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Effects of DNA Inserts on the Viscoelasticity of DNA Gel Composites

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Abstract

Understanding the viscoelastic phase separation of a DNA mixture will help model soft gels composed of discrete length distribution. The purpose of this study was to investigate the effects of DNA inserts on the viscoelasticity of DNA gel composites. The study focused on determining the viscoelasticity of mixed systems composed of longer double-stranded deoxyribonucleic acid (ds-DNA) solution as a background matrix re-constituted with shorter ds-DNA inserts of controlled lengths. Passive microrheology by video microscopy was employed to characterize shear moduli variations. The total concentration of each mixed system was 0.201 g/L. An increase in the viscous and elastic moduli of the three mixed systems was observed suggesting an enhancement in the viscoelastic properties of the mixed system upon the addition of the inserts. There was a greater enhancement on the viscous and elastic moduli of the system with longer inclusions. The findings of this study may shed light on biopolymer physics that can aid in examining diseases with genetic materials of resolvable length distributions.

Keywords: biopolymer, matrix, microrheology, moduli, shear

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Introduction

A composite material is a combination of two or more materials that work together to give the composite a new unique property. Most composites are made up of two materials: one is the 'matrix' that surrounds and binds fragments of the other material which is called 'reinforcement' (Moumene et al., 2016). Usually, the reinforcements are harder, stronger, and stiffer than the matrix. It often provides strength and stiffness of the composites.

Biocomposites are materials derived from a biological origin (Kuciel et al., 2010). Wood, bones, plant and animal cells are some examples of biological composites. The property of the biocomposite is usually dependent upon the properties of the constituents (Reddy et al., 2016). Many biological materials found in nature are nearly incompressible, that is, if they are under mechanical stress or strain, they will try to conserve their volume by changing their shape (Bacabac et al., 2010). Most of these materials have components that are fiber-based composites composed of soft and deformable elastic background, reinforced with stiff fibers (Das & MacKintosh, 2011). The components of these composites interact interdependently with each other. By varying the concentration of the fibers and the mechanical properties of the fiber and the matrix, the bulk properties of the composite can then be modified. Thus, understanding the mechanical properties of a composite biopolymer network, such as deoxyribonucleic acid (DNA) gel with inclusions, will help elucidate the mechanics of a living cell.

The biopolymer DNA is known to be the carrier of genetic information in an organism (Renneberg & Berkling, 2017). It governs the production of proteins and other molecules essential to life (Travers & Muskhelishvili, 2015). It has to be manipulated by other biomolecules to regulate, edit, copy and transcribe the molecule. A slight difference in DNA viscosity may affect the overall physical properties of the nuclear region as well as the transport of nuclear proteins (Lammerding, 2011).

One of the key parameters for the quantitative interpretation of the conformational properties of a DNA molecule is to understand its viscoelastic properties at different concentrations. At low concentration, DNA forms mostly viscous structures, and the transport of non-binding proteins is purely diffusional (Goodman et al., 2002). At high DNA concentration, overlapping and entanglement may occur, generating an elastic polymer mesh (Kundukad & Van der Maarel, 2010). Elasticity may, in turn, slow down the transport of nuclear materials. Genomic DNA forms a relatively rigid macromolecular assembly, which has the potential to form a stiff network (Gáspár & Csermely, 2012). The quantity that measures the rigidity of a molecule is called persistence length (Mantelli et al., 2011). The widely accepted experimental value of the DNA's persistence length is 50 nm or ~150 base pairs (bp) (Mitchel et al., 2017). A DNA segment shorter than the persistence length behaves like a straight, stiff rod (Nelson, 2012).

Studies on viscoelastic properties of DNA polymers have been reported. Zhu et al. (2008) obtained the viscoelastic moduli of lambda (λ) phage DNA through the entanglement transition with the help of particle tracking microrheology. Price et al. (2015) studied the mechanical properties of DNA using the DNA motion-capture technique. Chu et al. (2015) conducted microrheology studies with peptide nucleic acid-DNA complexation. Garai et al. (2015) determined the elastic properties of short DNA and nucleosomal DNA. Bravo-Anaya et al. (2016) also examined the rheological properties of DNA molecules in solution such as the molecular weight and entanglement influences. Stojković et al. (2015) determined the viscoelastic properties of levan-DNA mixtures by macro- and microrheology measurements. Bentley et al. (2018) characterized the microscale mobility and viscoelasticity of entangled blends of DNA of varying lengths and topologies.

The differences in the rheological properties of the different polymers can be emphasized as the polymers are mixed. In a binary mixture, when the behavior of a single polymer does not seem to be influenced by the presence of a second polymer, the constituents are

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then called phase separated. Understanding the viscoelastic phase separation of a DNA mixture will help model soft gels composed of discrete length distribution. This study aimed to investigate the effects of DNA inserts on the viscoelasticity of DNA gel composites. Specifically, this study determined the viscoelastic properties of a mixture of two lengths of DNA solutions. The diffusivity of the embedded beads into the DNA solutions was also measured. The findings of this study may shed light on biopolymer physics that can aid in examining diseases with genetic materials of resolvable length distributions.

Materials and Methods

DNA preparation

This study attempted to harvest DNA samples from *Escherichia coli* 1106 (purchased from Philippine National Collections of Microorganism, Biotech Acc. No. B1634, University of the Philippines, Los Baños, Laguna) using the standard protocols in commercially available extraction kits. However, designing length scales with *E. coli* using the available restriction enzyme was not possible. After performing gel electrophoresis, the DNA bands were very faint, and the gel cutting cannot be completed. Hence, this study used the ready to use λ-DNA solution as the alternative which was isolated from bacteriophage lambda (λcl857 Sam 7) purchased from Roche Diagnostics GmbH, Mannheim Germany. As received from the manufacturer, the λ-DNA stock solution has a concentration of 0.263 μg/μL. The λ-DNA molecule is a linear molecule which has 48490 bp and 12-base, single-stranded overhangs at both sides that are complementary. At ambient temperatures, in a solution containing purified λ-DNA, these single ten stranded overhangs may pair and form the so-called ‘cos-site’ (cohesive end site). As a consequence, the DNA is circularized.

The λ-DNA was digested with an EcoR1 restriction enzyme with restriction sites of GAATTC. Three different DNA lengths (21226 bp, 7421 bp, and 5643 bp) were successfully cut. A wizard genomic DNA purification kit purchased from Promega TM (USA) was used to isolate the DNA immediately after cutting and undergoing gel electrophoresis. The DNA samples were then purified and quantized using the UV-spectrophotometer.

Initially, the three cut DNA lengths were of different concentrations; the lowest was 0.201 g/L. The other two lengths were diluted with distilled water to obtain the same concentration for the samples. A series of sample DNA composites were prepared such that the composite contains a longer length DNA solution as the background matrix added with a DNA solution of shorter length as an insert. The composites prepared were of mixtures 21226 bp + 5643 bp; 21226 bp + 7421 bp; and 7421 bp + 5643 bp. The total concentration of all mixed systems was equal to 0.201 g/l. A droplet of the composite solution, added with a polystyrene microsphere of diameter 1.0 μm, was deposited on a microscope slide and sealed with a cover slip separated by a spacer (15 mm x 10 mm x 0.12 mm).

Video microscopy instrumentation

The samples were viewed through an inverted microscope with a 100x oil-immersion objective using the bright field microscopy. The motion of the beads in the sample was imaged and recorded at 30 frames per second using a CCD (charge-coupled devise) camera (WATEC, Japan) attached to the side port of the microscope (Axiovert 200Series, USA) using an image acquisition program in LabView (Laboratory Virtual Instrument Engineering Workbench, USA). The video of the motion of the beads was then processed for multi-particle tracking, which was done using a freeware in programming language IDL (Interactive Data Language) developed by Crocker et al. (2000).

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Particle tracking

The series of images acquired were analyzed using the IDL particle tracker made freely available through a maintained website for free tutorial and download of the software. The images were processed by minimizing the first unwanted spots through smoothing and thresholding. The rytrack.sav program would prompt the user to adjust parameters for image processing, particle identification, and tracking. The program produced ".gdf" (graph data format) files that contain the information on the position of the particle in each image. The particle positions were associated with the position in the later images to produce trajectories. Another .gdf file format was obtained to compute for the MSD (mean squared displacement) and the frequency-dependent complex shear modulus. Passive microrheology (Mason et al., 1997) by video microscopy was employed to characterize shear moduli variations in individual solutions and gel composites.

The Takayanagi model (Takayanagi, 1963) was used to predict the elastic modulus (G') of the mixed DNA lengths in a solution from the G' of the individual constituents at their effective concentration. Calculations of G' were made with the assumption that both polymers were restricted into their separate phases (Lundin et al., 2000).

Results and Discussion

DNA concentration and purity measurement

The ratio of the absorption coefficients at wavelengths 260 nm and 280 nm of the three lengths of DNA samples is shown in Table 1. The absorbance ratio obtained implied that the isolated DNA samples are free from contamination since it is within the range of 1.60 - 1.80 for pure DNA (Weising et al., 2005).

Table 1. Absorbance readings using a UV-spectrophotometer.

DNA lengths (bp)	Absorbance		DNA purity 260 nm/280 nm ratio
	260 nm	280 nm	
21226	0.124	0.072	1.72
7421	0.40	0.237	1.69
5643	0.15	0.086	1.74

Viscoelastic properties of the mixture

Figure 1 shows the comparison of the predicted G' of the mixed solutions from separate phases with the G' of the actual mixed solutions. The actual G' of the mixed system shows an anomalous elasticity compared to the predicted G' of the phase separated DNA solutions. The predicted G' of the mixed system 21226 bp + 4878 bp with shorter inserts appears to be more elastic than the predicted G’ of the mixed system 21226 bp + 7421 bp. On the contrary, the actual G’ of the mixed system 21226 bp + 7421 bp appears to be more elastic than the system with shorter inserts (21226 bp + 5643 bp). The actual mixed systems do not lie within the predicted G’ bound for both of the mixed systems. Thus, the mixed systems 21226 bp + 7421 bp and 21226 bp + 5643 bp appear to be a non-phase separated system. The constituents of the mixed system (DNA background with DNA inserts) were of the same material which could explain the result. Several studies showing anomalous elasticity were using a single DNA instead of composite (Cong et al., 2015; Salari et al., 2015; Le & Kim, 2014; Mazur & Maaloum, 2014).

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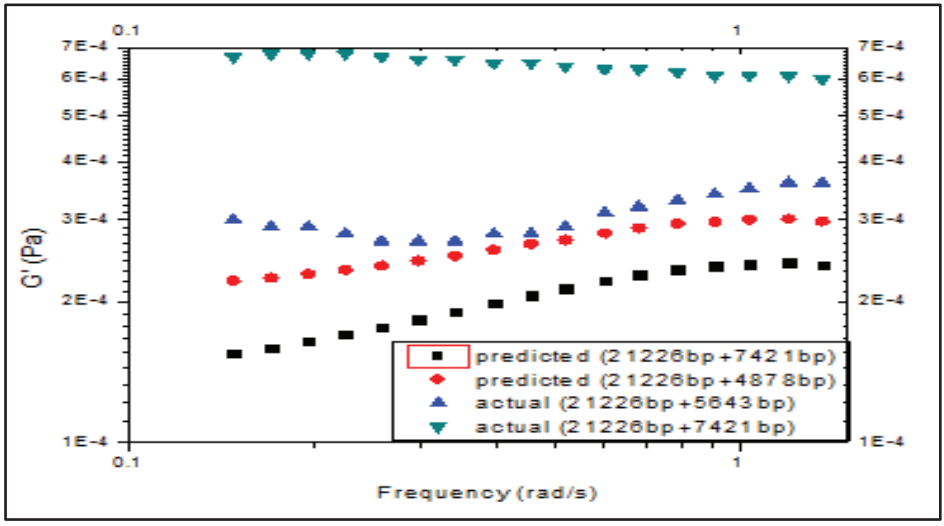
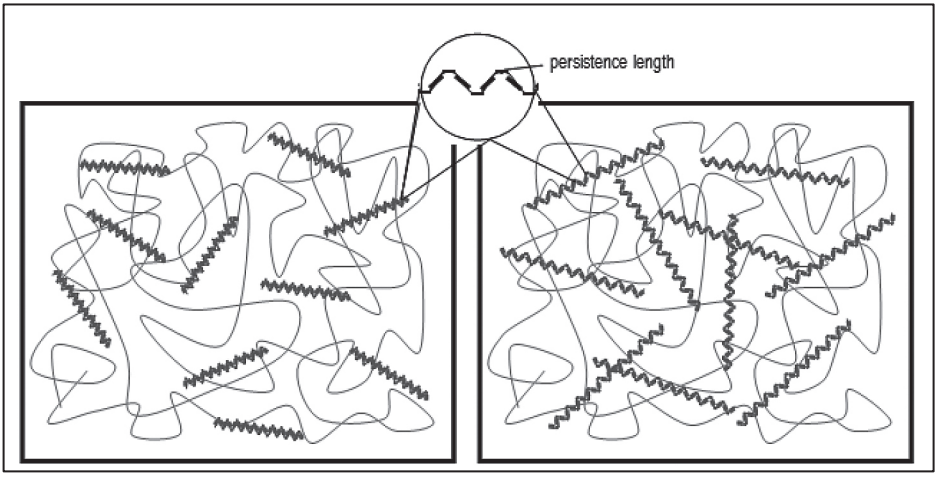


Figure 1. The plot of elastic modulus vs. frequency in logarithmic scale of the actual mixed systems of length combinations (21226 bp + 7421 bp and 21226 bp + 5643 bp) and the predicted mixed systems of length combinations (21226 bp + 7421 bp and 21226 bp + 4878 bp).

A single DNA sample may experience self-entanglement since DNA tends to bend or twist beyond its persistence length (Renner, 2015). Entanglement occurs in a DNA sample when the DNA concentration is above the critical concentration, the point where individual polymer overlaps each other (Bravo-Anaya et al., 2016). Critical overlap concentration of λ-DNA is determined to be 0.030 g/L. In this study, DNA concentrations for all mixed systems were equal to 0.201 g/L. A single DNA with a length of 5643 bp is approximately 37 times longer than the persistence length. The DNA with 7421 bp is about 49 times longer than the persistence length. It only implies that a single DNA of size 5643 bp may experience maximum self-entanglement of 37 times and the DNA of size 7421 bp may experience maximum entanglement of 49 times as shown in Figure 2.



(a) (b)

Figure 2. Cartoon sketch illustrating the mixed system with long (gray) and short (black) DNA. (a) mixed system with shorter DNA inserts, (b) mixed system with longer DNA inserts.

The background matrix of DNA size 21226 bp may also encounter self-entanglement of about 141 times. It implies that longer DNA inserts contribute more to the entanglement of the composite. This entanglement is because DNA molecules cannot cross through each other (Kundukad & Van der Maarel, 2010). As a result of entanglements, concentrated solutions of DNA are viscoelastic. Viscoelasticity implies an elastic response at higher frequencies, whereas for lower frequencies and corresponding longer times, the solutions are viscous-fluid-like (Larson, 1999). As a result of these entanglements, the motion of the DNA molecules is strongly hindered by the presence of the neighboring smaller molecules and the relaxation time may become very long (Kundukad & Van der Maarel, 2010). The G' of the actual mixed system with longer inserts (21226 bp + 7421 bp) displays greater elasticity than the actual mixed system with shorter DNA inserts (21226bp + 5643 bp).

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A. Mean-Square Displacement (MSD)

The plot of MSD as a function of lag time for each mixed system was fitted to distinguish the diffusion of the embedded particle (Figure 3). The slope of the graph gives the diffusion coefficient of the sphere in the solution. The observation time of the MSD of the mixed system was 10.34 seconds. Beyond this time, the 1.0 μm beads tend to move away very quickly from the facial plane. Thus, the beads tend to settle down longer than the observed time. It can be seen from the graph that for DNA composites 21226 bp + 5643 bp and 21226 bp + 7421 bp, the graph begins to deviate from linear behavior at a later time. The diffusion coefficient of the mixed system 21226 bp + 7421 bp is 0.6957 while the mixed system 21226 bp + 5643 bp has a diffusion coefficient of 0.7169. It shows that as the insert increases in length, the diffusion coefficient decreases. The beads diffuse slower in the mixed system with longer inserts. Diffusion of DNA molecules is dependent on length (Robertson et al., 2006).

The presence of the longer DNA length will make the mixed system stiffer, in consequence, impedes the motion of the beads. On the other hand, the composite 7421bp + 5643 bp retained its linear behavior. Hence, the composite of similar lengths 7421bp + 5643 bp behaves as viscous fluid for longer times.

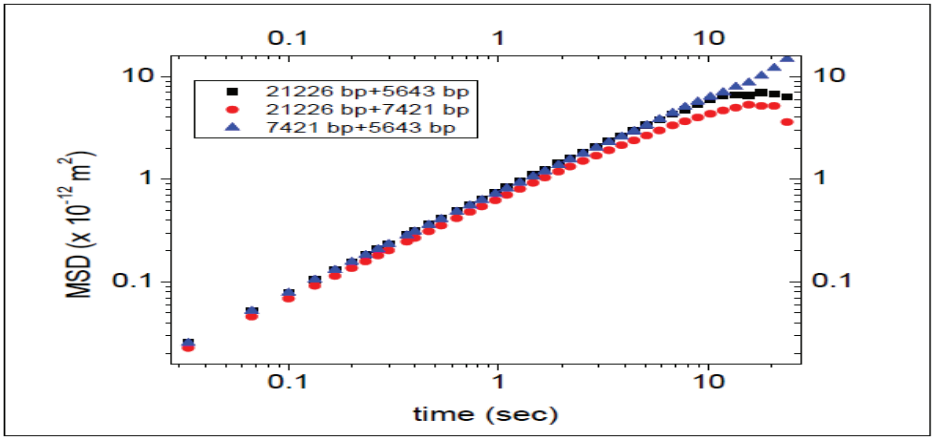

Figure 3. The plot of MSD against time for different DNA mixtures of different lengths in double logarithmic scale.

B. Viscous modulus

All the three solutions were approximately equal magnitude-wise (Figure 4). The shorter DNA length shows greater viscosity compared to the longer DNA lengths. Billones (2013) also revealed that the single-length DNA solutions have less dependence on the viscous modulus of the solution.

In a mixed system with the same background of 21226 bp but with different inserts of 7421 bp and 5643 bp, the composite with longer inserts seems to be more viscous than the system with shorter inserts (Figure 5). Hence, for the mixed system with same background size but a different length of inserts, the mixed system with longer insert shows greater viscous modulus.

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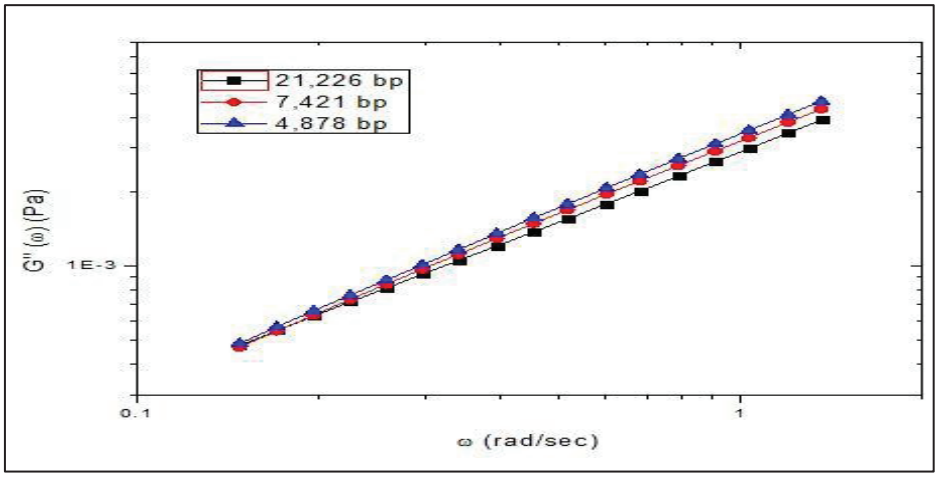

Figure 4. **The plot of the viscous modulus G"(ω) Pa vs. frequency (rad/sec) in logarithmic scale of the DNA lengths (21226 bp, 7421 bp, 4878 bp) of equal concentration (0.201 g/L).**

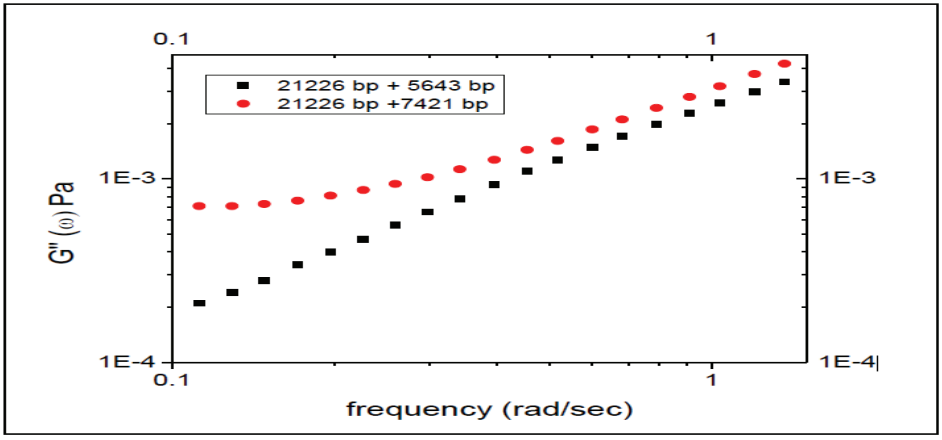

Figure 5. **The plot of the viscous modulus G"(ω) Pa vs. frequency (rad/sec) in logarithmic scale of the DNA mixed system with the same DNA length background of 21226 bp: the mixed system 21226 bp + 5643 bp and 21226 bp + 7421 bp.**

On the other hand, in a mixed system with the same insert size of 5643 bp but different DNA background lengths of 21226 bp and 7421 bp, the composite with shorter background length is likely to be more viscous (Figure 6). Hence, for the mixed system with the same size of insert and different background length, the shorter background seems to be more viscous and behaves more like a viscous fluid. This result is also supported by the MSD versus time previously shown in this study where the mixed system 7421 bp + 5643 bp behaves like a viscous fluid for a longer time. Results show that the viscous modulus of the mixed systems tends to approach equal magnitudes as frequency increases.

All the results in this study are found to be new and still to be studied further since there is no existing similar study in the literature. Most studies were of composites of different materials, and the reinforcement is a rod-like inclusion (Chu et al., 2015; Garai et al., 2015; Stojković et al., 2015). The study can be used to model soft gel composites with "soft" DNA inserts instead of a "rod" insert.

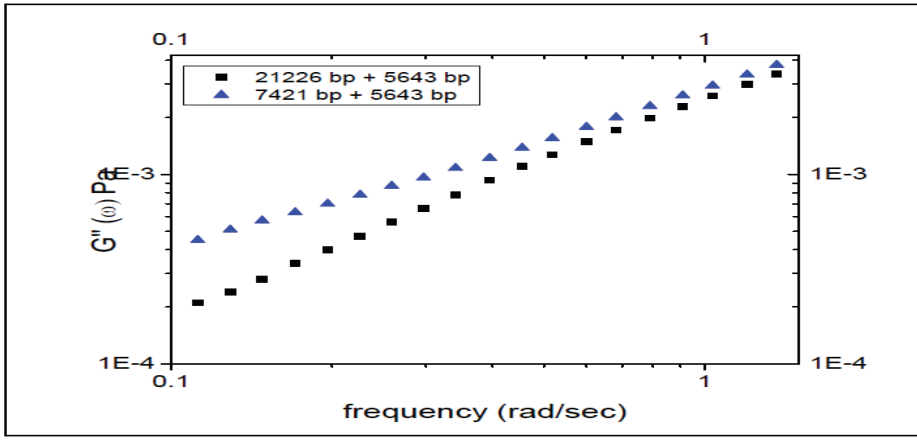

Figure 6. The plot of the viscous modulus G"(ω) Pa vs. frequency (rad/sec) in logarithmic scale of the DNA mixed system with the same DNA insert length of 5643 bp: the mixed system 21226 bp + 5643 bp and 7421 bp + 5643 bp.

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C. Elastic modulus

Figure 7 shows the dependence of elastic modulus on the DNA length. The shorter the DNA length present, the stiffer the solution becomes. The DNA molecule exhibits maximum stiffness at its persistence length. In the previous study of Billones (2013), it is shown that for single length DNA solutions, smaller DNA of 4878 bp has greater elastic modulus than the longer DNA with 21226 bp.

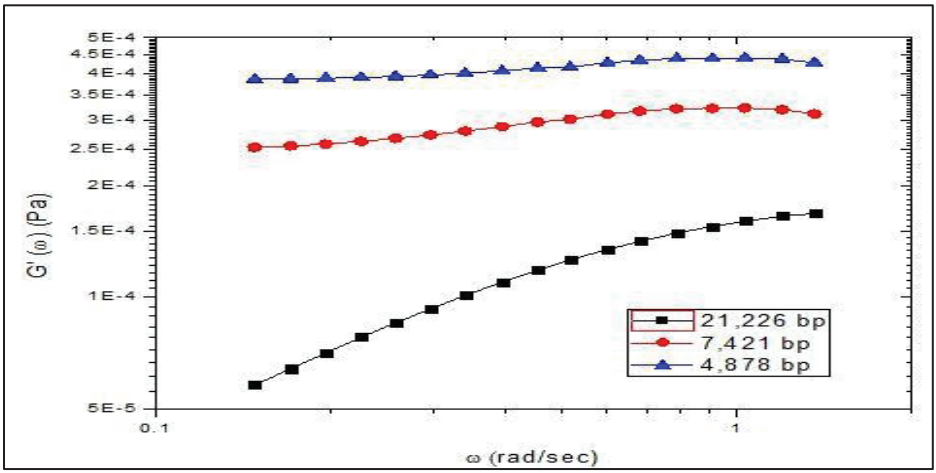

Figure 7. The plot of the elastic modulus G'(ω) Pa vs. frequency (rad/sec) in logarithmic scale of the DNA lengths 21226 bp; 7421 bp and 4878 bp of equal concentration (0.201 g/L).

The mixed system with DNA inserts tends to have an unlikely result compared to single DNA length in a solution. Figure 8 shows the effect of inserts into the elastic modulus of the mixed system. The DNA mixture with longer inserts has the higher elastic modulus compared to the mixed system with shorter DNA inserts. Hence, for the mixed system with same DNA length background matrix but different insert length, the longer inserts have greater elasticity. Unlike the study of Das and Mackintosh (2011) on rod inserts, the addition of soft DNA inserts increases the elasticity of the mixed system as the inserts become longer.

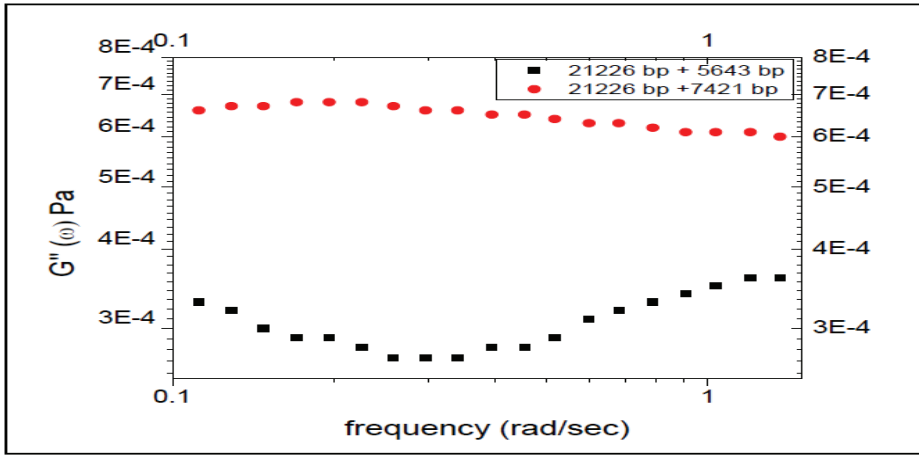

Figure 8. The plot of the elastic modulus G'(ω) Pa vs. frequency (rad/sec) in logarithmic scale of the DNA mixed system with the same DNA length background 21226 bp: the mixed system 21226 bp + 5643 bp and 21226 bp + 7421 bp.

Similar results can be seen comparing the mixed system with different background lengths but the same DNA inserts sizes. Figure 9 shows the elastic modulus vs. frequency of the mixed systems 21226 bp + 5643 bp and 21226 bp + 7421 bp. As the background DNA length increases, the elasticity of the mixed system also increases. It implies that longer DNA background also contributes to the elasticity of the composite system. Hence, for the mixed system with same size inclusion but different background length, the mixed system with longer DNA background has greater elastic modulus.

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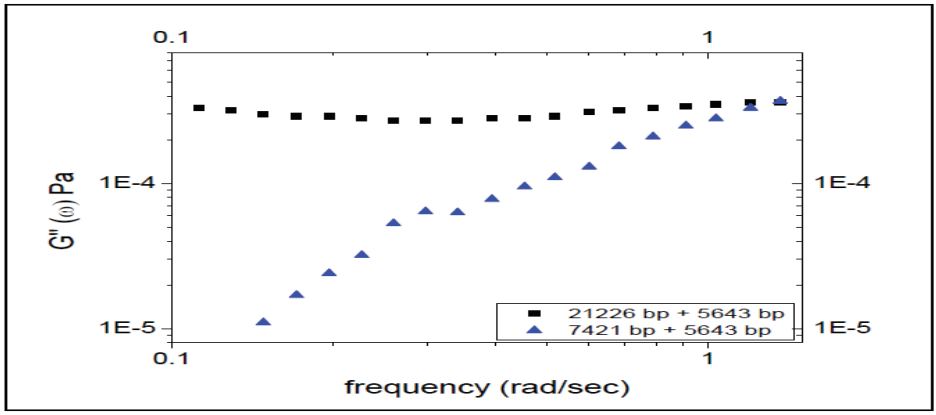

Figure 9. The plot of the elastic modulus G'(ω) Pa vs. frequency (rad/sec) in logarithmic scale of the DNA mixed system with the same DNA insert length of 5643 bp: the mixed system 21226 bp + 5643 bp and 7421 bp + 5643 bp.

Conclusion and Recommendations

The addition of DNA inserts into the DNA background increases the overall entanglement of the mixed system. The longer the DNA inserts, the greater the entanglement of the system, and the relaxation time becomes very long. This increase in entanglement and relaxation time contributes to the increase in the viscoelasticity of the mixed system with longer inserts. The viscous and elastic modulus of the three mixed systems enhance upon the addition of the inserts, but more for the latter. Further study on the DNA gel composites with varying lengths and concentration of DNA inserts is recommended. Studying across different frequency range using optical tweezer is also recommended.

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