizes when using similar electrophoretic conditions. Although the latest technological innovations such as automated documentation systems and capillary electrophoresis have higher capacities [22,26], slab gel electrophoresis is the backbone method due to its simplicity, cost effectiveness, and flexibility [2]. A hybrid of conventional gel electrophoresis and the current image analysis would be the best combination of the ease of use and the analysis capabilities required in routine DNA analysis.

5. Conclusion

This study offers detailed quantitative experiment on DNA fragment separation using agarose gel electrophoresis to come up with definite associations between molecular weight, migration speed, and electrophoretic mobility. The 1 kbp DNA

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

fragments were analyzed by giving an inverse exponential relationship with velocities of $8.87x10^{-4}$ to $2.69x10^{-4}$ cm/sec and mobilities of $0.25x10^{-4}$ to $0.829x10^{-4}$ $cm^2/V.s$. The frictional coefficient to charge ratio expanded exponentially with the fragment size, which agreed with theoretical expectations of DNA-gel matrix interactions [1,28].

The comparative analysis of DNA φX174 showed that structural conformation greatly determines the migration behavior. Topographical considerations of DNA induced by changes in DNA topology were confirmed through the trials conducted on circular single-stranded and linear double-stranded DNA forms with significantly different electrophoretic properties (f/q^*equals to $21.69x10^7$ $V.s/cm^2$) regardless of their molecular weights (5.4 kbp). The excellent arrangement of known fragment sizes with the observed migration positions confirms the experimental procedure and the quantitative analysis using ImageJ.

The quantitative correlations determined in this case can serve as important reference data to estimate the DNA size using modern technologies in molecular biology and evidences the efficiency of gel electrophoresis (traditional techniques) with the latest digital image analysis (modern digital technologies).

Acknowledgments

The author gratefully acknowledges Nathan Walsh, Laboratory Manager, for providing experimental protocols, guidance with laboratory procedures, and technical assistance. Special thanks to Professor Jiali Li, Department of Physics, University of Arkansas, for supervision, access to laboratory facilities, and valuable discussions. This work was conducted as part of the introduction to biophysics and biophysical techniques course at the University of Arkansas.

References

1. P. Y. Lee, J. Costumbrado, C. Y. Hsu, and Y. H. Kim, “Agarose Gel Electrophoresis for the Separation of DNA Fragments,” Journal of Visualized Experiments, no. 62, p. e3923, 2012, doi: 10.3791/3923.
2. M. R. Green and J. Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed. Cold Spring Harbor Laboratory Press, 2012.
3. J. Sambrook and D. W. Russell, Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, 2001.
4. J. Sowersby and T. W. Lewis, “A Sustainable, University Repository Method for Agarose Gel Electrophoresis Reduces Plastic Waste,” Biochemistry and Molecular Biology Education, vol. 52, no. 1, pp. 72–79, 2024, doi: 10.1002/bmb.21791.
5. R. Westermeier, Electrophoresis in Practice: A Guide to Methods and Applications of DNA and Protein Separations, 5th ed. Wiley-VCH, 2016.
6. F. Sarkozy and A. Guttman, “Agarose Gel Electrophoresis,” in Electrophoretic Separation of Proteins: Methods and Protocols, Springer, 2024, pp. 135–147.
7. S. Arnott et al., “The Agarose Double Helix and Its Function in Agarose Gel Structure,” Journal of Molecular Biology, vol. 90, no. 2, pp. 269–284, 1974, doi: 10.1016/0022-2836(74)90372-6.
8. R. B. Helling, H. M. Goodman, and H. W. Boyer, “Analysis of Endonuclease R EcoRI Fragments of DNA from Lambdoid Bacteriophages and Other Viruses by Agarose-Gel Electrophoresis,” Journal of Virology, vol. 14, no. 5, pp. 1235–1244, 1974.
9. N. C. Stellwagen, C. Gelfi, and P. G. Righetti, “The Free Solution Mobility of DNA,” Biopolymers, vol. 42, no. 6, pp. 687–703, 1997.
10. N. C. Stellwagen, “Electrophoresis of DNA in Agarose and Polyacrylamide Gels,” in DNA Sequencing II: Optimizing Preparation and Cleanup, Jones & Bartlett Learning, 2006, pp. 1–21.
11. N. C. Stellwagen, “Electrophoresis of DNA in Agarose Gels, Polyacrylamide Gels and in Free Solution,” Electrophoresis, vol. 30, no. S1, pp. S188–S195, 2009, doi: 10.1002/elps.200900052.
12. X. Wang et al., “Characterization of the Broad-Range Size Separation of DNA in Agarose Gel Electrophoresis,” Electrophoresis, vol. 41, no. 7–8, pp. 579–586, 2020, doi: 10.1002/elps.201900413.
13. N. C. Stellwagen, “DNA Gel Electrophoresis,” in Encyclopedia of Analytical Chemistry, Wiley, 2018, doi: 10.1002/9780470027318.a1403.pub3.
14. A. G. Ogston, “The Spaces in a Uniform Random Suspension of Fibres,” Transactions of the Faraday Society, vol. 54, pp. 1754–1757, 1958.
15. O. J. Lumpkin, P. Dejardin, and B. H. Zimm, “Theory of Gel Electrophoresis of DNA,” Biopolymers, vol. 24, no. 8, pp. 1573–1593, 1985, doi: 10.1002/bip.360240812.
16. N. C. Stellwagen, “Anomalous Electrophoresis of dsDNA in Agarose Gels,” Electrophoresis, vol. 43, no. 17–18, pp. 1795–1810, 2022, doi: 10.1002/elps.202200072.
17. T. Bawane et al., “Comprehensive Assessment of DNA Fluorescent Dyes for Gel-Based Applications: Safety, Sensitivity, and Environmental Impact,” Analytical Biochemistry, vol. 687, p. 115441, 2024, doi: 10.1016/j.ab.2024.115441.
18. P. A. Sharp, B. Sugden, and J. Sambrook, “Detection of Two Restriction Endonuclease Activities in Haemophilus Parainfluenzae Using Analytical Agarose-Ethidium Bromide Electrophoresis,” Biochemistry, vol. 12, no. 16, pp. 3055–3063, 1973.
19. Y. X. Leong et al., “Plasmon-Enhanced Nucleic Acid Sensing with Gold Nanoparticles as a Safer Alternative to Ethidium Bromide for Gel Electrophoresis Visualization,” Analytical Chemistry, vol. 95, no. 14, pp. 6041–6048, 2023, doi: 10.1021/acs.analchem.2c05635.
20. Q. Huang and W. L. Fu, “Comparative Analysis of the DNA Staining Efficiencies of Different Fluorescent Dyes in

Preparative Agarose Gel Electrophoresis," Clinical Chemistry and Laboratory Medicine, vol. 43, no. 8, pp. 841–842, 2005, doi: 10.1515/CCLM.2005.141.
21. C. A. Schneider, W. S. Rasband, and K. W. Eliceiri, "NIH Image to ImageJ: 25 Years of Image Analysis," Nature Methods, vol. 9, no. 7, pp. 671–675, 2012, doi: 10.1038/nmeth.2089.
22. S. Pandey et al., "AutoGelAnalysis: An Automated Tool for Analysis of Gel Electrophoresis Images," Electrophoresis, vol. 45, no. 3–4, pp. 335–343, 2024, doi: 10.1002/elps.202300166.
23. L. Koontz, "Agarose Gel Electrophoresis," in Laboratory Methods in Enzymology: DNA, Elsevier, 2013, pp. 23–34.
24. G. W. Slater et al., "Theoretical and Experimental Studies of DNA Sequencing Gels," in Analysis of Nucleic Acids by Capillary Electrophoresis, Vieweg+Teubner Verlag, 1997, pp. 81–101.
25. S. J. Younger et al., "Forensic Quantification and Classification of Linearity for PCR Amplicons via Multiplexed Capillary Gel Electrophoresis," Electrophoresis, vol. 44, no. 11–12, pp. 1015–1025, 2023, doi: 10.1002/elps.202200261.
26. R. Kalendar et al., "DNA Extraction, Purification, and PAGE-Resolved High-Salt Electroelution for Next-Generation Sequencing," Methods in Molecular Biology, vol. 2672, pp. 57–72, 2023, doi: 10.1007/978-1-0716-3226-0_3.
27. J. R. Brody and S. E. Kern, "History and Principles of Conductive Media for Standard DNA Electrophoresis," Analytical Biochemistry, vol. 333, no. 1, pp. 1–13, 2004, doi: 10.1016/j.ab.2004.05.054.
28. E. M. Southern, "Measurement of DNA Length by Gel Electrophoresis," Analytical Biochemistry, vol. 100, no. 2, pp. 319–323, 1979, doi: 10.1016/0003-2697(79)90235-5.

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo