Scherrheologie von λ-DNA-Lösungen bei hohen Scherraten

The Shear Rheology of λ-DNA Solutions at High Shear Rates

H. Dakhil[1], S. Steiner[1], S. K. Basu[1], A. Soller[1], S. Pan[2], N. Germann[2], B. Kappes[3], A. Wierschem[1,4]

[1] Lehrstuhl für Strömungsmechanik, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Cauerstr. 4, D-91058 Erlangen, Germany
[2] Fluid Dynamics of Complex Biosystems, Technische Universität München (TUM), Maximus-von-Imhof-Forum 2, D-85354 Freising, Germany
[3] Lehrstuhl für Medizinische Biotechnologie, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Paul-Gordan-Str. 3, D-91052 Erlangen, Germany
[4] LSTME Busan, 1276 Jisa-Dong, Gangseo-Gu, Busan 46742, South Korea

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Abstract

The rheology of polymer solutions has been studied intensively during decades. Yet, due to constraints in the accessibility of high shear rates, studies were restricted to the zero viscosity and the shear thinning regime. The infinite shear viscosity could not be reached. We have designed a narrow-gap rotational rheometer that allows studies at shear rates up to about 10^5 s^{-1} [Dakhil et al. 2014]. Recently, we have shown that it enables to study the infinite-shear viscosity plateau of semi-flexible polyelectrolytes [Dakhil et al. 2019]. Here, we present now a study on the shear rheology of DNA solutions. In rheology, DNA are paradigmatic polymer systems. They are large polymers with an excellent monodispersivity. Like for other polymers, zero shear viscosity and shear thinning have been well characterized [Pan et al. 2014, Pan et al. 2018]. We show that an infinite-shear viscosity plateau of DNA exists. We studied buffered aqueous solutions of λ-DNA at shear rates up to about 10^5 s^{-1}. At low shear rates, our data matches nicely with literature values. Well beyond formerly accessed shear rates, we find that the viscosity of the solutions levels off and finally reaches an infinite-shear viscosity plateau; hence, making available now the viscosity functions of DNA for the entire range of shear rates from the first Newtonian plateau to that of infinite-shear viscosity.

Introduction and Background

Rheology deals with the deformation and flow behavior of materials under the influence of mechanical stress. Rheology determines material properties such as viscosity, viscoelastic behavior or normal stress differences [Mezger T G 2006]. The application of rheology is extensive and can be applied in different areas of life such as in the polymer and chemical industry, foams, emulsions, suspensions, polymer melts, solutions etc. But there are also many applications of rheology in the field of pharmacy, food, cosmetics and biotechnology industries. Nowadays, rheology is also being used in medicine [Steffe J F 1996, Chhabra R P and Richardson J F 2008], blood, DNA or even cells [Dakhil et al. 2018, Dakhil et al. 2016, Kokkinos et al. 2016].

For the present work, the focus is on the measurement of λ-DNA in buffered solutions with high salt concentration. Along with the great biological importance, DNA has been discussed, for several times, as to whether it can be used a very good model in the field of polymer rheology [Robertson et al. 2006, Smith D E and Chu S. 1996, Valle F et al. 2005, Ross P

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D and Scruggs R L 1968]. Pan et al. showed that DNA can be used as a model polymer, in presence of sufficient salt [Pan et al. 2014]. Since DNA is a polyelectrolyte, sufficient salt in solution results in all charges being neutralized at the backbone of the DNA, making it look like a neutral polymer [Pan et al. 2014].

DNA (deoxyribonucleic acid) is very crucial as it is genetic material of living cells. The two most important functions are coding for protein production and self-replication for exact copies in new cells [Păun G et al. 2005]. In its double helix structure, DNA is a long, thin, and cylindrical chain that is also a polyelectrolyte [Bravo-Anaya L et al 2016, Anderson C F and Record M T 1982]. As a polynucleotide, the DNA is composed of several, different nucleotides, each consisting of a phosphate residue on the backbone, a sugar (deoxyribose) [Watson J D and Crick F H 1953] and a base. The differentiation of the nucleotides is possible only on the basis of the bases, which are divided into two groups: the purines (adenine and guanine) and the pyrimidines (cytosine and thymine) [Păun G et al. 2005]. DNA can be present in different confirmations: as a linear chain, as circular DNA in the form of a closed ring or as twisted DNA (also: supercoiled) in the form of extremely packed molecules.

The DNA of λ phage has a single strand of 12 nucleotides at both ends of the double helix, which is also called "sticky ends". This single stranded but complementary ends of two DNA molecules are called sticky ends. The respective single strands lead to the linear DNA merging into a circular DNA or with other DNA molecules. It is assumed that the cohesive ends are complementary to one another. Both guanine and cytosine can form a complementary pair with a triple hydrogen bond, as well as adenine and thymine with a double hydrogen bond [Păun G et al. 2005]. Depending on the content of guanine-cytosine and adenine-thymine bonds, the denaturation temperature and flexibility of the chain change. In general, DNA can be described as a semi-flexible molecule [Bravo-Anaya L et al 2016].

For rheological studies, a wide variety of DNA confirmations can be used. DNA is monodisperse, it can be stained easily for visualization, it has medium molecular weight and commercially available. These properties have made DNA a model polymer system for investigating polymeric liquids [Pan et al. 2014]. It can be distinguished between twisted, circular or even linear DNA. DNA can also cover a wide range of different molecular weights. λ-DNA, which has been used in this work, is linear genomic double stranded DNA of bacteriophage lambda virus.

In industry, polymers are subject to high deformation and shearing in many processes (e.g., production of plastic bottles, coating, lubrication, etc.) [Pipe C et al 2008]. Basically, it can be said that shear occurs as soon as a fluid flows over a surface [Smith D E et al. 1999]. Double-stranded DNA has been used as a model polymer in various experimental studies. The reason for this is the great advantage that DNA molecules are monodisperse [Pecora R 1991]. Negative charges are present on the backbone of the DNA. These lead to rejections among each other and stretch the DNA in length. In the presence of salt, the entire charge can be neutralized. The DNA is then twisted and can be considered as a neutral synthetic polymer. Furthermore, a great advantage is that DNA can be inherently linear, closed circular, and highly twisted. It can also cover a wide span of lengths (about 6 to 290 kbp) [Robertson R M 2006].
In some studies, it has already been shown that single DNA molecules show correlations between diffusion coefficients D and length L. Adjusting a fit results in an exponent of 0.6 in the power law, which agrees with linear polymers in the good solvent [Pan et al. 2014].

The main aim of the present work is rheological investigation of the λ-DNA solutions at different concentrations with the help of a narrow gap rheometer. The primary goal is to measure the second Newtonian plateau at high shear rates. Furthermore, the concentrations and conditions are chosen such that a comparison with data provided by Pan et al. 2014 is possible.

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The aim here is to obtain at low shear rates and a successfully determined zero viscosity of Pan et al. to supplement with the data of the high shear rates and thus to represent an entire viscosity curve. The overall experimental data of the shear tests should also serve to determine possible regimes of infinite viscosity as a function of concentration.

Materials and Methods

The following sections provide an overview of the materials and methods used in this work.

Chemicals:

The λ-DNA (New England Biolabs, # N3011L) has a length of 48.5 kilobasepairs (kbps) and the stock is supplied in a buffer of 0.5 mg of DNA mL^{-1}, which is dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA). This TE buffer is commonly used as a solvent to dilute the desired concentration from stock for DNA applications [Mezger T G 2006, Steffe J F 1996]. For rheological experiments, sodium chloride was also added (working concentration 0.5 M) to obtain charge neutrality of the DNA [Chhabra R P and Richardson J F 2008]. The chemicals used were purchased from Sigma-Aldrich: Tris-HCl (# T3038, Trizma® hydrochloride solution, pH 8.0, 1 M), ethylenediaminetetraacetic acid (EDTA, # 03690, 0.5 M), sodium chloride (# S6546, 5 M), water (# 00612, water for ion chromatography). The chemicals were sterile filtered through a syringe filter (Acrodisc® 0.2 µm pore size syringe filter, # Z259942, Sigma-Aldrich). All the buffer or solutions were prepared under the safety workbench.

Preparation of DNA Solutions

The DNA, supplied from New England Biolabs is stored at -20°C and thawed in a water bath at about 60°C for about 15 minutes, which is followed by sudden cooling at ice cold atmosphere with mild shaking. The structure of the double helix seems to remain intact at this temperature range of 60°C [C F Anderson and J. M T Record 1982]. At a salt concentration of 0.5 M sodium chloride, the denaturation temperature of DNA is above 90°C [Schildkraut, C., and S. Lifson 1965], but 60°C is high enough to dissociate the sticky ends of the DNA molecules and lead them to remain into single and linear entity. In this way, aggregations of the DNA molecules can be avoided [Pan et al. 2014, Pan et al. 2014]. Five minutes after cooling down in ice, under a clean bench the DNA vials should be opened to avoid contamination and diluted with TE buffer to obtain the desired concentrations for experiments. For further usage, DNA should be stored at -20°C for longer period of time. To avoid contamination, the DNA solution was prepared under a clean bench. In order to prepare a DNA solution, all sterile filtered components are pipetted with final concentrations into an Eppendorf tube with which an optimum amount of DNA was added. The fast pipetting or rapid release of DNA leads to high shear loads, which may trigger damaging of the sample. To avoid that, it is sometimes better to use pipette tips with an enlarged opening. Notwithstanding, it has been observed that careful pipetting, with usual pipette tips, leads no damage to the DNA sample. To dissolve and mix DNA properly into buffer, it is better to triturate the same solution for several times. A laboratory mixing device (e.g. vortexer) should not be used here to avoid damage to the DNA. After thorough mixing with pipette, DNA sample is ready for rheology experiment.

Experimental Set-Up:

The rheological experiments were performed using a modified UDS 200 rotational rheometer from Physica. Experiments were carried out in a parallel-disk configuration. In order to study the infinite-shear viscosity plateau at shear rates up to 10^5 s^{-1}, we worked at gap widths as low as 20 µm. To this end, the disks were aligned perpendicular to the axis of rotation to reduce zero-point errors, which are usually about 25 µm and larger [Davies, G. A., and J. R. Stokes, 2005, Davies, G. A., and J. R. Stokes 2008, Pipe, C. J et al. 2008], down to less

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than ±1 µm [Dakhil H and A. Wierschem 2014]. Therefore, we used a bottom plate made from glass with a diameter of 75 mm and an evenness of λ/4 (Edmund Optics), where λ is the testing wavelength (633 nm); the rotating upper plate is of 50 mm diameter with an evenness of λ/10 was from glass (Edmund Optics). The upper plate was attached to a measurement head of the rheometer with a diameter of 25 mm. For aligning the bottom plate perpendicular to the axis of rotation, it was fixed to a tripod, which was mounted on the rheometer table. The tripod was aligned with three micrometer screws, fixed to the rheometer. For disk alignment, the gap width was measured with a customized confocal interferometric sensor (STILSA). The sensor was placed underneath the fixed glass plate to measure the gap width at three different locations. For details on the disk alignment, refer to [Dakhil H and A. Wierschem 2014].

The experiments were carried out at gap widths with a variation of about ±0.5 µm. The temperature was fixed at 25.4±0.1°C using a hood equipped with a heating foil, which was controlled with an H-Tronic TSM 125 temperature control system. Evaporation of the fluid was minimized by placing wet tissue inside the hood. The setup is sketched in Fig.1.

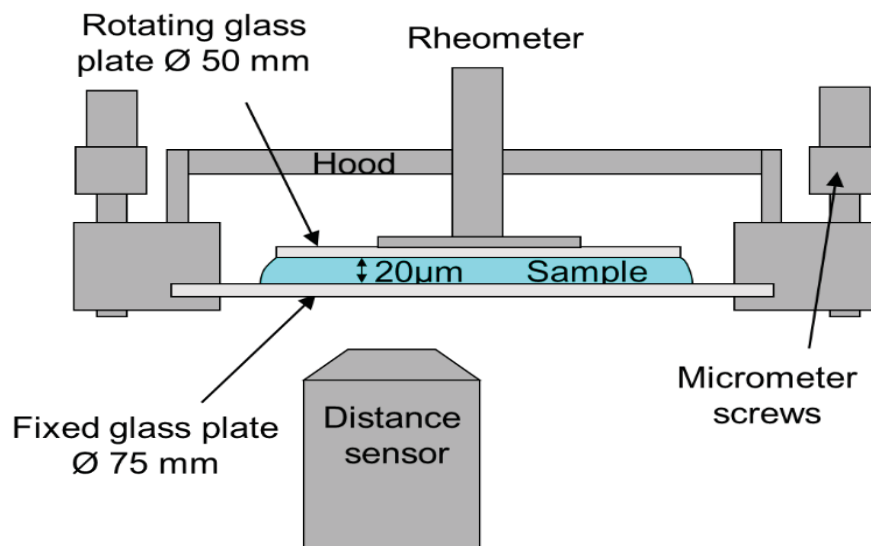

Figure 1 The diagram of modified narrow-gap rheometer with temperature control.

Application in shear flow

Just before going for any rheology measurement, the rotational rheometer must be initialized and parallel plates should be aligned. For the alignment, both the upper and lower plates are thoroughly cleaned with detergent (20 % SDS), 70 % ethanol and distilled water, respectively. Lint-free tissue papers as well as lint-free compressed air are used to clean the dusts.

The zero gap is set with the help of the rheometer software and the upper plate is placed at 20 µm gap. The motor, the drive and the inertia of the rheometer are configured. The free rotational movement of the upper plate is checked and is rotated at a constant speed of 0.04 revolutions per second. The rheometer software requires an adjustment of the number of data points and the duration per data point. Just to ensure that the upper plate remains in motion throughout the alignment process, high values are chosen. (viz. 3000 data points with 10 s measurement duration per data point).

The optical sensor measures 2000 data points over the duration of two revolutions (50 seconds total). The sensor data is imported into Excel. From this data, an average plate distance can be determined with the maximum and minimum distance. The maximum distance minus the minimum distance, i.e. the peak-to-peak amplitude, indicates the amount of tilt of

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the upper plate. With the help of Matlab software, the average gap width is directly calculated at three points and entered to control the actuators. The controller are able to set the plates to the desired 20 µm gap. Although the rheometer assumes a plate distance of 20 µm, the measurement of the sensor shows a deviation. The deviation is compensated by adjusting the lower plate. Once the alignment has the desired precision, the upper plate can be moved to the desired measuring position.

The DNA solutions of desired concentration and volumes can be applied between the plates using a pipette. Before a test is started, the sample rests under the rheometer for 5 minutes. A heating chamber is installed around the plates of the rheometer to reach the optimum temperature (here 25°C). In addition, a wet paper-towel is placed in an around the heating chamber to minimize the evaporation of the buffer.

After reaching a constant temperature, the sample is pre-sheared for 30 seconds at a constant shear rate of 100 s^{-1}. After pre-shearing, the sample rests for 5 minutes. The measurement time per data point is adjusted according to the shear rate. If data is to be produced for continuously increasing shear rates, the measurement time per data point should be at least 30 seconds and the shear rate should be logarithmically increasing.

Results and Discussion

We carried out measurements at different gap widths. In some experimental runs, we successively reduced the gap width stepwise, for instance, from 80 µm to 50 µm and finally to 20 µm to access shear rate up to 100000 s^{-1}. As an example, Figure 2 shows the viscosity function of the buffered λ-DNA solution at a concentration 0.125 mg/ml in the shear-rate range between 10 s^{-1} to 100000 s^{-1} determined at gap widths of 50 µm and 20 µm.

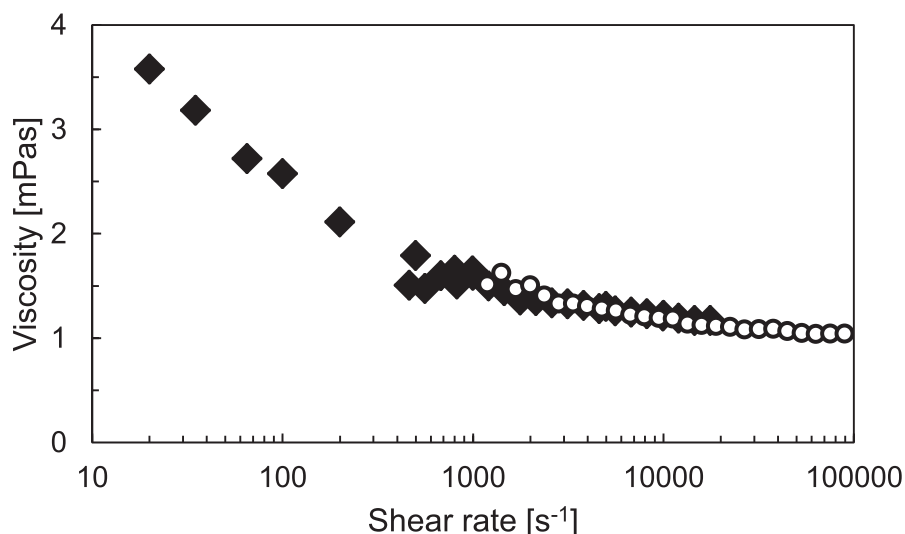

Figure 2: Viscosity Function of a λ-DNA solution (concentration 0.125 mg/ml). Diamonds and circles indicate measurements at 50 µm and 20 µm gap width, respectively.

As is apparent from the figure, the data measured at different gap widths, which was determined with different samples, nicely overlaps. This is possible due to the high precision of the plate alignment. The DNA solution is shear thinning: In the studied shear-rate range, the viscosity of the DNA solution decreases down to about 1 mPas, where it levels off into the infinite-shear viscosity plateau.

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References

Bansky A S, 2011: "Rheology and Its Applications in Biotechnology Comprehensive Biotechnology" Elsevier pp 189–201

Bravo-Anaya, L., Rinaudo, M., Martínez, F., 2016: "Conformation and Rheological Properties of Calf-Thymus DNA in Solution“, Polymers, 8, 51

C. F. Anderson and J. M. T. Record, 1982: ‘‘Polyelectrolyte theories and their applications to DNA’’, Annual Review of Physical Chemistry 33(1), 191-222

Chhabra, R. P. and Richardson, J. F., 2008: "Non-Newtonian Flow and Applied Rheology: Engineering Applications" 2nd edn, Oxford: Elsevier Science

D. Kokkinos, H. Dakhil, A. Wierschem, H. Briesen, A. Braun, 2016: "Deformation and rupture of Dunaliella salina at high shear rates without the use of thickeners", Biorheology 53, 1-11

Dakhil, H., and A. Wierschem, 2014: ‘‘Measuring low viscosities and high shear rates with a rotational rheometer in a thin-gap parallel-disk configuration’’, App. Rheol. 24(6), 63795-63801

Davies, G. A., and J. R. Stokes, 2005: ‘‘On the gap error in parallel plate rheometry that arises from the presence of air when zeroing the gap’’, J. Rheol. 49(4), 919-922

H. Dakhil, A. Wierschem, 2014: "Measuring low viscosities with a rotational rheometer in a thin-gap parallel-disk configuration", Applied Rheology 24, 63795

H. Dakhil, H. Do, H. Hübner, A. Wierschem, 2018: "Measuring the adhesion limit of fibronectin for fibroblasts with a narrow-gap rotational rheometer", Bioprocess and Biosystems Engineering 41, 353-358

H. Dakhil, D. Auhl, A. Wierschem, 2019: "Infinite-shear viscosity plateau of salt-free aqueous xanthan solutions", Journal of Rheology 63, 63-69

H. Dakhil, D. F. Gilbert, D. Malhotra, A. Limmer, H. Engelhardt, A. Amtmann, J. Hansmann, H. Hübner, R. Buchholz, O. Friedrich, A. Wierschem, 2016: "Measuring average rheological quantities of cell monolayers in the linear viscoelastic regime", Rheologica Acta 55, 527-536

Ikhomirova, I., Malysheva, Y., Kislov, N., Ryabov, M. and Muravyov, A., 2018: "Blood rheology and blood clotting in cancer", Thrombosis Research 164, S228

Mezger, T. G., 2006: "The rheology handbook: For users of rotational and oscillation rheometers", 2nd edn (Hannover: Vincentz Network)

Pan, S., D. A. Nguyen, B. Dünweg, P. Sunthar, T. Sridhar, and J. R. Prakash, 2018: ‘‘Shear thinning in dilute and semidilute solutions of polystyrene and DNA’’, J. Rheol. 62(4), 845-867

Pan, S., D. Ahirwal, D. A. Nguyen, T. Sridhar, P. Sunthar, and J. R. Prakash, 2014: ‘‘Viscosity radius of polymers in dilute solutions: Universal behavior from DNA rheology and brownian dynamics simulations,’’ Macromolecules 47(21), 7548-7560

Păun G, Rozenberg G and Salomaa A, 2005: "DNA computing: New computing paradigms" (Texts in theoretical computer science) 2nd edn (Berlin: Springer)

Pecora, R., 1991: " DNA: a model compound for solution studies of macromolecules", Science, 251, 893–8

Pipe, C. J., T. S. Majmudar, and G. H. McKinley, 2008: ‘‘High shear rate viscometry’’, Rheol. Acta 47(5-6), 621-642

Robertson, R. M., S. Laib, and D. E. Smith, 2006: ‘‘Diffusion of isolated DNA molecules: Dependence on length and topology,’’ Proceedings of the National Academy of Sciences 103(19), 7310

Ross, P. D. and Scruggs, R. L., 1968: "Viscosity study of DNA. II. The effect of simple salt concentration on the viscosity of high molecular weight DNA and application of viscometry to the study of DNA isolated from T4 and T5 bacteriophage mutants", Biopolymers 6, 1005–18

S. Pan, D. A. Nguyen, T. Sridhar, P. Sunthar, J. R. Prakash, 2014: "Universal solvent quality crossover of the zero shear rate viscosity of semidilute DNA solutions", Journal of Rheology 58, 339-368

Schildkraut, C., and S. Lifson, 1965: ‘‘Dependence of the melting temperature of DNA on salt concentration’’, Biopolymers 3(2), 195-208

Smith, D. E., 1999: "Single-polymer dynamics in steady shear flow", Science, 283, 1724–7

Smith, D. E., and S. Chu, 1998: ‘‘Response of flexible polymers to a sudden elongational flow,’’ Science 281(5381), 1335

Steffe, J. F., 1996: "Rheological methods in food process engineering", 2nd. edn, Freeman Press

Valle, F., Favre, M., Los Rios P de, Rosa, A., and Dietler G., 2005: "Scaling exponents and probability distributions of DNA end-to-end distance", Physical review letters 95, 158105

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Watson, J. D., Crick, F. H., 1953: "Molecular structure of nucleic acids; a structure for deoxyribonucleic acid", Nature, Apr 25; 171(4356):737-8

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