https://doi.org/10.70882/qsprkc76

SCIENCE FORUM (JOURNAL OF PURE AND APPLIED SCIENCES) 26, (4) (2026), 756 - 769

Science Forum (Journal of Pure and Applied Sciences)

Journal Homepage: https://atbuscienceforum.com.ng/index.php/jpas

Agarose Gel Electrophoresis of DNA: Quantitative Biophysical Study of Mobility and Size Dependence

Zeina Salman

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

Abstract

Separation of complex mixtures of biomolecules via agarose gel electrophoresis is a cornerstone molecular technique. Yet quantitative data on electrophoretic parameters, including migration velocity and mobility across a wide range of DNA fragment sizes and structures, remain scarce in the primary literature. In this work, we present detailed biophysical analysis of DNA fragment migration in 1% agarose gel under defined experimental parameters (90 V, 80 minutes, 1× TBE buffer). As a size standard, 1 kbp DNA ladder was used to compute migration distances from UV-illuminated gel images using ImageJ software, and to determine electrophoretic velocities and mobilities for 13 fragments ranging from 250 to 10,000 bp within the ladder. Three samples of double-stranded DNA that included 750 bp, 2.7 kbp, 4.4 kbp (pBR322-BstNI digest) were examined. Also, circular single-strand and linear double-strand DNA of the bacteriophage φX174 DNA were examined. The migration velocity was inversely exponential with size over the 250-10,000 bp size range (R^2 = 0.99), decreasing from 8.87 x 10^{-4} to 2.69 x 10^{-4} cm/sec. The range for electrophoretic mobility (μ) was from 0.25 × 10^{-4} to 0.829 × 10^{-4} $cm^2/V{\cdot}s$. Circular ssDNA φX174 with μ of 0.249 × 10^{-4} $cm^2/V{\cdot}s$ migrated significantly faster than the linear dsDNA form with μ of 0.162 × 10^{-4} $cm^2/V{\cdot}s$, even though the molecular weight of both is identical. This result reveals the strong influence of DNA structure on migration. These data agree with established electrokinetic theory and provide calibration references for DNA sizing in molecular biology and biophysics laboratories.

Keywords

DNA Fragment Separation;
DNA Topology;
Electrophoretic Mobility;
ImageJ Analysis;
Migration Velocity

EISSN: 3156-5190 (Online)
ISSN: 1119-4618 (Print)
JPAS
Faculty of Science, ATBU Bauchi

Corresponding Author: Zeina Salman, *Department of Physics, College of Science, Wasit University, Al-Kut, Wasit, Iraq.*
Email: zsalman@uowasit.edu.iq

INTRODUCTION

Agarose gel electrophoresis of nucleic acids is a fundamental method in contemporary molecular biology and biophysics. Agarose gel electrophoresis has offered scientists a simple yet effective method for separating DNA and RNA fragments according to size, which facilitates its use in a broad range of applications from restriction fragment mapping to confirmation of PCR products, molecular cloning, DNA fingerprinting, and medical diagnosis (Lee et al., 2012; Stellwagen, 2009). Its continued significance is not only due to its experimental simplicity, but also its broad range of applications in the separation of small DNA fragments with 50 bp to large DNA molecules with several hundred kilobase pairs (Green and Sambrook, 2012).

Agarose gel electrophoresis relies on the principles of charged macromolecular transport in a static electric field. DNA is uniformly negatively charged along its phosphate-sugar backbone where an elementary charge is contributed by a nucleotide (Brody and Kern, 2004). Under the influence of an electric field, DNA fragments move towards the positive pole at a speed determined by the balance between the electrostatic force and the frictional drag of the gel (Stellwagen et al., 1997). The size-dependent retardation gives rise to the typical band pattern observed in gel electrophoresis with smaller fragments migrating further from the original location than larger fragments over the same time period of electrophoresis (Sárközy and Guttman, 2024; Stellwagen, 2009).

DNA motion in agarose gel has been explained by two different theories depending on the size of the fragment (Lumpkin et al., 1985; Ogston, 1958). For small fragments that are below about 2,000 bp, the Ogston sieving model is applicable where the DNA molecule passes through pores in a random network of fibers with an average pore diameter larger than the molecular radius of the DNA (Ogston, 1958). For larger fragments, this model fails and reptation theory applies where the DNA molecule takes on a snake-like structure and slips through the gel matrix end-on, and its mobility is less dependent on molecular weight for very large molecules (Lumpkin et al., 1985; Stellwagen, 2018). Overlapping of these models and consequent changes in the relationship between velocity and size give insight into how DNA interacts with the gel.

The quantitative resolution of gel electrophoresis data has been greatly improved by the use of digital image analysis software, such as ImageJ, an open-source software developed by the National Institutes of Health (Schneider et al., 2012). ImageJ provides a means for accurate and repeatable determination of band migration distances, replacing subjective visual estimates and systematic extraction of mobility and velocity information from gel images (Schneider et al., 2012; Stellwagen, 2006). This conveys agarose gel electrophoresis from an inequitable to a quantitative biophysical assay.

Although agarose gel electrophoresis is widely used, few peer-reviewed publications report complete internally consistent quantitative sets of electrophoretic parameters - such as velocity, mobility, and frictional coefficient-to-charge ratios - measured

under cautiously defined experimental conditions for a wide range of DNA fragment sizes and conformations. These data sets are practically useful to researchers planning to calibrate their own gel systems or to interpret mobility data based on electrokinetic theories.

This current study contributes to this by offering a quantitative biophysical analysis of DNA fragment migration in 1% agarose gel electrophoresis. ImageJ was used to analyze the gel image and determine migration distances and electrophoretic velocities and mobilities of 13 fragments of a 1 kbp DNA ladder (250-10,000 bp), three sized dsDNA fragments (750 bp, 2.7 kbp, 4.4 kbp) and both major conformational forms of bacteriophage φX174 DNA (5.4 kbp circular ssDNA and 5.4 kbp linear dsDNA). The inclusion of both forms of φX174, which have the same molecular weight but different conformation, allows direct quantitative examination of conformation-dependent electrophoretic properties - an issue of both theoretical and practical interest.

MATERIALS AND METHODS

Experimental Setting and Biological Materials

The experiments described here were performed at the Biophysics Laboratory, University of Arkansas, under the guidance of Professor Jiali Li, and with the help of the lab's manager, Nathan Walsh. Biological and chemical materials were obtained from New England Biolabs (NEB). Biological materials used in this study involved a 1 kbp DNA ladder (NEB #N3232) for molecular weight markers; a 2.7 kbp liner dsDNA fragment; a 750 bp linear dsDNA fragment; a 4.4 kbp dsDNA preparation which consisted of pBR322 plasmid digested with restriction endonuclease BstNI to produce four defined fragments. Those were a 5.4 kbp circular ssDNA from bacteriophage φX174 (hereafter referred to as φX174 ssDNA), and 5.4 kbp linear dsDNA from bacteriophage φX174 (hereafter referred to as φX174 dsDNA). Chemicals used in the experiments were molecular-grade agarose, 10× Tris-Borate-EDTA (TBE) buffer, 6× DNA loading dye containing glycerol and tracking dyes, 10 mg/mL stock solution of ethidium bromide (EtBr) and TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Instrumentation

The electrophoresis apparatus was a horizontal gel tank with an 8.4 cm distance between the electrodes, a Bio-Rad PowerPac power supply set to constant-voltage mode, a gel casting tray tailored with sample combs, a UV transilluminator of 312 nm to excite the fluorescence of ethidium bromide, and a digital camera for capturing gel images. Micropipettes ranging from 2 to 20 µL with disposable sterile tips were used for liquid handling. Personal protective equipment, such as UV safety goggles and gloves, was used.

Preparation of Agarose Gel

The 1% (w/v) agarose gel was prepared as follows. Mixing 5 mL of 10× TBE buffer with 45 mL of deionized water was used to make 50 mL of 1× TBE. Agarose solution was made by dissolving 0.5 g agarose powder in 50 mL 1× TBE buffer. This was completely dissolved by heating in a microwave for about 70 seconds, occasionally swirling the liquid to ensure a clear solution. The agarose solution was subsequently cooled to around 60°C and 5 µL of EtBr stock solution was added and mixed gently to a final concentration of

0.5 µg/mL of EtBr. The gel casting tray closed with rubber gaskets was placed in the horizontal position in the electrophoresis chamber and a suitable sample comb was placed into the tray. The agarose solution with EtBr was poured into the tray avoiding the formation of bubbles and left to polymerize for 30-60 minutes at room temperature. Once the gel had solidified completely, the comb was removed creating clean undamaged wells to load the DNA samples into them.

DNA Sample Preparation

All DNA samples were mixed to a final volume of 12 µL that included 10 µL DNA in TE buffer + 2 µL 6× loading dye and the amount of DNA mass loaded was approximately 50 ng DNA per lane. The following DNAs were loaded into lanes. Lane 1 was the 1 kbp ladder with 5 µL stock + 5 µL TE; Lane 2 was 2.7 kbp dsDNA with 2.5 µL stock + 7.5 µL TE; Lane 3 was 750 bp dsDNA with 2 µL stock + 8 µL TE; Lane 4 was pBR322-BstNI digest with 2 µL stock + 8 µL TE; Lane 5 was φX174 circular ssDNA with 2 µL stock + 8 µL TE; and Lane 6 was φX174 linear dsDNA with 4 µL stock + 6 µL TE. Loading dye was added to the samples and mixed by pipetting before loading onto the gel.

Electrophoresis

Once the gel had solidified, the gel tray was rotated 90° to the left and relocated into the electrophoresis chamber with the loading wells facing the cathode. The running buffer, 1× TBE, was prepared by mixing 30 mL of 10× TBE buffer with 270 mL of deionized water and filled both of the chambers until the gel was submerged. The DNA samples were loaded into the wells using a micropipette and the electrophoresis was operated at a constant voltage of 90 V and 80 mA current limit for 80 minutes at approximately 22°C. The electric field strength, E, was 10.7 V/cm that is voltage applied between electrodes divided by the distance between electrodes, i.e., 90 V ÷ 8.4 cm. The progress of the dye markers, especially bromophenol blue representing 300 bp was observed occasionally during the run.

Gel Visualization and Imaging

After electrophoresis, the gel was transferred to a UV transilluminator at 312 nm in a protective chamber. The orange-red fluorescence of DNA-EtBr intercalation complexes under UV illumination allowed all DNA bands to be visualized against a dark background. The gel was imaged with a digital camera under controlled exposure settings to image bands spanning the complete range of DNA concentrations in the gel.

Quantitative Image Analysis

Digital gel images were loaded into ImageJ (version 1.54f; National Institutes of Health, Bethesda, MD, USA) (Schneider et al., 2012) and converted to 8-bit images to improve the resolution of measurements. The distance between the electrodes, 8.4 cm, was used as a spatial reference scale bar. The migration distance, d, for each DNA band was measured as a straight line from the base of the sample loading well to the intensity center of the band of interest, using lane-by-lane intensity profile plots created by ImageJ.

Distances were measured in centimeters to three decimal places, and each band was measured three times from separate analyses. The coefficient of variation between replicates was kept below 2%; bands with variation above 5% or that

were smeared were labeled and re-inspected. The average was used for further calculations.

Calculations

Each DNA fragment's velocity, *v*, was calculated using the following equation (Lee et al., 2012; Stellwagen et al., 1997):

$$v = d / t \quad (1)$$

where; d is the migration distance in cm and t is the total electrophoresis time, equal to 4,800 s.

To calculate the electrophoretic mobility, μ, the below equation was used (Lee et al., 2012; Stellwagen, 2006; Stellwagen et al., 1997):

$$\mu = v / E \quad (2)$$

where; E is the applied electric field strength, equal to 10.7 V/cm.

The ratio of frictional coefficient *(f)* to net charge per base *(q*)* was measured using the following relationship (Lee et al., 2012; Stellwagen, 2006; Stellwagen et al., 1997):

$$f / q^* = N / \mu \quad (3)$$

where; N denotes the total number of phosphate backbone charges, and it is equal to the same number of base pairs for ssDNA and twice the number of base pairs for dsDNA.

RESULTS

Gel Image Quality and Band Resolution

Migration of the six DNA samples in 1% agarose gel yielded well-resolved bands in Lanes 1-4 with variations in band intensity that were amenable to band image analysis as shown in Figure 1. Lane 1 for 1 kbp DNA ladder produced 13 sharp bands in the 250 to 10000 bp range. Lanes 2 and 3 contained single clear bands for the 2.7 kbp and 750 bp dsDNA samples, respectively. Lane 4 displayed four distinct bands from the restriction digest of pBR322 with BstNI at 1857, 1058, 929, and 383 bp.

The fifth expected band of 121 bp was not observed, either because the concentration of the band was below the detection limit for staining, or it migrated beyond the end of the gel. Lane 5 for φX174 circular ssDNA displayed a major band migrating between 5000 to 6000 bp on the ladder with a minor band remaining near the sample well, suggesting the presence of supercoiled or aggregated molecules. Lane 6 for φX174 linear dsDNA resulted in a smear with migration above the 10000 bp ladder band, suggesting heterogeneity that may be due to partial denaturation or degradation (Figure 1).

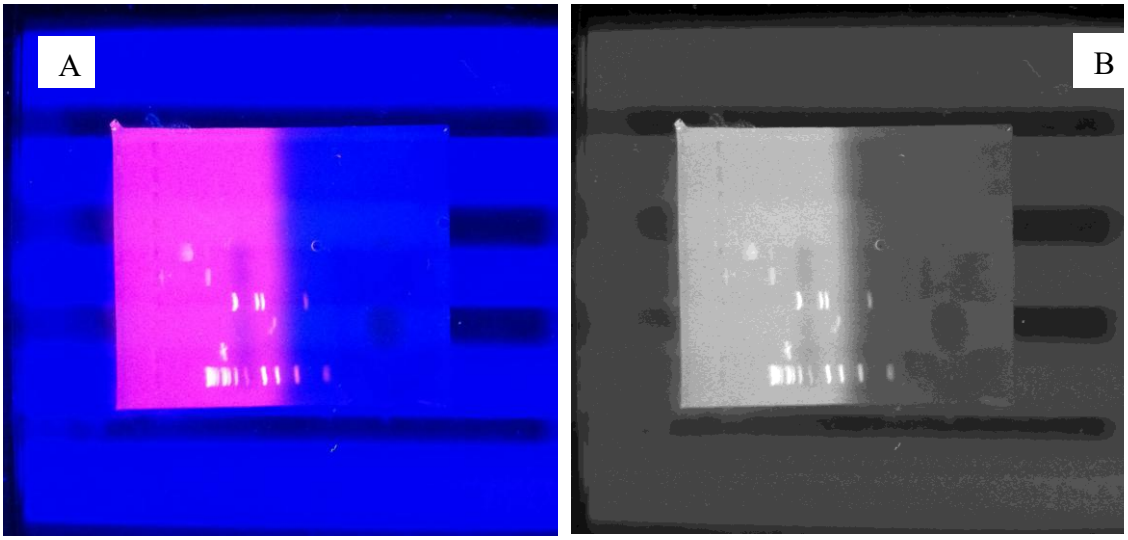

Figure 1. Agarose gel electrophoresis of DNA samples. (A) UV-illuminated gel image showing ethidium bromide-stained DNA bands. (B) ImageJ-processed grayscale image used for quantitative distance measurements. Lane 1: 1 kbp DNA ladder (13 bands, 250–10,000 bp); Lane 2: 2.7 kbp dsDNA; Lane 3: 750 bp dsDNA; Lane 4: pBR322-BstNI digest (4 fragments); Lane 5: φX174 circular ssDNA; Lane 6: φX174 linear dsDNA. Conditions: 1% agarose gel, 1× TBE buffer, 90 V, 80 minutes, EtBr staining, UV transillumination at 312 nm.

Migration Parameters of the 1 kbp DNA Ladder

Image processing of the 1 kbp ladder produced a linear negative correlation between the size of the fragments and the distance migrated with measurements ranging from 1.289 cm with 10,000 bp to 4.256 cm with 250 bp. The corresponding migration velocities ranged from 2.69 × 10^{-4} cm/sec (largest) to 8.87 × 10^{-4} cm/sec (smallest), a 3.3-fold change across the size range. Similarly, electrophoretic mobility, μ, increased from 0.250 × 10^{-4} $cm^2/V{\cdot}s$ with the 10,000 bp fragment to 0.829 × 10^{-4} $cm^2/V{\cdot}s$ with the 250 bp fragment.

The frictional coefficient-to-charge ratio *(f/q*)* also decreased with decreasing size from 40.0 × 10^7 $V{\cdot}s/cm^2$ at 10,000 bp to 0.302 × 10^7 $V{\cdot}s/cm^2$ at 250 bp, as expected from a size-dependent frictional resistance to migration in the gel matrix. All data for the 13 bands of the 1 kbp ladder are given in Table 1. Velocity and mobility decrease exponentially with increasing fragment size with R^2 equal to 0.99 as can be seen in Figure 2.

Table 1. Electrophoretic parameters for 1 kbp DNA ladder fragments.

Fragment (bp)	Distance d (cm)	Velocity v (×10⁻⁴ cm/s)	Mobility μ (×10⁻⁴ cm²/V·s)	*f/q** (×10⁷ V·s/cm²)
10,000	1.289	2.69	0.250	40.0
8,000	1.385	2.89	0.270	29.6
6,000	1.440	3.00	0.280	21.4
5,000	1.445	3.01	0.281	17.8
4,000	1.509	3.14	0.294	13.6
3,000	1.683	3.51	0.328	9.15
2,500	1.807	3.77	0.352	7.10
2,000	1.972	4.11	0.384	5.21
1,500	2.238	4.66	0.430	3.49
1,000	2.697	5.62	0.525	1.90
750	3.013	6.28	0.587	1.28
500	3.499	7.29	0.680	0.735
250	4.256	8.87	0.829	0.302

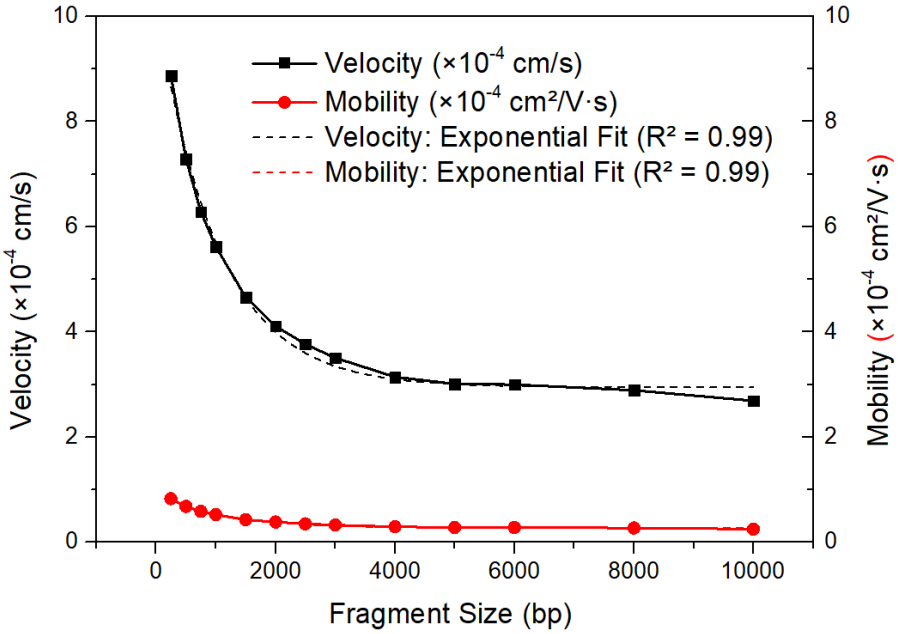

Figure 2. Migration velocity and electrophoretic mobility as functions of DNA fragment size. Migration velocity (black squares, left y-axis, ×10–4 cm/sec) and electrophoretic mobility (red circles, right y-axis, ×10–4 cm²/V·s) for 1 kbp ladder fragments (250–10,000 bp). Both parameters follow inverse exponential decay (dashed lines, R^2 = 0.99). Error bars represent standard deviation of triplicate measurements (CV < 2%).

The linear relationship between electrophoretic mobility and size of certain DNA fragments was also explored by plotting log(μ) vs. log (Fragment size) for 13 ladder fragments (Figure 3). The data were found to be linearly related when plotted with a log-log scale, with the scaling exponent, or slope, of −0.345 ± 0.015 and an adjusted R^2 of 0.979, meaning the mobility was found to be power law dependent on the fragment size. Larger fragments (≥ 5,000 bp) exhibit some deviation from a linear fit, and there is an expectation for a gradual trend toward the reptation migration-dominated regime at higher molecular weights.

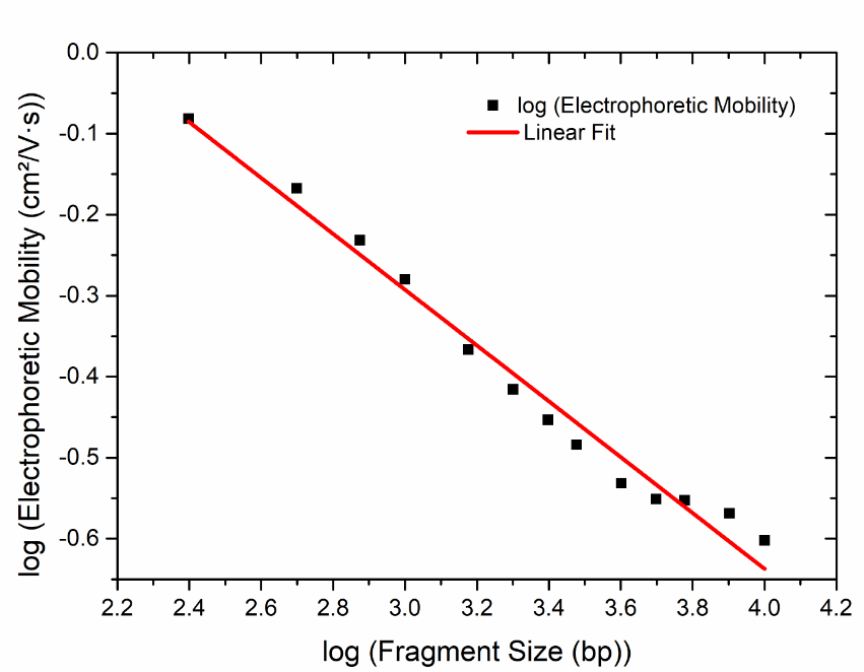

Figure 3. Electrophoretic mobility versus DNA fragment size on a log-log plot for the 13 fragments of the DNA ladder, ranging from 250 to 10,000 bp. A linear fit to the data is represented by the solid red line, which gives a scaling exponent of −0.345 ± 0.015 and an adjusted R^2 of 0.979. Small variations from linearity (for fragments ≥ 5,000 bp) are in line with the transition towards reptation-dominated migration.

The applicability of the Ogston sieving model to the experimental mobility data was examined by plotting log(μ/μ₀) vs. the radius of gyration for each of the 13 ladder fragments (Figure 4), in which μ₀ is the free solution mobility of the DNA fragments with $\mu_0 = 4.2 \times 10^{-4}$ $cm^2/V{\cdot}s$ (Stellwagen et al., 1997).

The data exhibited a good linear relationship ($K = 0.00178$ $nm^{-1} \pm 5.17 \times 10^{-5}$ and adjusted $R^2 = 0.990$) with the predictions of the Ogston model. The fragments had a slight deviation from linearity when $r > 200$ nm, which corresponds to molecular sizes ≥ 5,000 bp, suggesting the start of reptation-dominated migration, i.e., separation, at larger molecular sizes.

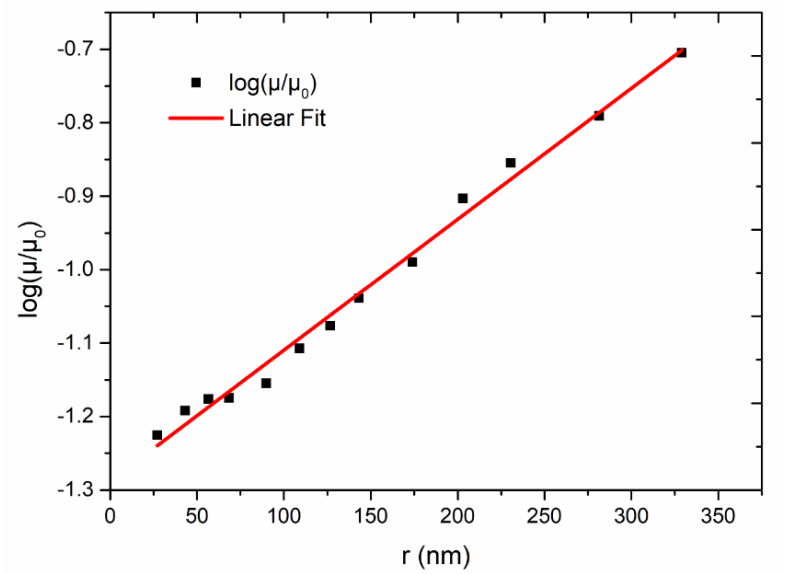

Figure 4. The standardized electrophoretic mobility log(μ/μ₀) as a function of the radius of gyration r for the 13 ladder fragments using the Ogston model fit. The free solution mobility of DNA is $\mu_0 = 4.2 \times 10^{-4}$ cm²/V·s. The linear fit is described by the solid red line, producing a retardation coefficient $K = 0.00178$ nm^{-1} ($\pm 5.17 \times 10^{-5}$) and a refined $R^2 = 0.990$. Divergence of fragments with r > 200 nm (≥ 5,000 bp) from linearity shows the initiation of reptation-dominated migration.

Migration Parameters of Individual dsDNA Samples

The 2.7 kbp dsDNA fragment moved 1.651 cm from the well with a velocity of 3.44×10^{-4} cm/sec and mobility of 0.320×10^{-4} cm²/V·s. It migrated slightly above the 2,500 bp and below 3000 bp bands of the ladder, as expected for its size. The *f/q** ratio, calculated using N equals 5,400 that is twice the base-pair count for dsDNA, was 16.875×10^7 V·s/cm².

The 750 bp dsDNA fragment co-migrated precisely with the 750 bp ladder band, producing a migration distance of 2.912 cm, a velocity of 6.067×10^{-4} cm/sec, a mobility of 0.567×10^{-4} cm²/V·s, and an *f/q** of 2.646×10^7 V·s/cm². The ability to superimpose on the marker confirms the precision of the gel system and measurement.

The digestion of pBR322 DNA with BstNI in Lane 4 yielded four bands. The 1,857 bp fragments fell between the 1,500 and 2,000 bp ladder bands; the 1,058 bp fragment was just above the 1,000 bp markers; the 929 bp band was just below this marker, indicating that the gel has a resolution to distinguish between bands that are only 129 bp apart; and the 383 bp band fell between the 250 and 500 bp ladder bands. The migration parameters for each of these four bands are shown in Table 2.

Table 2. Electrophoretic parameters for pBR322-BstNI digest fragments.

Fragment (bp)	Distance d (cm)	Velocity v ($\times 10^{-4}$ cm/s)	Mobility μ ($\times 10^{-4}$ cm²/V·s)	f/q^* ($\times 10^7$ V·s/cm²)
1857	1.935	4.03	0.377	9.85
1058	2.486	5.18	0.484	4.37
929	2.632	5.48	0.512	3.63
383	3.701	7.71	0.721	1.06

Conformation-Dependent Migration: φX174 DNA

The circular ssDNA of bacteriophage φX174 in Lane 5 migrated 1.280 cm from the sample well with a travel speed of 2.667×10^{-4} cm/sec and a mobility of 0.249×10^{-4} cm²/V·s. With N equals 5,400 with ssDNA, the f/q^* ratio was 21.69×10^7 V·s/cm². The major band was located between the 5,000 and 6,000 bp bands of the ladder, corresponding to its expected molecular weight. We also noticed a minor band at the well origin due to a proportion of highly supercoiled or associated molecular structures that were unable to penetrate into the gel.

Completely opposite to the ssDNA form of φX174, the linear dsDNA form in Lane 6 only migrated 0.832 cm with a velocity of 1.733×10^{-4} cm/sec and a mobility of 0.162×10^{-4} cm²/V·s, being the smallest values of all the samples. The f/q^* ratio at 66.67×10^7 V·s/cm² was 3.1-fold greater for N equals 10,800 dsDNA compared to that observed for the circular form of the ssDNA genophile type. This sample was not detected as a single sharp band, but as a smear above the 10,000 bp size ladder, reflecting possible sample degradation or a high degree of molecular variability.

The side-by-side analysis of these two forms of φX174 - equivalent in molecular weight that is 5.4 kbp but not in strand architecture or topology - emphasizes that DNA structure can have a significant, measurable impact on mobility. The rod-like structure of the linear dsDNA conformer leads to higher friction coefficients in the agarose matrix, compared to the circular ssDNA with 2.5-fold difference in mobility in agarose, despite equal molecular weight.

DISCUSSION

The size-velocity and -mobility profiles presented here for the 1 kbp ladder follow the expected theoretical models of the influence of fragment size on DNA migration in agarose gels. The exponential decrease in migration velocity with increasing molecular weight with R^2 equal to 0.99 in the 250-10,000 bp range suggests a shift from one to another mode of migration (Lumpkin et al., 1985; Ogston, 1958). The lower part of this range, less than 2,000 bp, is expected to migrate by Ogston sieving with the gel acting as a molecular sieve, and mobility inversely proportional to the radius of the molecule (Ogston, 1958). Larger fragments, larger than 2,000 bp, are likely to migrate by reptation where the DNA fragment adopts a stretched linear configuration

and moves through the gel end-on with a concomitant lessening of the decline in velocity with size (Lumpkin et al., 1985; Stellwagen, 2018). This slight change in slope of the velocity curve occurring at 2,000-3,000 bp is indicative of such a transition in this region.

The power-law scaling exponent (–0.345) derived from the log-log analysis of the data provides an additional mechanistic picture of the migration of DNA in 1% agarose gel. This value is in the theoretically predicted range of –0.3 to –0.5 obtained for DNA fragments which experience a transition between the Ogston sieving and reptation regimes (Lumpkin et al., 1985; Ogston, 1958). The exponent of –0.345 that was observed and the slight deviation of the fragments size above 5,000 bp suggest an interpretation that supports both regimes—large size and small size—in these fragments ranging from 250 bp to 10,000 bp.

The best fit yielded a retardation coefficient K of 0.00178 nm^{-1}, quantifying the resistance of the gel matrix to which the DNA fragments are subject during electrophoresis. This is a measure of the effective pore structure of the 1% agarose gel used under the experimental conditions. The R^2 value of 0.990 again verifies that the Ogston model is suitable to cover the size range tested, which is very good as sieving becomes the main mechanism for DNA fragments to migrate across the gels for smaller fragments. The divergence of the larger size of the fragments from the linear fit at r>200nm is also consistent with the transition to reptation-dominated migration, which is theoretically predicted for DNA in agarose gel (Lumpkin et al., 1985; Ogston, 1958) and confirmed by the previously mentioned log-log scaling analysis.

The ratio of the friction coefficient to charge (f/q^*) presents a convenient measure of the size-dependent retardation. Its exponential increase with fragment size, which is over two orders of magnitude in the 250-10,000 bp size range, reflects the increasing importance in dominating over the electrostatic driving force of the gel matrix interactions with the larger effective molecular sizes. This is consistent with analyses of DNA in porous media (Stellwagen, 2022; Wang et al., 2020).

Close agreement between observed positions of the 2.7 kbp and 750 bp dsDNAs and the bands based on the marker ladders to which they correspond offers further confirmation of the reproducibility of the system. The separation of the four pBR322-BstNI fragments including the near-comigrating 1,058 and 929 bp pair, which are only a 129 bp difference apart, shows that the DNA sizing methods employed here are also highly accurate and would be required for applications of restriction analysis.

The comparison of interest in this physical analysis is between the two forms of φX174 DNA. The fact that the circular ssDNA form has a greater mobility than the linear dsDNA, as does the linear form of pBR322, clearly demonstrates the impact of molecular topology on gel mobility (Helling et al., 1974; Stellwagen, 2006). The comparatively stiff double-helix structure of the linear dsDNA has a larger hydrodynamic cross-section as well as greater anisotropy that interacting with the matrix more significantly than the relatively floppy structure of the

circular ssDNA (Helling et al., 1974; Stellwagen, 2006). This 3.1-fold difference in f/q^* between these two forms, which are 66.67×10^7 and 21.69×10^7 V·s/cm², respectively, quantifies this effect.

The broad distribution of the linear dsDNA form above the 10,000 bp marker is likely due to sample degradation, strand denaturation or conformational variability as a result of sample's storage history. This finding is a reminder of the need for high integrity DNA samples in generating meaningful electrophoretic patterns.

The quantitative analysis using ImageJ software was efficient and replication was excellent where the coefficient of variation for replicate measurements less than 2%. The adoption of user-friendly, open-source software also adds to the ease and replicability of the approach in resource constrained laboratories (Schneider et al., 2012).

A single agarose concentration (1%) was used in this study, and the mobility values reported should not be extrapolated to other agarose gel concentrations widely used in molecular biology laboratories. Degradation of the sample produced band-smearing instead of a discrete band and compromised the accurate measurement of electrophoretic mobility, thus limiting the accurate quantitation of migration parameters of the linear dsDNA form of φX174.

Temperature was not explicitly controlled throughout the electrophoresis as only an approximation of 22°C was made; it is known that DNA electrophoretic behavior is temperature-sensitive, which could lead to slight shifts in the mobility of the DNA in the experiment. Finally, ImageJ-migration distance measurements were based on manual identification of the bands, adding some subjectivity and measurement uncertainty, although these were performed in triplicate, with a coefficient of variation < 2%.

CONCLUSION AND RECOMMENDATION

Our approach offers a comprehensive quantitative biophysical analysis of DNA fragments in 1% agarose gel electrophoresis. The dependence of flow velocity and electrophoretic mobility on the 40-fold range of fragment size ranging from 250 to 10,000 bp was determined with high accuracy and agrees with the expected size-dependent retardation behavior in agarose gel. Comparison of the different conformational states of the φX174 DNA demonstrated the significant influence of DNA structure on its electrophoretic migration which is independent of fragment size. The approach, using widely accessible laboratory facilities and open-source image processing, is readily reproducible, offering insight that can be used in numerous molecular biology and biophysical applications.

DATA AVAILABILITY

This study and its tables report all the data on which it is based. No additional data are available.

ETHICS STATEMENT

The study did not involve experiments with human subjects, animal experimentation or use of social media data to perform an analysis. Consequently, there was no need for ethical approval regarding the study presented in this article. The DNAs used in this study are

all products readily available for commercial use, so that they do not pose ethical concerns.

ACKNOWLEDGEMENTS

The author is thankful for Professor Jiali Li, Department of Physics, The University of Arkansas for supervision, and access to the Biophysics Laboratory, as well as the Laboratory Manager, Nathan Walsh for technical advice and help with the protocols. This was a project for a biophysics course at the University of Arkansas.

COMPETING INTERESTS

The author declares no competing financial or nonfinancial interests or personal relationships with other people or organizations that could have influenced the work covered in the current article.

FUNDING

No specific funding was received for this research.

REFERENCES

Brody, J. R., and Kern, S. E. (2004). History and principles of conductive media for standard DNA electrophoresis. *Analytical Biochemistry, 333*(1), 1–13. https://doi.org/10.1016/j.ab.2004.05.054

Green, M. R., and Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Helling, R. B., Goodman, H. M., and Boyer, H. W. (1974). Analysis of endonuclease R·EcoRI fragments of DNA from lambdoid bacteriophages by agarose-gel electrophoresis. *Journal of Virology, 14*(5), 1235–1244. https://doi.org/10.1128/JVI.14.5.1235-1244.1974

Lee, P. Y., Costumbrado, J., Hsu, C. Y., and Kim, Y. H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*, (62), e3923. https://doi.org/10.3791/3923

Lumpkin, O. J., Déjardin, P., and Zimm, B. H. (1985). Theory of gel electrophoresis of DNA. *Biopolymers, 24*(8), 1573–1593. https://doi.org/10.1002/bip.360240812

Ogston, A. G. (1958). The spaces in a uniform random suspension of fibres. *Transactions of the Faraday Society, 54*, 1754–1757. https://doi.org/10.1039/TF9585401754

Sárközy, F., and Guttman, A. (2024). Agarose gel electrophoresis. In: Kurien, B. T. and Scofield, R. H. (eds). *Electrophoretic Separation of Proteins*. Springer, Pp: 135–147.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Stellwagen, N. C. (2006). Electrophoresis of DNA in agarose and polyacrylamide gels. In: Kieleczawa, J. (ed). *DNA Sequencing II*. Jones and Bartlett Learning, Pp: 1–21.

Stellwagen, N. C. (2009). Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution. *Electrophoresis, 30*(S1), S188–S195. https://doi.org/10.1002/elps.200900052

Stellwagen, N. C. (2018). DNA gel electrophoresis. In: Meyers, R. A. (ed).

Encyclopedia of Analytical Chemistry. Wiley. https://doi.org/10.1002/9780470027318.a1403.pub3

Stellwagen, N. C. (2022). Anomalous electrophoresis of dsDNA in agarose gels. *Electrophoresis, 43*(17–18), 1795–1810. https://doi.org/10.1002/elps.202200072

Stellwagen, N. C., Gelfi, C., and Righetti, P. G. (1997). The free solution mobility of DNA. *Biopolymers, 42*(6), 687–703. https://doi.org/10.1002/(SICI)1097-0282(199712)42:6<687::AID-BIP7>3.0.CO;2-Q

Wang, X., Veeraraghavan, N., Poorey, K., Gillispie, J., Kappeler, K. V., Maher, C. A., et al. (2020). Characterization of the broad-range size separation of DNA in agarose gel electrophoresis. *Electrophoresis, 41*(7–8), 579–586. https://doi.org/10.1002/elps.201900413