An experimental investigation of shear properties of porcine brain tissue

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An Experimental Investigation of Shear Properties of Porcine Brain Tissue

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Contents

1 Introduction 3

2 Methods and Materials 4
- 2.1 Experimental Setup . . . 4
- 2.2 Sample Preparation . . . 5
- 2.3 Testing . . . 5
  - 2.3.1 Dynamic Experiments . . . 5
  - 2.3.2 Relaxation Experiments . . . 7

3 Results 9
- 3.1 Dynamic Strain Sweep Experiments . . . 9
- 3.2 Small Deformation DFS Experiments . . . 12
- 3.3 Stress Relaxation Experiments . . . 12
- 3.4 Large Deformation DFS Experiments . . . 14

4 Discussion 18

5 Conclusions and Recommendations 21

Bibliography 23

A Experiment Preparation 25

B Sample Information 27

Chapter 1

Introduction

The head is identified as the body area most frequently involved in life-threatening injuries in traffic accidents [1]. Injuries of the human head can be divided in two groups. First, the head and brain can be injured by impact, deforming the skull and the brain. The second group of injuries are the injuries caused by fast and large rotations or translations of the head (characteristic duration between 1-50 ms [2]). These movements are regarded responsible for injuries like acute subdural hematoma and diffuse axonal injury. Victims may be disabled for life or die without superficial wounds on the head. To estimate the probability for internal damage, mechanisms that cause these injuries have to be known, and knowledge of the mechanical response of the contents of the head during impact must be acquired. With this, head protection criteria can be drawn up. Since it is impossible to determine the response of the brain to impact *in vivo*, an experimentally verified mathematical model for the human head is used. For such a model constitutive behaviour of the different tissues that constitute the brain has to be known.

This report deals with an experimental investigation of shear properties of porcine brain tissue. It is assumed (as commonly accepted in the literature) that, for finite strains, brain tissue behaves as a nonlinear viscoelastic material [3, 4, 5, 6], while in the limit of small strains, but for any strain rate, it behaves as a linear viscoelastic material. The material parameters are determined by means of dynamic torsional shear experiments and large deformation stress relaxation experiments. In Chapter 2, the methods and materials are discussed. Next, in Chapter 3, the results of dynamic and relaxation experiments are presented. Finally, the results are discussed and some conclusions and recommendations for future research are given in Chapters 4 and 5.

Chapter 2

Methods and Materials

The constitutive behaviour of solid materials is usually investigated by means of tensile experiments. Brain material, however, is very soft, which results in difficulties where clamping is concerned. Shear experiments are a common method to characterise viscous and viscoelastic fluids but can also be used to investigate the behaviour of soft solid tissues such as brain material. Margulies [7] conducted fast rotation experiments with physical head models and justified the assumption that brain tissue undergoes large shear strains when the head suffers fast rotations. For these reasons shear experiments are an excellent method to discover the constitutive behaviour of brain material.

2.1 Experimental Setup

Shear material properties of porcine brain were investigated with a rotational viscometer (Advanced Rheometric Expansion System (ARES), Rheometric Scientific^{TM}) using the plate-plate configuration. A schematic view of the experiment configuration is shown in Figure 2.1.

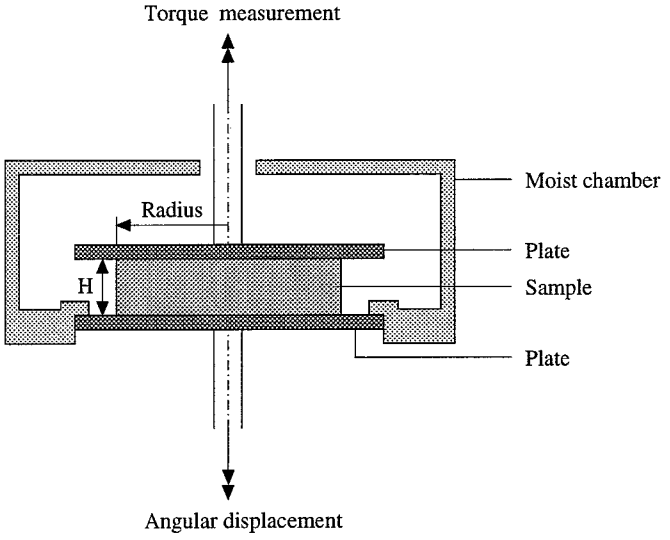


Figure 2.1: Schematic view of the experiment setup

A cylindrical sample is loaded by means of a prescribed angular dispacement of the lower plate. The upper plate is kept stationary and here the resulting torque is measured. The temperature was kept constant during testing and a moist chamber was used to prevent dehydration of the sample.

2.2 Sample Preparation

Cylindrical samples with a diameter of 12-13 mm and a height of 2-3 mm were prepared from tissue out of the pons of porcine brain. The brains were harvested from adult pigs, aged approximately six months, immediately following sacrifice by electrical shock at a local abattoir. The two hemispheres of the cerebrum were separated down the longitudinal fissure. The brain tissue was transported in an expanded polystyrene box and was wetted with a physiological saline solution (Merck, Darmstaat, Germany). A more accurate description of the preparation method is given in Appendix A. Information about the different samples is given in Appendix B.

2.3 Testing

Three different experiments were conducted:

1. Dynamic strain sweep test,
2. Dynamic frequency sweep test,
3. Stress relaxation test.

These will be discussed in the following sections.

2.3.1 Dynamic Experiments

Theory

In dynamic experiments the applied load varies in time. Often harmonic loading functions are used. In case of the ARES this means that a harmonically varying displacement is applied to the bottom plate, which imposes a sinusoidal strain $\gamma(\omega, t)$ on the sample, according to:

$$\gamma(\omega, t) = \gamma_0 \sin(\omega t) \tag{2.1}$$

where γ_0 is the strain amplitude and ω the angular frequency. As mentioned before, it is assumed that for small strain amplitudes the tested material is linear viscoelastic. Therefore, in steady state, the stress will also oscillate sinusoidally, with the same angular frequency. However, the viscous behaviour dissipates energy, which causes a phase shift $\delta(\omega)$ (also called loss angle) between the stress and the enforced strain. Without phase shift (i.e. $\delta(\omega) = 0°$) the material behaves purely elastically, whereas when $\delta(\omega) = 90°$, the material behaviour can be described as being purely viscous. The relation between the shear stress τ and the strain γ gives the parameters we are interested in:

$$\begin{aligned} \tau(\omega, t) &= \gamma_0(G'(\omega)\sin(\omega t) + G''(\omega)\cos(\omega t)) \\ &= G_d(\omega)\gamma_0 \sin(\omega t + \delta(\omega)) \end{aligned} \tag{2.2}$$

G' is called the elastic (or storage) modulus, which accounts for the elastic part of the stress, in other words the storage of mechanical energy. G'' is the viscous (or loss) modulus, which determines the viscous part of the stress, accounting for the dissipation of mechanical energy. $G_d(\omega, T)$ is the dynamic modulus and is defined as:

$$G_d = \sqrt{(G')^2 + (G'')^2} \tag{2.3}$$

These material parameters can be calculated using the measured torque values at the upper plate:

$$M(\omega, t) = M_0(\omega)\sin(\omega t + \delta(\omega)) \tag{2.4}$$

where $M_0(\omega)$ is the torque amplitude.

The storage modulus and the loss modulus are related to the measured quantities $M_0(\omega)$ and $\delta(\omega)$ according to:

$$G'(\omega) = \frac{2HM_0(\omega)}{\pi R^4 \alpha_0}\cos(\delta(\omega)) \tag{2.5}$$

$$G''(\omega) = \frac{2HM_0(\omega)}{\pi R^4 \alpha_0}\sin(\delta(\omega)) \tag{2.6}$$

where H is the sample height, R the sample radius, $M_0(\omega)$ the amplitude of the measured torque, and α_0 the amplitude of the angular displacement of the lower plate [8].

The enforced strain is the strain at the outer edge of the sample, which is given by:

$$\gamma_R = \frac{R\alpha_0}{H} \tag{2.7}$$

where H is the sample height, R the sample radius, and α_0 the amplitude of the angular displacement of the lower plate.
Using equations (2.5), (2.6) and (2.7), the dynamic modulus can be written as follows:

$$G_d(\omega) = \frac{2M_0(\omega)}{\pi R^3 \gamma_R} \tag{2.8}$$

Experimental Protocol

All experiments in this project were performed at a temperature of 38°C, since this is the body temperature of a pig. Three dynamic experiments were executed.

Dynamic Strain Sweep Test

The first was a dynamic strain sweep test (further referred to as DSS), during which an increasing strain amplitude was imposed while the angular frequency was kept constant. The samples were tested for two different frequencies: in the first test the frequency was kept constant at 10 rad/s (1.59 Hz) and in the second test the frequency was 200 rad/s (31.8 Hz). The strain was varied between 0.1% and 10% in both tests. These tests were done to investigate the validity of the assumption that brain material shows linear viscoelastic

behaviour for sufficiently small shear strains. If this is true, the dynamic modulus and the loss angle are no function of these small shear strains. Furthermore, the limit of the linear viscoelastic region can be found. Sampling was done logarithmically in the strain domain, with 10 measurements per decade.

Dynamic Frequency Sweep Test, linear region
Secondly, small deformation dynamic frequency sweep tests (further referred to as DFS) were used to investigate the visco-elastic properties within the linear regime. During these tests the angular frequency was increased from 0.1 rad/s up to 200 rad/s (0.0159 - 31.8 Hz), while the strain level (1%) was chosen within the linear regime, as found in the dynamic strain sweep tests. The sampling for this test was logarithmically in the frequency domain, also with 10 measurements per decade.

Dynamic Frequency Sweep Test, non-linear region
The third dynamic experiment was a DFS test also. Here, however, the strain amplitude was chosen higher than in the previous experiment, respectively 2, 5, 10, 20, 50 and 100%, in order to investigate the visco-elastic properties in the non-linear regime. The angular frequency was increased from 0.1 rad/s up to 100 rad/s, and sampling again was done logarithmically in the frequency domain, with 10 measurements per decade.

2.3.2 Relaxation Experiments

Theory

Information in the non-linear regime can also be acquired by performing large deformation stress relaxation tests. Stress relaxation is achieved by imposing a step strain γ_0 on the sample at $t = 0$ by rotating the bottom plate at a prescribed angle. This strain is kept constant for $t > 0$ and the resulting torque is measured and used to calculate the relaxation stress. This is illustrated in Figure 2.2.

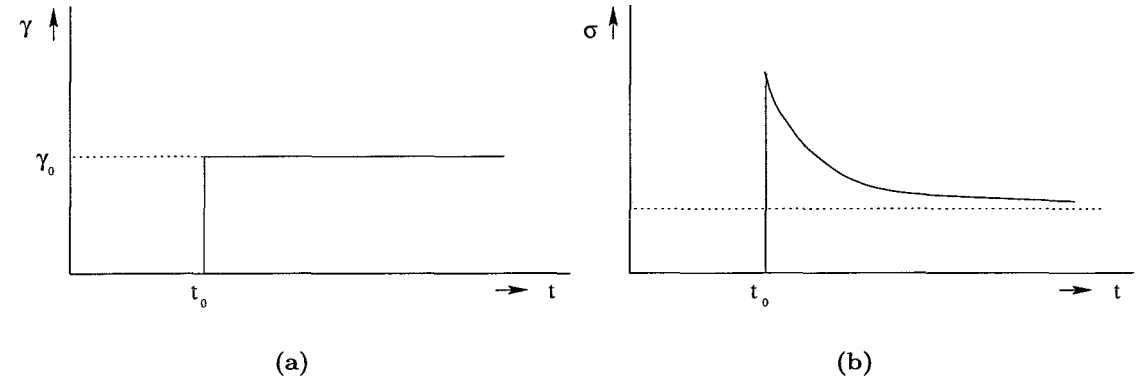


Figure 2.2: (a) Imposed strain step γ_0. (b) Typical relaxation curve.

For small strains the relation between the strain and the resulting stress is given by:

$$\tau(t) = G(t)\gamma_0 \tag{2.9}$$

in which $G(t)$ is the relaxation modulus. For larger strains the relaxation modulus becomes a function of the strain:

$$\tau(t, \gamma_0) = G(t, \gamma_0)\gamma_0 \tag{2.10}$$

When, on a logarithmic scale, the relaxation curves are nearly parallel, the stress relaxation modulus $G(t, \gamma_0)$ can be split into time- and strain-dependent terms.

$$G(t, \gamma_0) = G(t)h(\gamma_0) \tag{2.11}$$

In the linear regime, the stress relaxation modulus $G(t, \gamma_0)$ must reduce to the linear viscoelastic modulus $G(t)$ and $h(\gamma_0)$ must approach unity. The function $h(\gamma_0)$ is called the damping function and can be obtained at each strain by determining the amount of vertical shift on a log-log plot.

Experimental Protocol

The motivation to use stress relaxation experiments is twofold. Firstly, when after a while the relaxation modulus no longer changes as a function of time, the commonly used Maxwell parameter G_∞ can be found. Secondly, when the relaxation curves are (nearly) parallel, the damping function $h(\gamma_0)$ can be determined, which makes it possible to split the relaxation modulus $G(t, \gamma_0)$ into a time- and a strain-dependent term (equation 2.11).
The relaxation experiments were done with strain steps of 20, 40, 60 and 80%, and lasted 300 seconds. The sampling was done logarithmically; 200 measurements were taken during the first twenty seconds and 100 during the remaining 280 seconds.

Chapter 3

Results

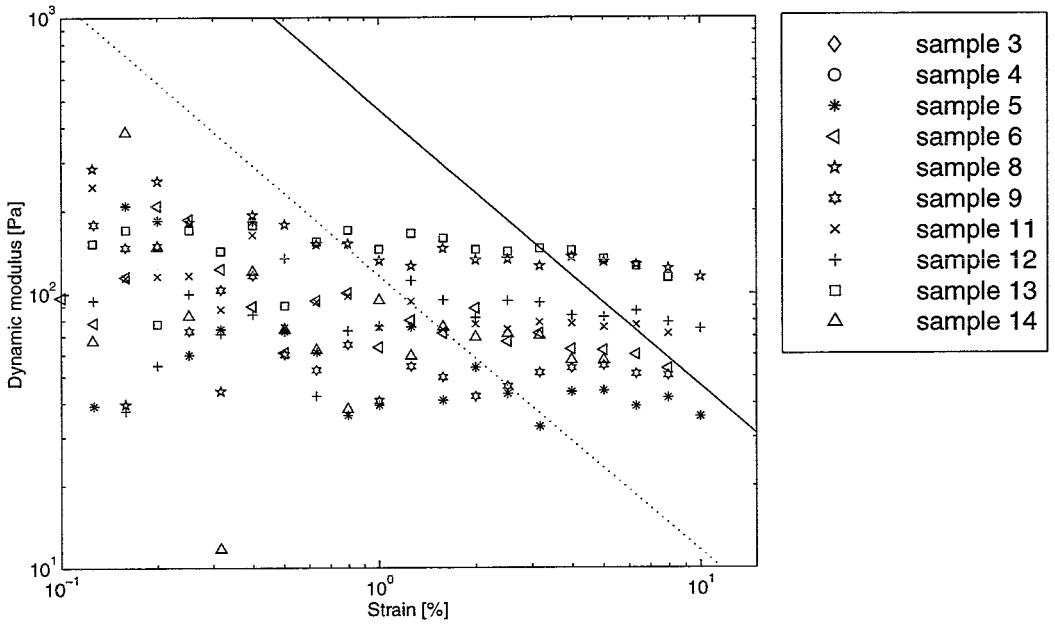
In this chapter the results of the experiments that were described in the previous chapter are discussed. Firstly, the assumption that the mechanical behaviour of brain tissue can be considered linear viscoelastic for small strains is investigated. The results of these tests are used to investigate the frequency dependency of the material by means of dynamic frequency sweep tests in the linear regime. Finally, the outcome of stress relaxation experiments in the non-linear regime are discussed. In total, fourteen samples were tested and the results of ten samples were considered useful for further analysis.

3.1 Dynamic Strain Sweep Experiments

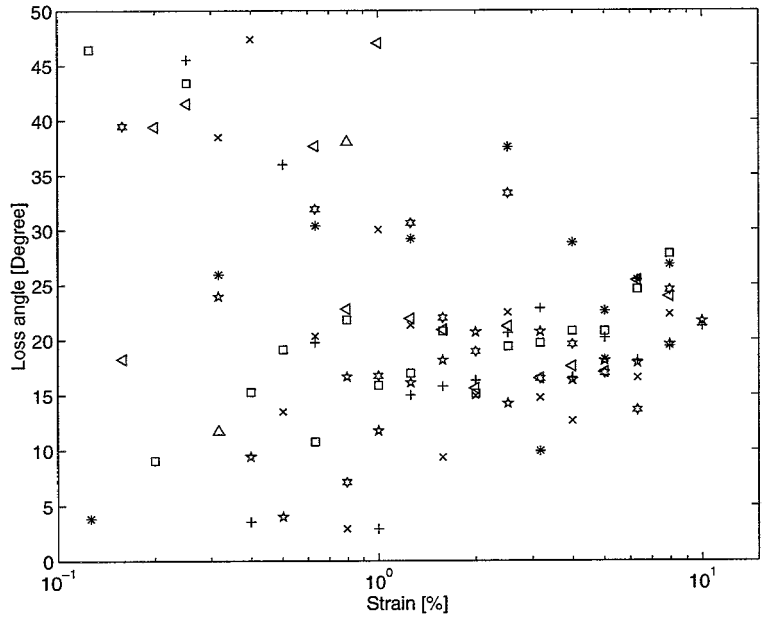
The existence of a linear viscoelastic regime was investigated with DSS tests. As mentioned in section 2.3.1 the samples were tested at two different frequencies: 10 rad/s and 200 rad/s. The results of these tests are shown in Figure 3.1 and Figure 3.2 respectively. The legend in Figure 3.1 is also valid for Figure 3.2.

In these figures the solid line represents the resolution of the torque transducer of the ARES. It has been determined by substituting the minimal torque transducer resolution $M_0 = 2 \times 10^{-6}$ Nm into equation (2.8). After visual inspection of the data points with respect to this line one could conclude that the resolution line can be shifted to the dotted line, increasing the number of data points within the accuracy of the transducer. The resolution that constitutes this dotted line is $M_0 = 5 \times 10^{-7}$ Nm. The subsequent discussion is limited to data points above this resolution line.

Figure 3.1 shows that the dynamic modulus lies in the range of 40-150 Pa and the loss angle in the range of 15-28 degrees. The dynamic modulus and the loss angle appear to be independent of the imposed shear strain up to approximately γ_0=5%, which means that the constitutive behaviour of porcine brain material can be considered as linear viscoelastic for shear strains upto 5% at a frequency f of 10 rad/s. It should be said that for several samples the loss angle was smaller than zero for one or more strains.
At a test frequency of 200 rad/s the dynamic modulus lies within the range of 140-600 Pa. In the tested strain range (γ_{max}=10%) no dependency of the dynamic modulus on the enforced strain was found. The loss angle showes an abnormality: the largest part of the loss angle data lies in the range of 220-255 degrees, which is theoretically impossible.

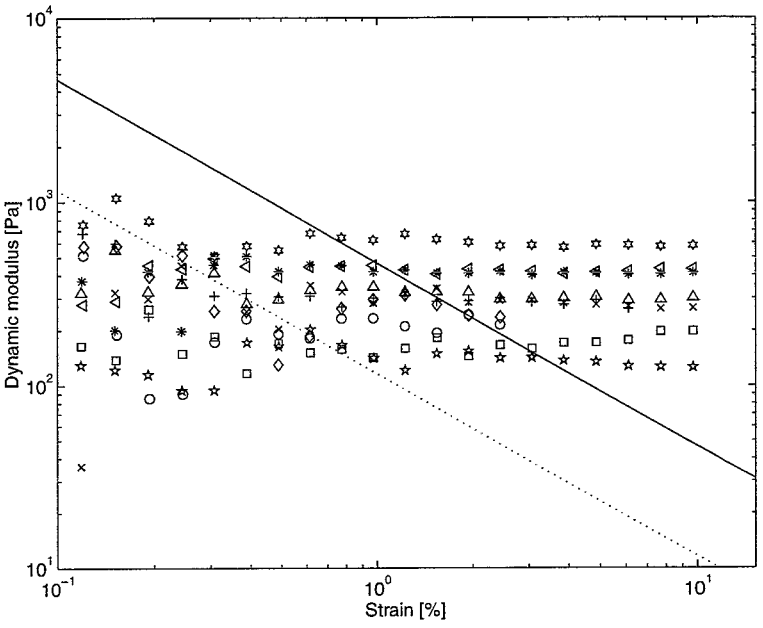


(a) Dynamic modulus

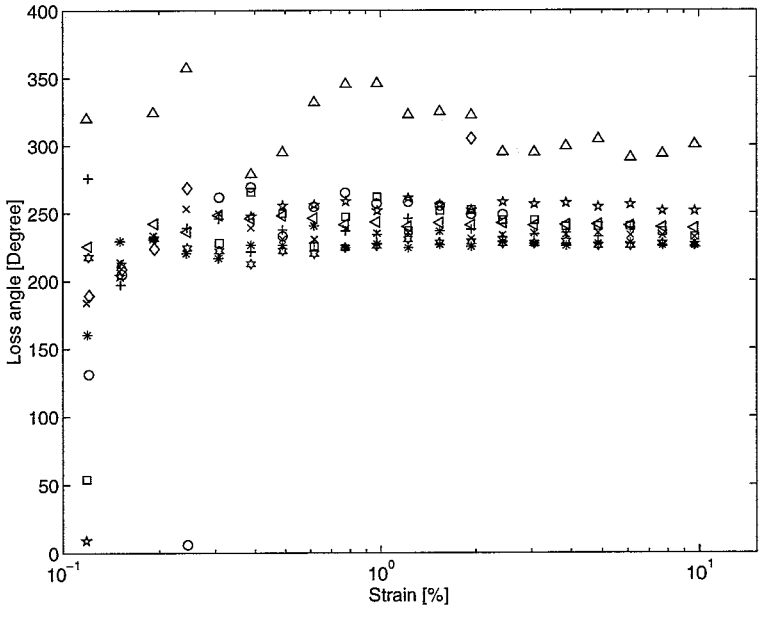


(b) Loss angle

Figure 3.1: Dynamic modulus and loss angle as a function of the strain γ_0 at a frequency f=10 rad/s and temperature T = 38°C for 8 different samples (the lines represent the resolution of the ARES).



(a) Dynamic modulus



(b) Loss angle

Figure 3.2: Dynamic modulus and loss angle as a function of the strain γ_0 at a frequency f=200 rad/s and temperature T = 38°C for 10 different samples (the lines represent the resolution of the ARES). See Figure 3.1 for legend.

Comparing Figures 3.1 and 3.2 it can be noted that the linear regime appears to be larger when the test frequency is higher. Furthermore, it can be seen that the modulus increases when the frequency increases. This increase, however, is very different for the seperate samples and is illustrated in Figure 3.3. An incidental circumstance is that for higher test frequency more data points fall within the accuracy of the ARES, since the dynamic modulus lies in a higher range.

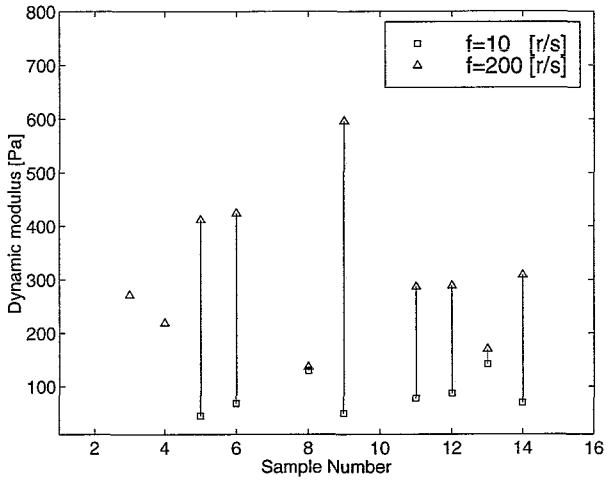


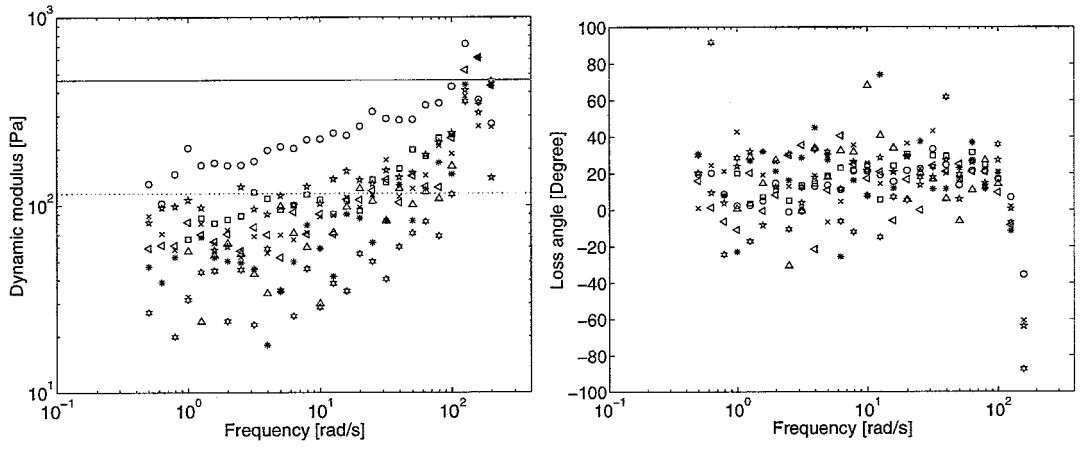
Figure 3.3: Average dynamic modulus taken from DSS tests results for shear strains larger than 1% (lines drawn for clarity).

3.2 Small Deformation DFS Experiments

The DSS tests showed that the linear viscoelastic behaviour of porcine brain material depends on the test frequency: the linear regime decreases with decreasing test frequency. The test frequencies for the DFS test varied between 0.1 and 200 rad/s, whereas the lowest frequency at which the linear regime was tested was 10 rad/s. At this frequency non-linear behaviour was found at a strain amplitude of approximately 5%. A smaller transition strain can be expected at a frequency of 0.1 rad/s. To be on the safe side, the frequency sweep tests were executed at a shear strain amplitude γ_0 of 1%. The results of these tests are recapitulated in Figure 3.4 (see Figure 3.1 for legend).
The dynamic moduli increase as a function of the frequency. They were in the range of 20-150 Pa at 1 rad/s and 100-320 Pa at 100 rad/s. However, only a small part of the data lies above the resolution line. Most loss angle data points were in the range of 3-35 degrees, but negative loss angles were found in this test as well.

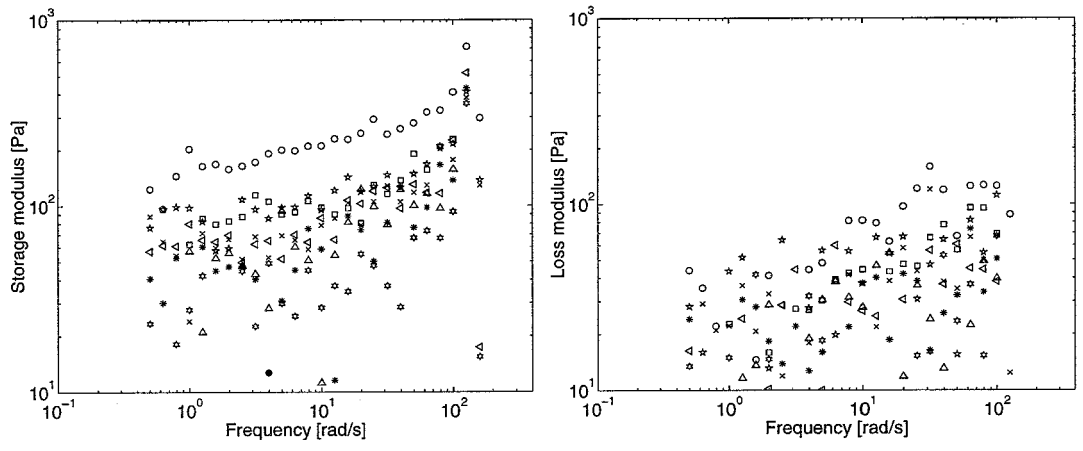
3.3 Stress Relaxation Experiments

Stress relaxation experiments are the simplest method to find non-linear behaviour of the tested material. Unfortunately, the results of the stress relaxation experiments in this project did not suffice. A typical example of a resulting stress relaxation curve is shown in Figure 3.5.



(a) Dynamic modulus

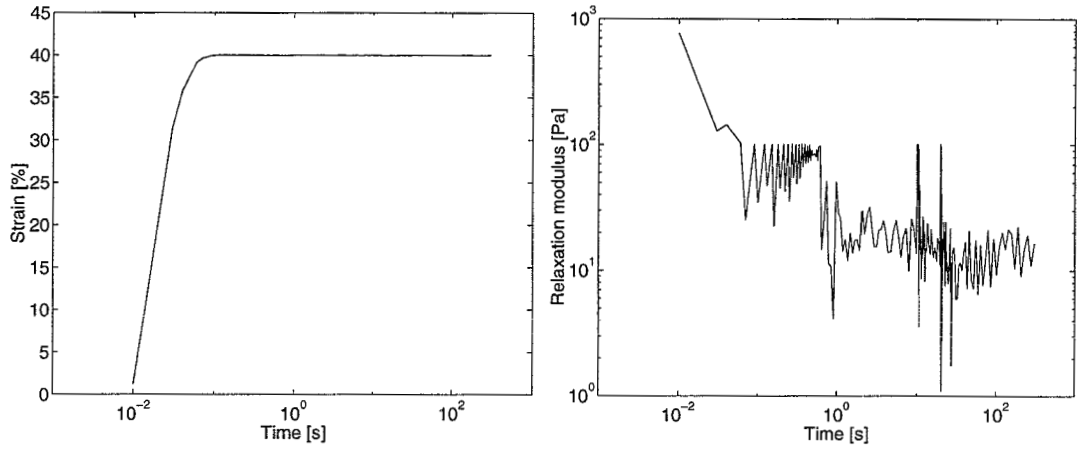
(b) Loss angle



(c) Storage modulus

(d) Loss modulus

Figure 3.4: Results of dynamic frequency sweep test at a shear strain amplitude γ_0=1% and temperature T = 38°C for 8 different samples (the lines represent the resolution of the ARES).



(a) Imposed strain γ_0

(b) Relaxation modulus $G(t)$

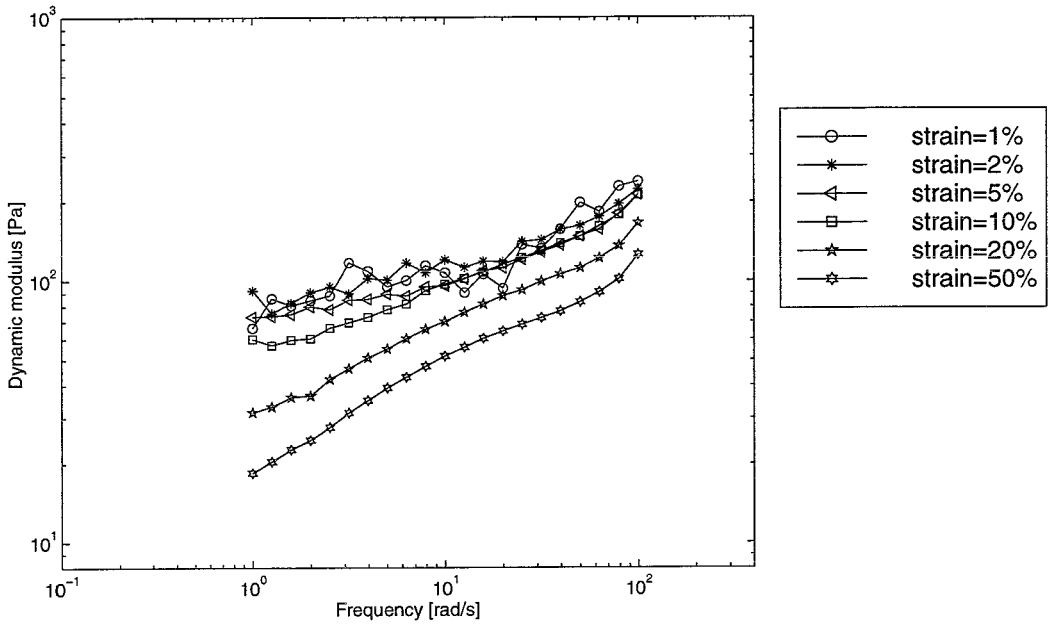
Figure 3.5: Imposed strain γ_0=40% and resulting relaxation modulus $G(t)$ for sample 9 as a function of the time at $T = 38°C$.

In this figure large, noisy, fluctuations in the relaxation modulus can be seen, which are considered to be not realistic. Much of these fluctuations can be explained by the fact that the measured torque values were of the same magnitude as the resolution of the torque transducer. An other contributing factor was the vibration of the upper (transducer) stem, which was caused by a mechanical defect of the ARES.

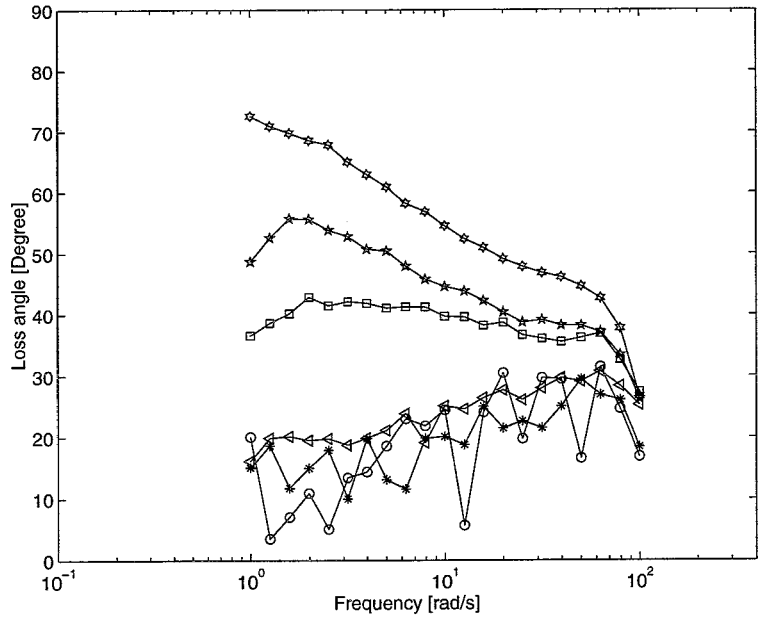
3.4 Large Deformation DFS Experiments

To get an impression of the non-linear behaviour of brain material large deformation DFS tests were done. Two samples were tested this way. The results are shown in Figure 3.6 and Figure 3.7.
Three observations can be made. Firstly, the results show more fluctuations when strain levels are small. Secondly, the dynamic modulus decreases with increasing strain amplitude. The opposite is true for the loss angle: increasing strain amplitude causes a larger loss angle. Finally, looking at the frequency dependency, it can be noted that for strains upto 5% the loss angle increases with increasing test frequency, whereas for larger strain levels the loss angle decreases with increasing test frequency.

In Figure 3.8 the dynamic modulus is shown as a function of the strain at a frequency of 10 rad/s. When the fluctuations at small strains are neglected, it is clear that the dynamic modulus decreases with increasing strain amplitude, as is expected in the non-linear regime.

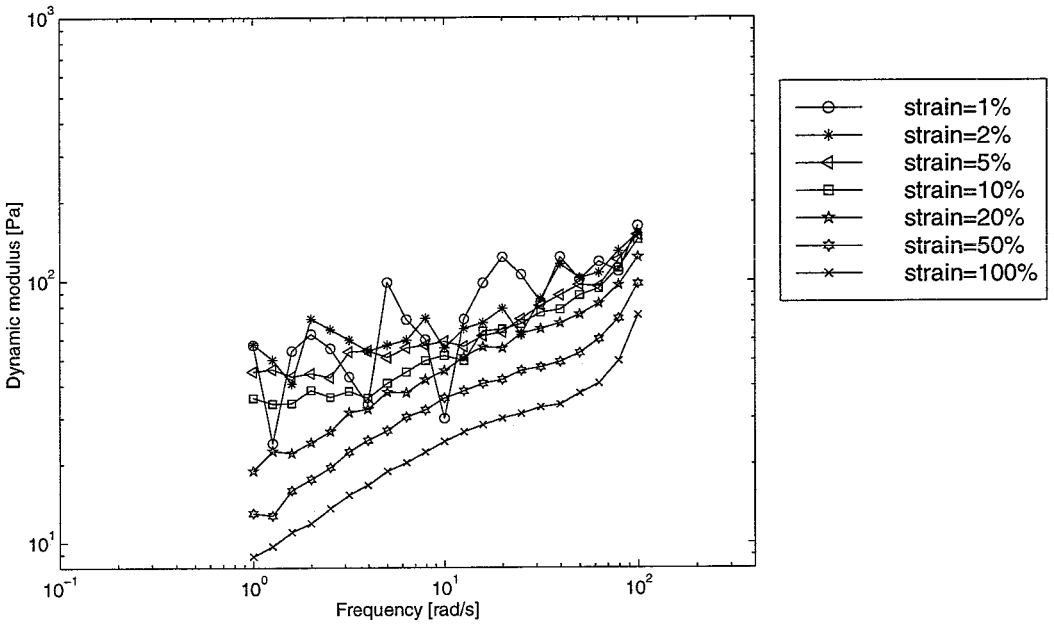


(a) Dynamic modulus

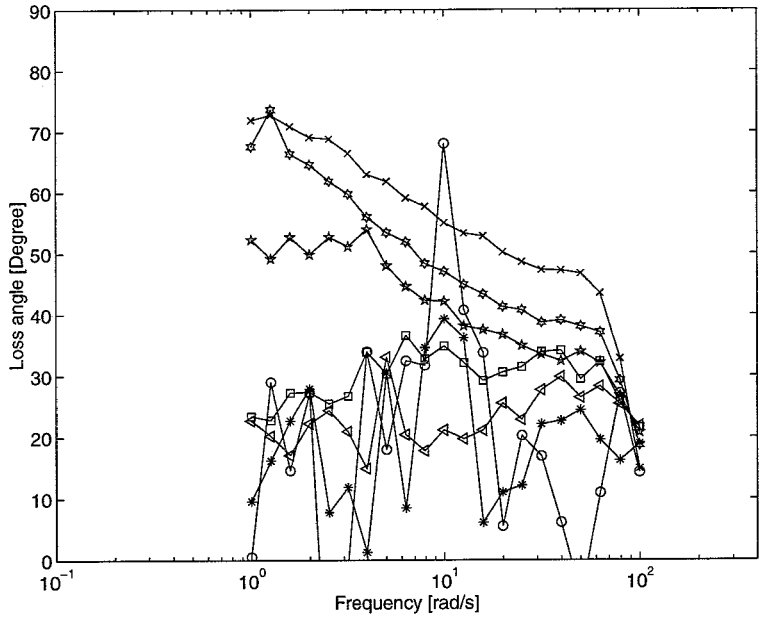


(b) Loss angle

Figure 3.6: Dynamic modulus and loss angle as a function of the frequency f at different strain amplitudes γ_0 and at $T = 38°C$ for sample 13.



(a) Dynamic modulus



(b) Loss angle

Figure 3.7: Dynamic modulus and loss angle as a function of the frequency f at different strain amplitudes γ_0 and at $T = 38°C$ for sample 14.

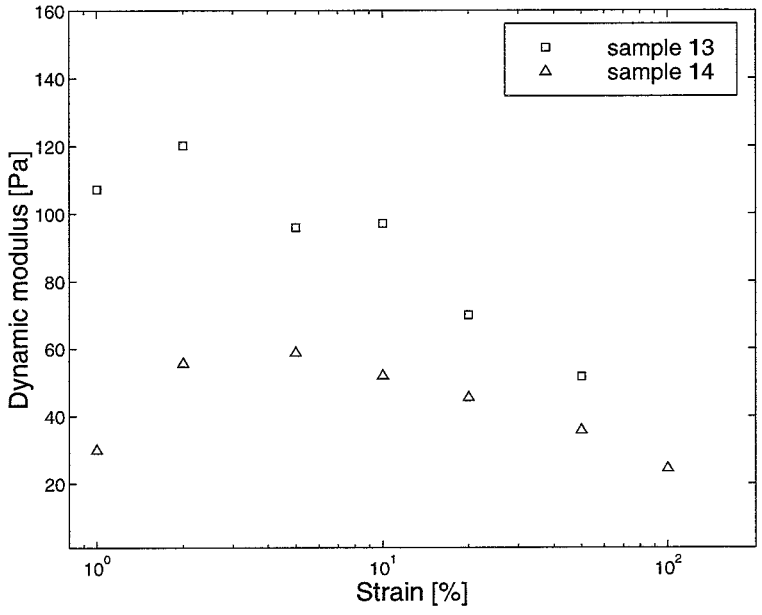


Figure 3.8: Dynamic modulus as a function of the strain γ_0 at a frequency f=10 rad/s for sample 13 and 14.

Chapter 4

Discussion

The measurements and calculations that are described in the previous chapters are influenced by several error sources that are difficult to control.

Concerning the samples

Firstly, large errors can be introduced by the preparation of the samples. Brain tissue is a soft tissue, and therefore it is difficult to cut samples with precisely the same dimensions. Since all samples are cut with the same cork bore, the differences in radius of the samples are minor. However, residual stresses may cause deviations from the assumed circular shape. The height of the different samples is not as uniform as the diameter, since it was cut by hand and without the use of a mould. This also made it very difficult to cut the upper and lower surface of a sample perfectly parallel and flat. The height of the samples is determined by measuring the distance between the upper and lower plate once the sample is in position. This distance is adjusted by measuring the normal force which is imposed upon the upper plate when it comes in contact with the sample. When the normal force increases, contact has been established. When the upper and lower surfaces of the sample are not parallel, the final height adjustment is reached by visual inspection. However, when the upper and lower surfaces of the sample are not flat, it is possible that parts of the sample surface are not in contact with the plates. This could result in lower moduli.
Furthermore, the positioning of the sample with respect to the plates is difficult. A good alignment of the center of the samples with the axis of symmetry of the viscometer is not easy to achieve.
All these factors can cause large deviations in the separate results. Some experiments and calculations on the effect of these uncertainties have been done by Meulman [8], who found a maximum error of 15% due to diameter uncertainties.

In addition to geometry errors, a spread in the results can be caused by tissue variations. A predominant direction of the fibres can introduce anisotropic behaviour in which case samples with different origin and orientation will have different material properties.

Another problem working with brain tissue is degeneration, which causes differences between *in vivo* and *post mortem* properties as well as changing *in vitro* properties as a function of time after sacrifice of the donor. Enzymes will break down proteins and cell walls and the mechanical behaviour is altered. The degree of degeneration is dependent on

tissue type, the method of storage and preservation, and the time after sacrifice. Grimm *et al.* [9] examined the influence of several storage solutions and found that prolonged storage of brain material is best accomplished by placing the samples dry in close-fitting, airtight containers in a refrigerator.

Concerning the experiments

Linearity can only be explained when an assumption for a model is made. A commonly used model to describe linearity is the generalised Maxwell model. In this model linearity is defined by stating that the stress is proportional to the strain. In this situation the frequency has no influence on the stress. However, one of the results of the DSS tests was that the linear regime increased with increasing frequency. If this is true, the assumption made in the linear Maxwell model might not hold for brain material.
Also, one should bear in mind that shear experiments alone will not suffice in order to completely characterise brian material, since the tissue volume does not change during these tests.

In this project negative loss angles were found in several experiments, which, in theory, have no physical meaning. An explanation for this phenomenon could be found in the fact that the measured torque values lay below the resolution of the torque transducer. The loss angles that were found in the DSS tests at a frequency of 200 rad/s were extremely high. No explanation for this abnormality has been found.

The stress relaxation experiments in this project had no useful outcome for two reasons. Firstly, measured torque values were too small. The second reason was a mechanical flaw of the ARES. The upper stem vibrated when the motor was in transient mode and this vibration is measured by the transducer. This noise can be reduced by adding weight to the stem.

It is impossible to jump the strain γ instantaneously to γ_0 because of inertial effects. A way to control the manner in which the strain is forced on the sample is with a rate sweep test. The rate sweep test imposes a prescribed strain rate on the sample during a commanded period of time. Different zones can be generated, each with their own strain rate and time span. By choosing a strain rate of zero s^{-1} in the second zone, a stress relaxation test can be simulated. This way a known strain level is reached in a known time interval. Also, the strain rate dependency of the material can be tested. This is illustrated by Figure 4.1.
This test, however, was not available on the ARES at the time of this project.

The DFS tests with larger strain amplitudes had some interesting results. The fluctuations at smaller strain amplitudes are, again, caused by torque values that lay below the resolution of the torque transducer.
The trend of the dynamic modulus is as expected (see [10]): the dynamic modulus decreases with increasing strain amplitude. According to the results of the DSS experiments (Figure 3.1) the results of the DFS experiments for strains of 1, 2 and 5% should overlap, since these strain levels are within the linear regime. This is true for all measurements that fall within the accuracy of the ARES.

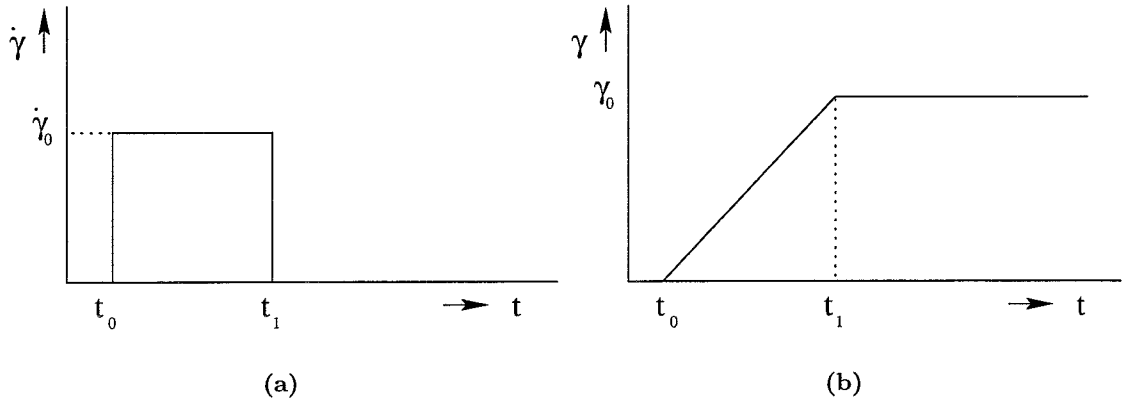


Figure 4.1: (a) Strain step γ_0. (b) Typical relaxation curve.

The increasing loss angle that occurs for increasing strain amplitude can be translated into an increase in viscous behaviour. Also, for strain levels of 1, 2, and 5% the loss angle increases with increasing test frequency, which indicates more viscous and less elastic behaviour as the frequency increases. For larger strain levels the loss angle decreases with increasing test frequency, which could be interpreted as a decrease in viscous behaviour. The results of this test can be used as validation of a chosen constitutive model.

When comparing the results of different experiments, it must be noted that all experiments are performed on separate samples. It was very difficult to cut the samples at a specific height and relatively large differences resulted. This may be one of the reasons for the perceived spread in data. The use of new samples in every experiment makes sure that no damaged or altered samples are used. Reproducibility of the measurements was tested for some samples by performing two identical DFS experiments consecutively with the same sample under equal test conditions and comparing the results. Visual comparison gave the impression that the measurements were not reproducible. However, since the measurements were too close to the torque resolution, no attempt was made to compare the results statistically and no conclusions could be drawn.

Unfortunately, nothing can be said about the adhesion between the samples and the plates of the viscometer. When slip occurs during oscillatory experiments the commanded strain will not be reached and the results will be affected.

Chapter 5

Conclusions and Recommendations

The objective of this project was an experimental investigation of shear properties of porcine brain tissue. Knowledge of these properties and of the constitutive behaviour of brain tissue must be acquired in order to design a numerical model that predicts the mechanical response of the brain to for instance impact. Material parameters are determined by means of dynamic torsional shear experiments. The assumed linear regime for small strains is found with dynamic strain sweep tests and the non-linear regime is investigated with stress relaxation experiments. In these experiments the measured torque values were of the same magnitude as the accuracy limit of the rheometer, which, especially in the case of the relaxation experiments, resulted in unreliable data. A second method used to investigate the non-linear behaviour was with dynamic frequency tests at higher strain amplitudes. Larger strain amplitudes resulted in a decreasing dynamic modulus, which was as expected (see [10]).

During the project several problems and questions were encountered and some points of attention are in place for future research:

Recommendations for the experimental setup:

- For future testing a torque transducer with a higher resolution should be used.
- To determine whether or not slip occurs during oscillatory experiments, an oscilloscope can be used to visually check the shape of the curves of the enforced angular displacement and the measured torque. In the case of slip the shape of the torque curve will not be sinusoidal. Slip may be prevented by using tissue glue that is thinly applied to the plates before positioning the sample.

Recommendations concerning the sample:

- Use a larger brain, for instance that of a calf, so that a sample with a larger diameter can be cut and a better diameter to height ratio can be obtained.
- Design a more accurate preparation method (especially where the height of the sample is concerned) in order to lower the spread in measured properties caused by geometry differences of the samples. A mould could be very useful (see [8]).

- Histological investigations should be performed to get an indication of the structural changes induced by the storage, the preparation method and the experiments. Also, it can be used to estimate any predominant direction of fibers in a sample.
- Direct contact of brain tissue with fluid should be minimised in order to reduce the effect of fluid absorption. Prolonged storage of brain tissue (longer than 30 minutes) may be best accomplished by placing the samples dry in close-fitting, airtight containers (e.g. plastic bags or plastic wrap) in a refrigerator. This will minimise both autolysis and water evaporation [9].

Recommendations for a future test protocol:

- Investigate the frequency dependency of the linear regime.
- Investigate the non-linear regime with more accurate stress relaxation experiments.
- Are DFS experiments with large strain amplitudes a useful method to investigate non-linear behaviour?
- Reproducibility of the experiments has to be further checked: in the case of reproducibility of small oscillatory shear experiments, the same sample can be used to measure non-linear behaviour.

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Appendix A

Experiment Preparation

- **Supplier**
  Slachthuis De Wit
  Industriepark 10
  5663 PG Geldrop
  040-2862290
  Contact: Bram de Wit
  Monday, Wednesday and Friday (coffee break: 9.30h-10.30h)

- **Transportation**
  The brain material is transported on a with **physiological saline solution** (0.15M NaCl (Merck, Darmstaat, Germany)) (cell lab.) wetted **diaper** (bio lab.) in a **polystyrene box** (cell lab.).
  Contact bio lab. WFW: Theo van Duppen
  Contact cell lab. W/BMT: Carlijn Bouten

- **Storage**
  The brain material is stored in a properly closing **jar** (cell lab.), filled with physiological saline solution. The jar is labelled with name, date and contents. Store the jar (no longer than three days) in a refrigerator ($T = 7°C$) (cell lab.).

- **Preparation**
  Before cutting the samples it is advisory to prepare the ARES for testing; mount the correct plates and set the correct temperature.
  The samples are cut in a fume cupboard in the cell lab. The following is a list of necessities:

  - **plastic gloves**
  - **petri dish with lid** (for transportation of the samples)
  - **small garbage bag**
  - **paper towels**
  - **overhead slide** (cutting surface)
  - **sharp knife**
  - **hollow bore** with the desired diameter

- **scalpel** with **spare blades**
- **blunt pair of tweezers**
- **physiological saline solution**
- **tape** (to close the garbage bag)
- **spatula**
- **jar with lid** (tissue storage)
- **freezer**, $-80°C$

- **Cutting samples**
  Always wear gloves. Wet the overhead slide with little physiological saline solution to prevent the brain tissue from sticking to it. Put the brain tissue on a overhead slide and find a homogeous part of tissue. The knife can be used to cut away tissue that obscures visibility. The scalpel is for finer cutting. Try not to stretch the tissue too much, since this may cause damage. Twist the hollow bore and at the same time apply pressure in order to cut a sample. With the scalpel the sample height can be adjusted. Put the sample in the petri dish with (little) physiological saline solution.

- **Cleaning**
  Gather the unused brain material, the diaper, the used tissues and the gloves in the garbage bag and store it in the freezer. Clean the used tools and disinfect the fume cupboard.

- **Experiments**
  For each experiment an experiment record should be made containing the following information:

  - date
  - animal
  - age of the animal
  - cause of death of the animal
  - the place where the sample is taken out of the tissue, possibly illustrated with pictures and/or photo's
  - sample identification
  - sample dimensions (diameter, height)
  - storage conditions (temperature, storage solution, storage time)
  - time between death and experiment
  - experiment type
  - experiment conditions (temperature, humidity, normal force, strain, frequency, etc.)
  - start and end time of experiment
  - file name of saved data
  - observations during experiment
  - the degree of adhesion of the sample to the plates

  Contact: Gerrit Peters

Appendix B

Sample Information

Some additional sample information is given in Table B.1. Here, D is the sample diameter, H is the sample height, t_d is the time between death and start of experiment and t_p the time between preparation of sample and start of experiment (both time indications in hours:minutes).

Table B.1: Sample information

	D [mm]	H [mm]	$t_d[h:m]$	$t_p[h:m]$
sample 3	12	3.336	4:55	0:45
sample 4	12	3.220	3:45	2:35
sample 5	12	2.440	23:16	0:51
sample 6	13	3.450	28:29	0:54
sample 8	13	2.240	70:22	0:32
sample 9	13	2.980	1:15	0:40
sample 11	13	2.470	1:02	0:32
sample 12	13	2.649	5:40	1:10
sample 13	13	2.519	27:40	0:25
sample 14	13	2.539	49:29	0:29