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ÚSTAV FYZIKÁLNÍ A SPOTŘEBNÍ CHEMIE

# UTILIZATION OF DYNAMIC MECHANICAL ANALYSIS AS AN ADD-ON MODULE OF OSCILLATORY RHEOMETRY FOR HYDROGELS

VYUŽITÍ DYNAMICKÉ MECHANICKÉ ANALÝZY JAKOŽTO DOPLŇKOVÉHO MODULU OSCILAČNÍ REOMETRIE  
PRO HYDROGELOVÉ SYSTÉMY

## BACHELOR'S THESIS

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Utilization of dynamic mechanical analysis as an add-on module of oscillatory rheometry for hydrogels

### Bachelor's Thesis:

1. Write the recent state of the art aimed on the utilization of dynamic mechanical analysis (DMA) to study hydrogels.
2. Suggest and optimize the workflow of DMA measuring for semi-solid materials.
3. Determine the mechanical properties of hydrogels with different ways of crosslinking using dynamic mechanical analysis.
4. Correlate the outputs from DMA with standard oscillation experiments.
5. Discuss the results and applicability of DMA in study of hydrogels.

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Student's signature

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## **Abstract**

The bachelor's thesis deals with the optimization of dynamic mechanical analysis and its use for characterization hydrogels with different types of cross-linking. During the optimization, problems with the correct preparation of samples for the best possible results were solved, the selection of the most suitable geometry for the measurement of the selected hydrogels, and then detailed instructions for using the rheometer and setting up the Trios software were written. Next, the results from measurements using oscillatory shear rheometry and subsequent comparison of results between Dynamic mechanical analysis and oscillatory shear rheometry were discussed.

## **Abstrakt**

Bakalářská práce se zabývá optimalizací dynamické mechanické analýzy a jejím využitím pro charakterizaci hydrogelů s různým typem síťování. Při optimalizaci byly řešeny problémy se správnou přípravou vzorků pro co nejlepší možné výsledky, výběr nejvhodnější geometrie pro měření vybraných hydrogelů a následně byl sepsán podrobný návod pro používání reometru a nastavení softwaru Trios. Dále pak byly diskutovány výsledky z měření pomocí oscilační smykové reometrie a následné porovnání výsledků mezi dynamickou mechanickou analýzou a oscilační smykovou reometrií.

## **Keywords**

Dynamical mechanical analysis, Oscillatory shear rheometry, Young modulus, Agarose hydrogels, Poly(ethylene-glycol) thiol, Semi-interpenetrated polymer networks.

## **Klíčová slova**

Dynamická mechanická analýza, Oscilační smyková reometrie, Youngův modul, Agarózové hydrogely, Poly(ethylen-glykol) thiol, částečně provázané polymerové síťování.

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# 1 Introduction

Hydrogels are highly water-containing substances composed of cross-linked polymer chains in a dispersion medium (water). These materials have gained significant attention due to their unique mechanical properties and are being widely investigated in various fields, particularly in medicine. They have potential applications in drug delivery systems and tissue engineering, where they can be used to create tissues similar to human tissues for transplantation. In order to select the appropriate hydrogel and active substance, it is imperative to have knowledge of the hydrogel's mechanical properties, which can be acquired through rheological measurements. Rheology, a branch of science concerned with the study of the deformation and flow of matter, plays a crucial role in determining the mechanical properties of hydrogels. Typically, oscillation tests are employed to determine the mechanical properties of hydrogels, specifically the viscoelastic moduli. The storage modulus, which reflects the energy absorption capacity of a material upon deformation, and the loss modulus, which indicates the energy dissipation capacity upon deformation (flow), are two important parameters that can be measured using oscillatory shear rheometry. However, this method is limited to measuring properties in one direction and is not suitable for anisotropic materials.

To overcome this limitation, dynamic mechanical analysis (DMA) can be used to measure the mechanical properties of hydrogels in different direction. The DMA method has not been utilized at the VUT Faculty of Chemistry, and the goal of this work is to optimize the method for use in multiple experiments. However, understanding the mechanical properties of anisotropic materials requires more than one method. Hence, it is essential to employ a combination of various techniques, such as the Dynamic mechanical analysis (DMA) and oscillatory shear rheometry, to obtain a comprehensive understanding of the mechanical behaviour of these materials.

The theoretical section of this work includes the characterization of hydrogel systems, preparation, and characterization of representative hydrogels used in the experimental work. The second section covers the fundamental principles of rheology and highlights the differences between dynamic mechanical analysis and oscillatory shear rheometry. Lastly, the theoretical portion focuses on recent research in the areas of hydrogels and rheology (state of the art).

The experimental part of this work is aimed on optimizing the DMA method. This involves preparing the sample, configuring the rheometer and trios measuring system, and evaluating various geometries. The study also investigates and discusses the differences between measurements obtained using DMA and oscillatory shear rheometry.

## **2 Theoretical part**

### **2.1 General characterization of hydrogel systems**

Hydrogels are a type of colloidal dispersion system, in which solid particles are dispersed in a liquid medium, with water being the typical liquid medium for hydrogels. The 3D polymer network formed by the solid particles within a hydrogel is responsible for its ability to absorb and retain water. This network structure allows for the hydrogel to swell in size as it absorbs water, while still maintaining its overall integrity and shape. The network chains in the gel are connected to each other with various types of bonding to form large molecules on macroscopic scale. Type of cross-linking, together with the 3D structure of the hydrogel, are the main reasons why hydrogels are able to absorb large amounts of water. The ability of hydrogels to absorb water arises from hydrophilic functional groups ( $\text{OH}^-$ ,  $\text{COO}^-$ ) attached to the polymer backbone while their resistance to dissolution arises from cross-links between networks chains [1, 2, 3].

Important property of hydrogels is their highly porous structure with a softer surface which makes them very similar to living tissue such as bone tissue [4]. The gel is type of dispersion phase when it is neither completely solid nor completely liquid and this phase is mostly called semi-solid. Thanks to this dispersion phase we can specify mechanical properties with elastic modulus and with viscous modulus which is considered to be smaller than elastic due to the fact that the gel usually holds the structure [1, 3]. These mechanical properties are further elaborated in the chapter 2.2 on the principles of rheology.

Some specific hydrogels are known as smart material for their behaviour responding to physical and chemical stimuli. In medical applications, we often encounter pH-responsive hydrogels that can change the degree of dissociation due to their functional groups. Such materials have potential for release and uptake for protons with almost zero changes in pH. This property makes them great delivery vehicles for drugs, peptides, and protein delivery [2, 5].

#### **2.1.1. Classification of hydrogel systems**

Hydrogels can be classified into categories due to different type of cross-linking, presence of electric charge and based on cross-linked junction. Depending on the chemical functionalities involved, physical and chemical cross-linking reactions can be distinguished. Covalent bonds are formed between at least one type of hydrogel forming macromolecule during chemical cross-linking. Physical cross-linking creates strong, non-covalent interactions between the constituent macromolecules. In chemical cross-linking strategies, macromer reactivity and associated cytotoxicity have to be carefully balanced [2, 6].

### *Based on type of cross-linking*

As already mentioned, hydrogels are divided into two categories i.e., physically cross-linked and chemically cross-linked.

#### *Physically cross-linked hydrogels*

It is the most common and easiest way for hydrogel formation by cross-linking through physical interactions. This includes weak bonding interaction in which hydrogen bonds have the greatest influence. There are numerous physical gelation mechanisms. Gelation is typically caused by a thermodynamic change in the polymer solution that increases polymer-polymer interactions. However, it is important to note that for this process to occur, the polymer fibres must also exhibit a strong hydrophilicity, which allows for interactions between the polymer and the surrounding solvent molecules. This balance between polymer-polymer and polymer-solvent interactions is essential for the formation and stability of hydrogels. Physical bonding makes hydrogels temporarily existing and reversible in nature. Example of physically cross-linked hydrogel is Agarose which is prepared by cooling hot solution of agarose to form physically cross-linked gel. Agarose as well as gelatine are physically cross-linked hydrogels created by the sol-gel temperature inducted process [2, 6, 7, 8, 9].

Physically cross-linked hydrogels can result from ionic interactions between polymers. The inclusion of di- or trivalent counter ions in an ionic polymer can lead to cross-linking between polymers. This approach is based on the principle of gelling polyelectrolyte solutions (e.g., Na<sup>+</sup> alginate-) with a multivalent ion of opposite charges (e.g., Ca<sup>2+</sup> + 2Cl<sup>-</sup>). Additional examples of such hydrogels include chitosan-polylysine, chitosan-glycerol phosphate salt, and chitosan dextran hydrogels [7].

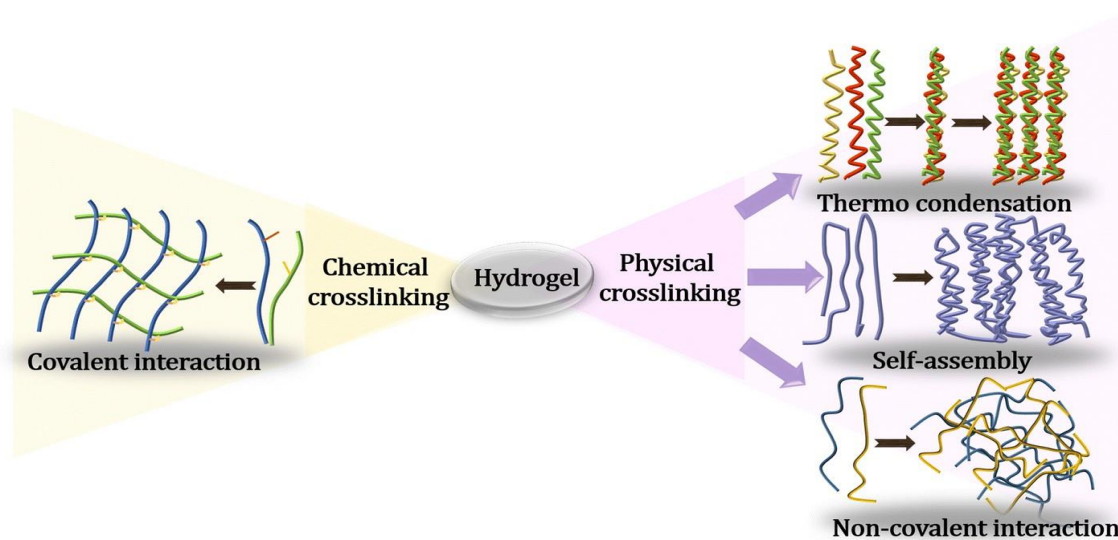
A hydrogen bond arises from the interaction between an electron-deficient hydrogen atom and a functional group with high electron density. Hydrogen bonding can serve as a cross-linking mechanism in polymers that contain multiple hydroxyl groups, such as poly (vinyl alcohol). This bonding can facilitate cross-linking between polymers that possess the same or different functional groups. A blend of poly (acrylic acid) and polyacrylamide, for instance, demonstrates partial insolubility in water due to hydrogen bonding between their respective carboxyl and amide groups, despite the high solubility of each polymer in water. Hydrogels with hydrogen bond can be affect with factors like molar ratio of each polymer, polymer concentration, type of solvent, temperature, and polymer structure [7, 9].

#### *Chemically cross-linked hydrogels*

Second category are chemically cross-linked hydrogels. This process involves the use of a crosslinking agent to connect two polymer chains or graft monomers onto the polymer backbone. The functional groups of natural and synthetic polymers, such as OH, COOH, and NH<sub>2</sub>, can be cross-linked using compounds such as aldehydes



(e.g., glutaraldehyde, adipic acid dihydrazide). Monomers that contain double bonds and are hydrophilic, such as acrylic acid, acrylamide, and hydroxyethyl methacrylate, can undergo polymerization and form chemical bonds with cross-linkers that also have double bonds. The resulting chemical-bonded hydrogels possess permanent properties due to the covalent nature of the cross-linking entity. Chemical cross-linking improves hydrogel's ability to absorb water through its hydrated porous structure [2, 7, 10].



**Fig. 1** Schematic illustration of hydrogels fabricated through chemically cross-linked or physically cross-linked [10].

### *Based on Cross-Linked Junctions*

This classification divides hydrogels into three major groups: blend hydrogels, block copolymeric hydrogels, and polyelectrolyte complex hydrogels [2].

#### *Blend hydrogels*

These are a combination of two or more hydrogels with same type of crosslinking that can be combined to form a new material with distinct physical properties. These hydrogels are used in a variety of fields and have received significant attention due to their new and superior properties such as electrical, thermal, and mechanical properties when compared to conventional hydrogels. Illustrative examples of such hydrogels include alginate-methylcellulose or alginate gelatine. [2, 11].

#### *Block Copolymeric hydrogels*

These hydrogels are constructed from blocks of various polymerized monomers. Polystyrene-b-poly (methyl methacrylate), for example, is typically produced by first polymerizing styrene and then polymerizing methyl methacrylate (MMA) from the reactive end of the polystyrene chains. Block copolymers have been widely used in the fabrication of nanostructured materials improve drug efficacy, reduce drug toxicity, and provide long-term therapy. In 2013 Lee et. al. produced a block copolymer hydrogel by

introducing positively charged polycarbonates with propyl and benzyl side chains and a vitamin E component into physically cross-linked networks of "ABA" polycarbonate and poly (ethylene glycol) triblock copolymers. [2, 12, 13].

#### *Polyelectrolyte complex hydrogels*

These hydrogels have repeating units with an (poly)electrolyte group, which can be polycations or polyanions. The interaction of these poly-ions results in the formation of interpolymer complexes that can be directed by a specific bonding system such as hydrogen bonding or electrostatic interaction between different polymer chains. The synthesis of a new polyelectrolyte complex hydrogel (PEC) from the self-assembly of salean and chitosan has been achieved. Importantly, these PECs offer a platform for intelligent delivery of vitamin C nutrients in the intestinal environment. [2, 12].

### **2.1.2. Synthesis of hydrogels**

Physically cross-linked hydrogels are synthesized by altering physical parameters such as solvent, pH, or temperature, as in the case of agarose. On the other hand, chemically cross-linked hydrogels can be produced using two established techniques: (a) polymerization of hydrophilic monomers, and (b) modification or functionalization of existing polymers [14, 15].

#### *Hydrogel synthesis from monomers*

Chain or step polymerizations are both effective methods for creating hydrogels. The process of making hydrogels most frequently involves free-radical chain polymerizations of hydrophilic monomers containing a carbon double bond [14, 15].

Beginning with the homolytic dissociation of a weak bond or a redox reaction, an initiating species will produce active radical centres to start the polymerization process. When active radical centres are formed, they quickly spread through the carbon double bonds of numerous monomer units to create polymer chains. The polymerization process can be stopped when two active radical centres come into contact with one another through combination or disproportionation. This free-radical reaction method is incredibly adaptable and can be used to create hydrogels with different structures [14].

The technique for producing active centres depends on the specific monomers, solvents, and reaction conditions to be used, but it can be based on heat (thermal initiators), light (photo initiators),  $\gamma$  radiation, or electron beams. Each initiation procedure produces free radicals, which react with the monomer molecules to form a polymerized gel [14].

#### *Hydrogel synthesis by polymer modification*

By modifying or functionalizing existing synthetic polymers, synthetic hydrogels can be created. PVA, which is easily made by hydrolysing poly (vinyl alcohol), is possibly the most typical example (vinyl acetate). Another hydrogel can be created from PVA by

adding hydroxyl moieties. Crosslinks can be created by combining condensation agents with bifunctional or polyfunctional properties with pending hydroxyl groups. Various aldehydes, maleic and oxalic acid, dimethyl urea, polyacrolein, diisocyanates, divinyl sulfate, and ceric redox systems are examples of potential crosslinking agents. Water or an alcohol like benzyl alcohol, methanol, or ethanol may be used as the reaction's solvent [14].

### **2.1.3. Characterization of used hydrogel systems**

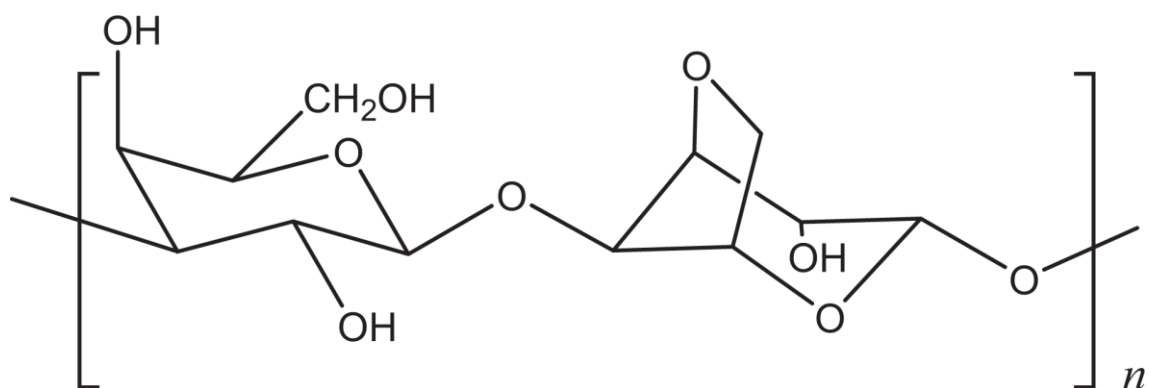
I have chosen agarose as a model for physically cross-linked polymers, and Poly(ethylene glycol) thiol (PEG thiol) as a representative for chemically cross-linked hydrogels for my experimental work.

#### *Agarose*

The polysaccharide known as agarose, which has an average molecular weight of 120,000 Da, is made up of 1,3-L-D-galactopyranose and 1,4-linked 3,6--anhydro-k-l-galactose units. In a hot solution, agarose chains exist in a rigid and unordered conformation. When the temperature drops below 40 °C, the coils organize into orderly helices that then group together to form thick bundles with sizable water pore spaces. Strong elasticity, high turbidity, an aqueous microenvironment, and bio affinity of the agarose gel matrix make it a perfect model system for immobilizing proteins on solid substrates. Comparing agarose gel to other gels based on polyacrylamide, alginate, and polymerized polyacrylamide hydrazide, it was found to be the most effective for building the bioreactor with cytochrome P450 [16].

Agarose exhibits mechanical properties that are comparable to those of tissues and are easily customized by changing the polymer concentration. An example of these properties is in a 2018 study written by G.R. López-Marcial [4]. When it is dissolved in water, it forms a gel with a rigid network, resulting in a three-dimensional porous structure that promotes cell adhesion, spreading, and proliferation. Agarose hydrogels can be polymerized *in situ*, which reduces the invasiveness of the procedure and also enables the hydrogel to take on the desired shape [17].

Another frequent application of agarose is to create a three-dimensional environment for *in vitro* studies of chondrocyte mechanotransduction pathways. Such studies typically involve encasing cells in agarose, exposing the cell-seeded hydrogel to predetermined levels of mechanical deformation or loading, and then observing the changes in cellular organization that result [18].



**Fig. 2** chemical structure of agarose [19].

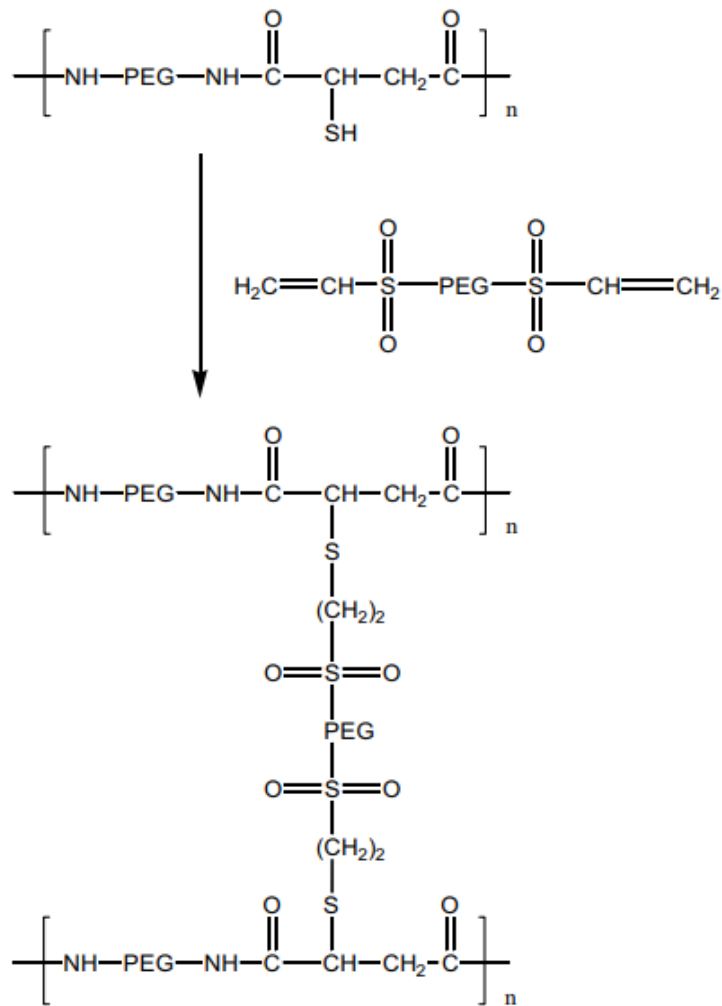
### *Poly(ethylene glycol)*

Poly(ethylene glycol) (PEG) is currently used in a wide range of established and emerging applications in pharmaceuticals because of its capacity to affect the pharmacokinetic properties of drugs and drug carriers. Longer blood circulation times are the result of the pharmacokinetics of drugs being administered changing when they are protected by or bound to PEG. As a result, there is a higher chance that the drug will act at its intended site before being recognized as foreign and removed from the body. Therefore, the majority of conjugated drugs, as well as liposomal and micellar formulations available on the market or in advanced clinical trials, contain PEG [20].

When injected subcutaneously, a copolymer made of poly(ethylene glycol) (PEG) can spontaneously transform into a hydrogel. Multiple thiol groups that are distributed along the polymer backbone at regular intervals are chemically cross-linked to form gels [21].

There are many different molecular weights of polyethylene glycol, ranging from 200 to tens of thousands g/mol. The water soluble and hygroscopic polymer has two states at room temperature: a colourless, viscous liquid with a molecular weight less than 600 g/mol and a waxy, white solid with a molecular weight greater than 800 g/mol [22]. PEGs are typically not monodispersing polymers, but their numerical designation (for example, PEG-2000) generally indicates their average molecular weight. Solid PEG is highly soluble in water, for instance, PEG-2000 has a solubility of about 60 % wt in water at 20 °C. Lower molecular weight liquid PEGs can be used as solvents without adding water [22].

Understanding hydrogels mechanical properties can significantly help to improve the formulation and applicability of them, which are becoming more and more significant from a pharmaceutical perspective [23].



*Fig. 3 Preparation of PEG thiol through chemical cross-linking using thiol groups [21].*

## 2.2 Basic principles of rheology

### 2.2.1. Rheology and rheometry

Professor **Eugene C. Bingham** and his colleague **Markus Reiner** of Lafayette College are credited with creating the term "rheology". It refers to the study of matter's flow and deformation. When the American Society of Rheology was established in 1929, this definition was accepted [24].

It is a branch of physics and physical chemistry because the most important variables are from mechanics: forces, deflections, and velocities. The word "rheology" is derived from the Greek verb "rhein," which means "to flow." Rheology is thus known as "flow science". Rheological experiments, however, also provide information on the deformation behaviour of solids, in addition to the flow behaviour of liquids. The relationship here is that many materials flow as a result of a significant deformation brought on by shear forces [25, 26].

Not until the early 20th century did rheology begin to be recognized as a separate branch of science. However, interest in the behaviour of liquids and solids has existed for a very long time among scientists and other practical users [25].

Rheometry is a commonly used method for determining the rheological properties of materials. Both rotational and oscillatory rheometers are utilized for analysing the viscoelastic behaviour of materials, including both liquids and solids. [25].

### *Rotational tests*

Rotational tests can be divided into two types. The first one is tests with controlled shear rate (CSR tests) and the second one is controlled shear stress (CSS tests) [25].

The rheometer sets and regulates the rotational speed or shear rate, as appropriate, during CSR tests. The controlled shear rate test method is typically chosen if the liquid under investigation exhibits self-levelling behaviour (i.e., no yield point) and if viscosity measurements need to be taken at a specific flow velocity or shear rate, respectively. This is the case when it is necessary to simulate specific process conditions, such as those that might arise during pipe flow or when painting or spraying [25].

The rheometer presents and controls the torque or shear stress during CSS tests. A "controlled shear stress test," also known as a "CSS test" or "CS test," is the name of this test procedure. This is the "classic" technique for figuring out the yield points of pastes, gels, and dispersions. Since any motion, whether it be flow or creep, is typically a reaction to an acting force, almost all flow processes in nature are shear stress controlled [25].

Any rheometrical study is frequently motivated by the expectation that behaviour observed in industrial settings can be related to a simple measurable rheometrical function. Rheometry could therefore be crucial for process and quality control. The usefulness of any proposed constitutive model for the test material, whether this is based on molecular or continuum concepts, may also depend on it [24].

### **2.2.2. Oscillatory rheometry**

The oscillatory tests fit into the larger category of dynamic measurements where stress or strain both change harmonically over time. The relevant strains are small enough to fall within the range of linear viscoelasticity, and the mathematical theory of linear viscoelasticity serves as the formal analytical framework for deriving the conclusions from the experimental data. Since this theory explains the precise relationships between the various material functions defined in the time and frequency domains, measuring just one viscoelastic function is equivalent to performing a full linear viscoelastic characterization [26, 27, 28].

### The complex shear modulus $G^*(\omega)$

One of the most important quantities is complex shear modulus. It is a complex number defined as:

$$G^*(\omega) = \frac{\text{complex stress}}{\text{complex strain}} = \frac{\sigma^*}{\gamma^*} = G'(\omega) + iG''(\omega) \quad (1)$$

A frequency sweep can be used to experimentally determine  $G^*(\omega)$  from oscillatory measurements. The storage modulus, or real part  $G'(\omega)$ , provides the in-phase stress to strain ratio. The storage module  $G'(\omega)$  characterizes the energy storage capacity of a material prior to deformation. A quarter cycle is when the most elastic energy is stored in terms of energy:

$$E_{st} = \int_0^{\gamma_0} G' \gamma d\gamma = \frac{1}{2} G' \gamma_0^2 \quad (2)$$

whereas a full cycle stores no elastic energy at all. The loss modulus is a measurement of viscous losses that is found in the imaginary portion of the complex shear modulus  $GI(\omega)$ . The loss modulus  $iG''(\omega)$  determines how much energy the material is able to release. The amount of energy lost steadily rises; the loss in a full cycle is:

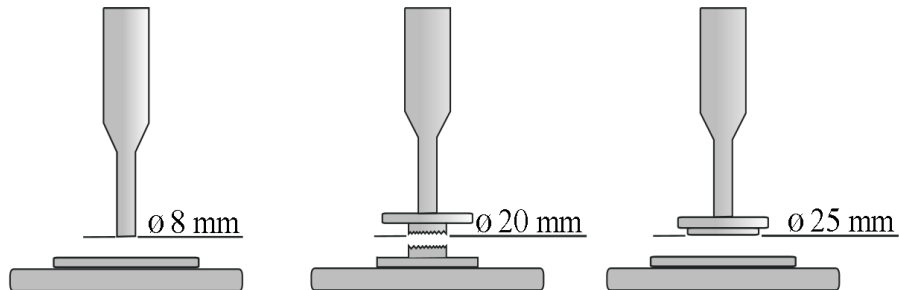
$$E_d = \oint G'' \left( \frac{\dot{\gamma}}{\omega} \right) = \pi G'' \gamma_0^2 \quad (3)$$

### Test methods

The majority of instruments, referred to as controlled strain rheometers, operate at controlled angular or linear displacement. Fewer instruments—known as controlled stress rheometers—operate by applying a controlled harmonic torque or force to a boundary surface [27].

### Controlled torque and controlled displacement rheometers

Fig. 4 displays the experimental setups. This figure defines various geometries that produce a shearing flow by rotating only the upper part of the cell, with the lower part remaining fixed. While a harmonic angular deformation corresponds to an oscillatory experiment that directly produces the complex rheological functions, continuous rotation at a constant rotational speed corresponds to a steady shearing flow [24,26].



**Fig. 4** The main geometries used in dynamic testing.

Parallel plate is the only type of geometry which were used in experimental part. This is the reason why other types of geometries are not described.

### *Parallel plates*

Because the shear rate is not constant throughout the material, it must be carefully verified that the input motion's amplitude is small enough to support the linearity hypothesis. The shear deformation ( $\gamma = \theta r/h$ ) is maximum at the plate edge ( $r = R$ ) and zero at the centre ( $r = 0$ ). However, the data can be adjusted in a steady shearing flow to account for non-Newtonian effects [27].

This geometry is the most widely used because it has many benefits. A parallel-plate geometry is frequently a practical alternative because it is simple to set up and control the sample but may be challenging to squeeze a highly viscous or filled material between a cone and plate and may take too long for the stresses to relax before the experiment [27].

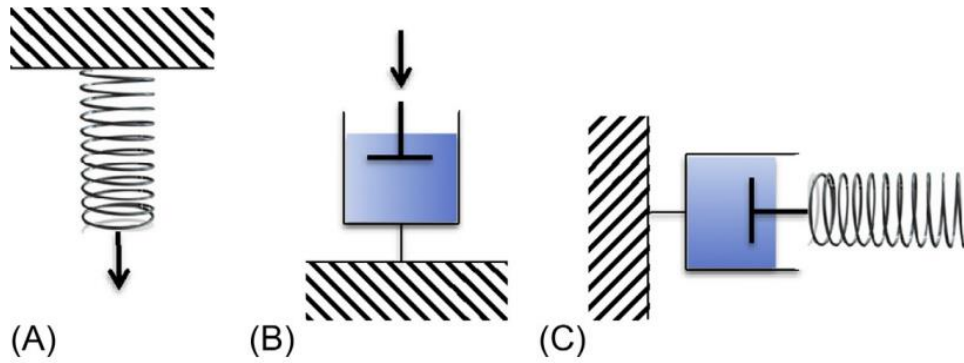
In this geometry, the plate edge's maximum strain amplitude  $\gamma_0$  is  $\theta_0 R/h$ , where  $\theta_0$  is the angular amplitude of the oscillatory motion [27].

### **2.2.3. Dynamical mechanical analysis (DMA)**

Dynamical mechanical analysis (DMA), a popular technique for characterizing the viscoelastic properties of materials, particularly polymers, has gained popularity in recent years [29]. The viscous and elastic contributions to the mechanical behaviour of the material can be studied by applying axial oscillating forces or deformations to a material and measuring the response. Furthermore, the relationship between viscoelastic properties and various variables, including temperature, frequency, or time, is possible. Thermal behaviour must be considered when examining polymer properties, especially in biomedical applications where a potential change in mechanical behaviour brought on by a change in temperature can significantly alter how the material interacts with the body and, therefore, its intended function. [29].

When using DMA, a sample is repeatedly subjected to a small amount of deformation (or stress). DMA, also known as DMTA (Dynamic Mechanical Thermal Analysis), should not be confused with thermomechanical analysis (TMA). TMA is a separate technique used for analysing the thermal and mechanical properties of materials, while DMA focuses specifically on the dynamic mechanical behaviour of materials under varying conditions. DMA deforms a sample with known geometry using a sinusoidal deformation. Both a controlled stress and a controlled strain can be applied to the sample. The sinusoidal wave is produced by a force motor and is transmitted to the sample by a drive shaft. One issue has always been the compliance of this drive shaft and how any stabilizing bearings used to hold it in place will affect it [29].





**Fig. 5** Models for the description of the different mechanical behaviour: (A) Hooke model for ideal elastic material, (B) Newton model for ideal liquid, and (C) Maxwell model for viscoelastic materials [29].

DMA offers crucial knowledge about the viscoelastic behaviour of substances, particularly polymers, as a function of frequency, temperature, or time when applying an oscillatory stress (or strain). The simultaneous existence of viscous (i.e., liquid-like) and elastic (i.e., solid-like) properties in a material is referred to as viscoelastic behaviour. Hooke's law (eq. 4), which states that the strain is directly proportional to the stress by the constant known as Young's modulus, describes the behaviour of a solid (Fig. 5 A). However, for a perfect liquid, the response to a stress is proportional to the rate of strain by a constant called viscosity (Fig. 5 B). Polymeric materials display an intermediate character that can differ somewhat from the two models (Fig. 5 C) (i.e., elastic-like and liquid-like). As a result, for viscoelastic materials, viscosity and Young's modulus do not remain constant over time or depend on the values of applied shear rate or strain [29].

$$F = k \cdot X \quad (4)$$

A sinusoidal strain (or stress) that varies with time is applied to the sample at the linear region of the DMA basis.

$$\varepsilon(t) = \varepsilon_0 \sin(\omega t) \quad (5)$$

Where  $\omega = \text{frequency}$

In fact, in the case of elastic behaviour (Fig. 6 A), Hooke's law predominates, and the stress is in phase with the applied strain, so the response of the material depends on its characteristic.

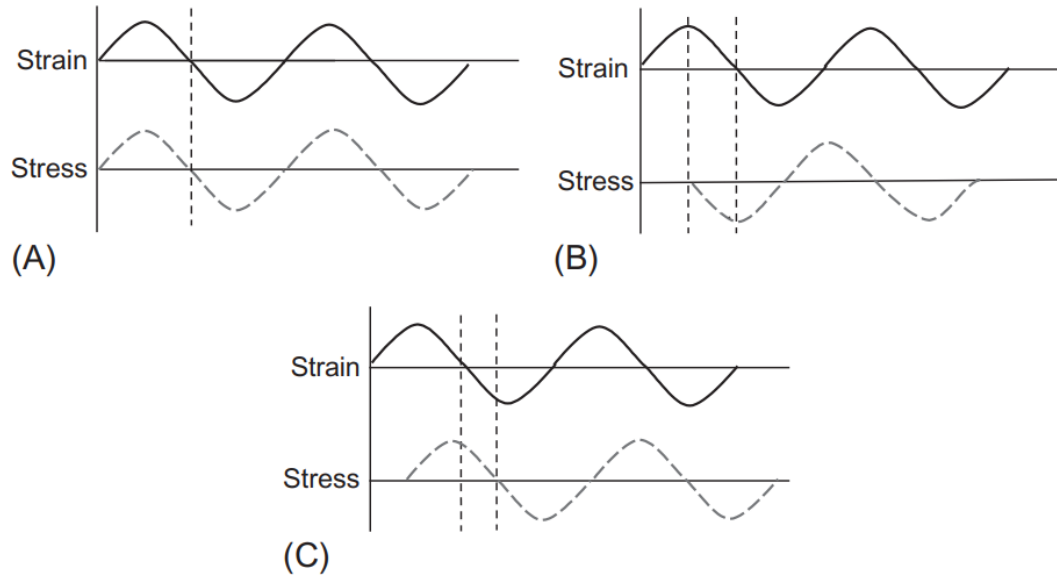
$$\sigma(t) = \sigma_0 \sin(\omega t) \quad (6)$$

The stress is  $90^\circ$  out of phase with the applied strain if the material behaves viscously (Fig. 6 B).

$$\sigma(t) = \sigma_0 \sin \left( \omega t + \frac{\pi}{2} \right) \quad (7)$$

The phase angle of viscoelastic materials (Fig. 6 C) will display an intermediate behaviour, falling between 0 (i.e., the ideal elastic solid) and 90° (i.e., ideal liquid).

$$\sigma(t) = \sigma_0 \sin(\omega t + \delta) \quad (8)$$



**Fig. 6** Relationship of the applied sinusoidal strain to stress: (A) ideal elastic solid, in-phase,  $\delta \frac{1}{4} 0^\circ$ , (B) ideal (Newtonian) liquid, out-of-phase,  $\delta \frac{1}{4} 90^\circ$ , and (C) viscoelastic materials, phase lag,  $0^\circ < \delta < 90^\circ$  [29].

A motor that exerts force on the sample (controlled stress) and a displacement sensor that measures the amplitude make up a controlled stress DMA (strain). A deformation in the form of amplitude is asked for when programming the instrument. The instrument applies force (its control parameter) until the desired amplitude is measured. The measured amplitude (strain) is also constant over the course of the test. To maintain a constant amplitude, the controlled force variable adjusts as the sample's stiffness does [29].

### *Compression mode*

DMA has many types of modes. Compression mode is the only mode which is used in the experimental part of this bachelor thesis. In the compression mode, a fixed flat surface is used for the sample, and force is applied by an oscillating plate. Materials with low to moderate modulus can benefit from compression (e.g., foams and elastomers). Additionally, measurements of expansion or contraction as well as adhesive tack testing can be done in this mode [29].

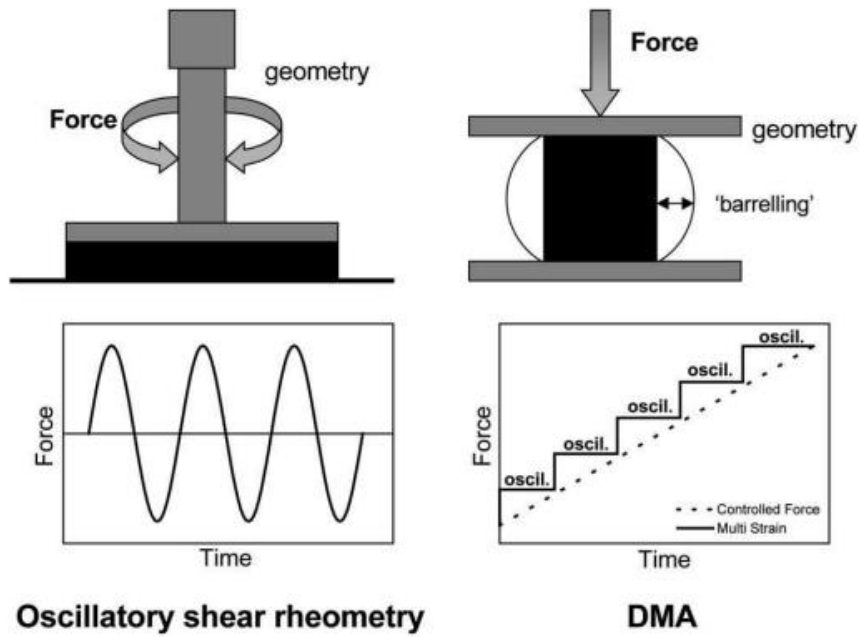
#### **2.2.4. Comparison between oscillatory shear rheometry and DMA measurements**

The mechanical analysis of hydrogels has been conducted using a variety of techniques. The most frequently used ones are oscillatory rheometry, dynamic mechanical analysis (DMA), and elongation/compression analysis [23]. These analyses offer valuable information regarding the gel's strength, which can be characterized in terms of viscosity or viscoelasticity. Additionally, these analyses establish the relationship between the gel's strength and its composition. [23].

The high-water content (up to 99 %) of hydrogels is what makes mechanical analyses of them the most challenging. Any type of hydrogel could be rheologically characterized using an oscillatory shear rheometer by following a specific methodology. However, this method encounters a challenge with the sample slipping down the wall during the analysis. It is common practice to use compressional DMA as a tool for mechanically analysing strong materials. It can, however, be used to characterize hydrogels [23].

Rheometers apply shear forces while DMA uses compression forces, which is the primary distinction between the two methods. The parallel-plate clamp was by far the best option given the properties of hydrogels. As a result, both instruments use essentially the same geometries. A thin water layer between the hydrogel surface and the geometry served as lubricant because of the high-water content of the hydrogels. The hydrogel would slide out from between the geometry's plates if the surfaces of the plates were not perfectly parallel to one another [23].

According to data on both swollen and non-swollen hydrogels, DMA in "multi-strain" MS mode works best for rheologically characterizing hydrogels in comparison to rheometers. The preferred accuracy, measurement speed (DMA is much faster than the method used on the rheometer), amount of sample available (much smaller amounts are necessary when DMA is used), and measurement range of  $G'$  all affect the method for mechanical characterisation of hydrogels, which is becoming more and more important in pharmaceuticals [23].



**Fig. 7** Schematic representation of the configurations for DMA (left) and oscillatory shear rheometry (right) [23].

A significant artifact called "barrelling" is also depicted in the drawing on the right (Fig 7). The graphs below each illustration display the forces used throughout the various experiments as a function of time. The graph also shows the vertical oscillations (oscil.) that were carried out in the MS-mode with a fixed amplitude at each static force level [23].

### 3 State of the art

Hydrogels are incredibly versatile and can be formed into almost any shape, size, or form. Hydrogels have the ability to imbibe water up to a thousand times their dry weight. In recent years, dozens of hydrogel medical products made from synthetic polymer and biopolymers have had a significant impact on patient care [30]. The following section of this work provides a detailed account of selected hydrogels and their respective applications.

In a 2018 study, **G.R. López-Marcial** and his team dealt with agarose-based hydrogels as proper bioprinting materials for tissue engineering. Whole study was about to find proper substitute in osteoarthritis treatment. Total joint arthroplasty, in which some of the underlying bone tissue and native cartilage are removed and replaced with metal and polymer components, is the standard treatment method for osteoarthritis. While this method is effective in reducing joint pain, the mismatch in material stiffness between native tissues (0.5-1.0 MPa for cartilage) and implanted materials (900 MPa for ultrahigh molecular weight polyethylene) leads to long-term issues, including accelerated degradation of the surrounding healthy tissue. They tested several types of agarose-based hydrogels and compared them with Pluronic F-127, a gel with known printing capabilities. Of the compared hydrogels, it was agarose with concentrations of 2,3 and 4 % weight by volume (Type VII powder, Sigma-Aldrich, St. Louis, MO). Alginate-agarose with concentration of 3.75 % or 5 % weight by volume) [4].

To assess mechanical properties that are affected by temperature and strain rate, four experiments were conducted. In the first experiment, a constant oscillatory stress was applied while raising the temperature of the Peltier plate by 5 °C/min from 25 to 70 °C (1 Pa at 1 Hz). In the second experiment, viscosity was measured at 37 °C while shear rate was raised from 0.01 to 10.00 s<sup>-1</sup>. In the third experiment, the extruder wall was subjected to the calculated shear rate ( $\dot{\gamma}_w$ ) for 1000 s at 37 °C. The highest shear stress recorded was referred to as the shear yield point  $\tau_0$ . There were three stages in the fourth experiment. In the beginning, a constant oscillation at 1 Hz was used for 100 s. Following the application of ( $\dot{\gamma}_w$ ) for 800 s to induce yield, a constant oscillation (1 Hz, 30 min, 37 °C) was then applied. The storage ( $G'$ ) and loss ( $G''$ ) moduli were measured. The amount of time required for the storage modulus to overcome the loss modulus during the second oscillation is known as the recovery time [4].

Temperature had a slight effect on storage modulus. There was no clear differentiation between Pluronic and the other agarose-based gels at 37 °C, but the storage modulus of 2 % agarose was noticeably lower than Pluronic. Compared to Pluronic, 3 % agarose had a significantly higher shear yield strength. No other noteworthy variations in yield strength were found in shear, which may be partially explained by the wide range of agarose-only gels (3 and 4 % agarose). All gels instantly recovered after yielding in

shear, according to relaxation experiments. Pluronic's hydrogel was too soft to test, so unconfined compression was not possible. Similar compressive yield strengths were found in 3 % and 4 % w/v agarose gels, which was higher than 2 % w/v agarose and the agarose-alginate mixtures [4].

They found out that a 5% w/v agarose-alginate is the ideal for the extrusion-based bioprinting of 3D. The mixture maintained excellent cell viability and supported matrix production while printing structures with high shape fidelity that were comparable to those made from Pluronic, a substance that is well-known to be ideal for extrusion-based printing. A further advantage was that the agarose-alginate mixture did not need any additional cross-linking steps or a sacrificial material for the printing process, which are methods that could have an impact on long-term performance [4].

In 2021 **Marta Ghebremedhin** and her team investigate physics of agarose fluid gels and its rheological properties and microstructure. In this study, agarose with concentration of 0.5 % wt, 1 % wt and 2 % wt was examined with Discovery HR-3 rheometer. All measurements were performed in triplicate. To investigate the fluid gels' viscoelastic properties, an amplitude sweep test was conducted. This technique involved measuring the storage ( $G'$ ) and loss ( $G''$ ) moduli to characterize the deformation behaviour of the samples in the non-destructive range known as the linear-viscoelastic (LVE) region. It was also evaluated to determine the boundaries of the non-linear viscoelastic range (NLVE), which is defined as the behaviour after this range has been exceeded. The measurements were carried out at various shear strain, and the impact of various geometries on the formation of agarose fluid gels was taken into account. This procedure was designed to find out how much the various geometries affect particle formation. Microscopic analyses of agarose fluid gels made with different geometries showed the presence of unordered chains on the surface and microstructures of gel particles with a similar shape, the parallel-plate geometry produced a larger particle size distribution. Cone-plate geometry produced lower viscosity and storage ( $G'$ ) and loss ( $G''$ ) moduli than parallel-plate geometry at 25 °C in the viscosity profiles and amplitude sweep tests. In conclusion, it is demonstrated that, as predicted, the storage and loss modulus rise with concentration, but a discrepancy arises when the LVE limit and the viscosity at low shear rate are taken into account. The LVE range and viscosity at low shear rates were both the shortest for the 1% wt fluid gel. Furthermore, analysis of the microstructure using microscopy and measurement of particle size revealed that a crucial factor in this nonlinearity with regard to the rheological properties is the presence of disordered, helically aggregated polymer chains on the particle surfaces. The size of the particles and the number of disordered chains on the surface of the particles were found to decrease with increasing agarose concentration [31].

In 2015 **Steven D. Reinitz** and his team tested Dynamic mechanical analysis and compared it with gravimetric gel swell technique to analyse the cross-linking density in different materials. Ultrahigh molecular weight polyethylene (UHMWPE) a hydrogel

commonly used by orthopaedics was tested. The purpose of this study is to compare the capabilities of gravimetric gel swell and DMA in the measurement of cross-link density in a variety of HXL (Highly cross-linked) UHMWPE materials and to establish the correlation between the two techniques' findings. The network properties of cross-linked UHMWPE are taken into account by DMA calculations of the cross-link density. A polymer's storage and loss moduli can be measured using DMA. The polymer's networked portion exhibits elastic behaviour because it prevents chain movement. The stiffness of this network is captured by the storage modulus. A stiffer network results from shorter chain segments between cross-links and entanglements. They found out that in comparison to gravimetric gel swell, DMA analysis of cross-link density may offer better depth resolution because of the thinner sample dimensions. The goal of this study was to ascertain whether gravimetric gel swell or DMA could be used to measure cross-link density in HXL UHMWPE. The findings imply that DMA can be used as a substitute for gel swell analysis and that both methods have benefits and drawbacks that make them suitable for different applications. DMA is preferred for depth dependent analysis because of the thinner specimen size it uses, such as when comparing cross-link density to oxidation throughout a sample's depth. Additionally, DMA is preferred when attempting to distinguish smaller differences between samples due to the observed moderate improvement in variation compared to gel swell. Unlike DMA, gel swell specimens can be created from even the most curved or thinnest devices, allowing for the analysis of every retrieved component. Furthermore, gel swell offers a significantly higher throughput; the current equipment in this laboratory can test up to eight materials per day using ten specimens over a period of two hours. All highly cross-linked devices that the laboratory has retrieved can be tested thanks to the higher throughput. With a comparable coefficient of variation, dynamic mechanical analysis provides a deeper depth resolution for measuring cross-links in highly cross-linked UHMWPE than the earlier developed gravimetric gel swell method. Before comparing the results of the two techniques, the magnitudes of the values produced by DMA and gel swell must be calibrated or converted, but the results imply that the difference is a straightforward linear conversion [32].

In a study written by **Sebastián Jaramillo-Isaza** and his team, dynamic mechanical analysis was used for agarose-based hydrogels that could be used in regenerative medicine. In this study there were six different types or compositions of agarose-based hydrogel and then all samples were tested with DMA. One type of hydrogel was chitosan combined with agarose. Chitosan has a crosslinked assembly that improves its chemical and structural stability and, as a result, the biological, functional, and mechanical properties of scaffolds [33]. By changing the agarose content, different sample compositions were produced for this study. This fact enables the evaluation of the type VII agarose's potential impact on the mechanical characteristics of these hydrogels. From the results we can see that DMA is useful method in biomechanical characterization of hydrogels. It was discovered that the mechanical characteristics of the nanostructured

hydrogel may be caused by molecular bonds among the materials' fibers; as a result, this property may promote cell migration, adhesion, or proliferation. It was possible to identify variations in the mechanical response of chitosan-agarose based hydrogels over time, despite the fact that this study did not take temperature and frequency changes into account. In order to deform these materials, less force is needed because both the storage modulus and the loss modulus have been found to decrease over time. On the other hand, these findings show how agarose affects chitosan-agarose hydrogel's mechanical response, particularly in terms of their resistance to deformation [33].

In 2015 **Hai Lin** with his team examined dynamical mechanical properties of maleated hyaluronic acid hydrogels. They were looking into a brand-new sodium hyaluronate (HAs) modification technique that produces a HAs derivative with better properties like higher storage modulus and a higher degree of substitution (DS). Hydrogels were also created using the obtained HAs derivatives, and their mechanical characteristics and swelling kinetics were studied. The NETZSCH DMA 242C was used for the DMA compression tests. Both frequencies of 1.0 Hz and 10 Hz were used, simulating the physiological stride frequency's normal and maximum ranges. The tests were conducted at a constant temperature of 25 °C. The test parameters were set to a 20 µm amplitude, a 0.001 N preload force, and a 120% force track. For each modification condition, three cylinder-shaped samples with an 8 mm diameter and 2 mm height were used. From the results we can see that the majority of the samples have storage moduli greater than 200 kPa, with the 25 series of maleated hyaluronan (MaHA) hydrogels having the highest, around 290 kPa. In earlier studies, their team found that ternary hydrogels made of collagen, chondroitin sulphate, and hyaluronic acid had compressive moduli between 45 and 54 kPa. Because the mechanical properties of different materials may result in noticeably different responses in biological systems, such as stem cell differentiation and bioactive molecule secretion, the range of the storage modulus may be another crucial factor. Between the hydrogel samples created for this study and those reported in the literature, there is a more than 100 kPa difference in storage modulus. Additionally, at higher concentrations and longer curing times, the range of storage modulus of hydrogels developed in their study may be even more significant. In comparison to natural cartilage, most hydrogels created for cartilage tissue engineering currently have relatively weaker mechanical properties. The DMA results also imply that the E' does not always rise in step with the DS. However, as anticipated, the MaHA hydrogels' loss moduli are significantly lower than their storage moduli, indicating that the elastic rather than the viscous characteristics of those hydrogels are more prominent. The storage moduli statistical analyses reveal a significant difference between each group. In general, the more double bonds grafted on the HAs, the higher the storage moduli of the developed hydrogels, provided that the double bonds are fully reacted during the photopolymerization [34].

In 2021 **Elizabeth Q. Contreras** published a study which deals with mechanical properties of new water based thermoset elastomer. Even when temperatures rise above



100 °C, the hydrogel is very stable. A polymer hydrogel based on poly(maleic anhydride-co-isobutylene) exhibits rapid and reversible strain in response to stress and offers elasticity, long-term durability, and thermal resilience over a wide range of temperature as a sealant resin. The study first determines the hydrogels' viscoelasticity through oscillatory tests in small spaces. Once this happens, Hooke's law was used to represent the elastic response of viscoelastic materials in one dimension. To determine viscoelasticity all samples were tested. Liquid resin oscillatory macro-rheological tests measure  $G'' = 0$  for all samples. A study demonstrates that loss modulus ( $G''$ ), where  $G'' > G'$ , persists for all liquid gels, does not increase over time as temperature increases.  $G'$  grows and surpasses  $G''$  as the gel solidifies and the amount of crosslinking increases, where  $G' > G''$ . With the help of the time and temperature tests made by rheometer, she finds out that this elastomer can be used as cement sheaths. The high polymer density, crosslinking, and elasticity of new thermoset gel thermally stable up to 200°C to form polyimide gels enable the optimization of a new water-based hydrogel for the recovery of broken cement sheaths at higher temperature ranges and pressures. With the help of elastic copolymers made to be resistant to chemical stimuli and aromatic tri-functionalized monomers, a balance between a soft and rigid gel is achieved [35].

## 4 Experimental Part

### 4.1 Chemicals

- Deionized water
- Agarose (Sigma-Aldrich, Type I, low EEO, CAS: 9012-36-6)
- Poly(ethylene-glycol) thiol (PEG-4SH) (Laysan Bio, Inc, Lot: 165-174)
- Poly(ethylene-glycol) diacrylate (PEGDA) (Laysan Bio, Inc, Lot: 163-94)
- Sodium alginate (Carl Roth, CAS: 9005-38-3)
- Calcium chloride anhydrous powder g.r. (CAS: 10043-52-4)
- Chitosan (Sigma-Aldrich, CAS: 9012-76-4)
- Poly(sodium 4-styrenesulfonate) (Sigma-Aldrich, CAS: 25704-18-1)
- Acetic acid glacial 99.8% (Penta chemicals unlimited, CAS: 64-19-7)
- DIY Ultrasound Phantom Gel (EPDM) (Huminic medical, 852844007390)
- Sodium hydroxide beads a. g. (Penta chemicals unlimited, CAS: 1310-73-2)
- Potassium phosphate monobasic (Sigma-Aldrich, CAS: 7778-77-0)
- Sodium phosphate dibasic dihydrate (Sigma-Aldrich, CAS: 10028-24-7)

### 4.2 Facilities

- Rheometer Discovery HR-2 (TA instruments) in oscillatory shear mode and Dynamic mechanical analysis mode
- Sensors:
  - 8 mm (Peltier plate Sandblasted 109411)
  - 20 mm (Peltier plate CrossHatched 115802)
  - 25 mm (Peltier plate – smooth)
- Magnetic stirrer with heating (Heidolph Instruments MR Hei-Tec)
- Analytical scale (Denver instruments 224A)
- Scale (Denver instrument S-603)
- TRIOS software (TA instruments)

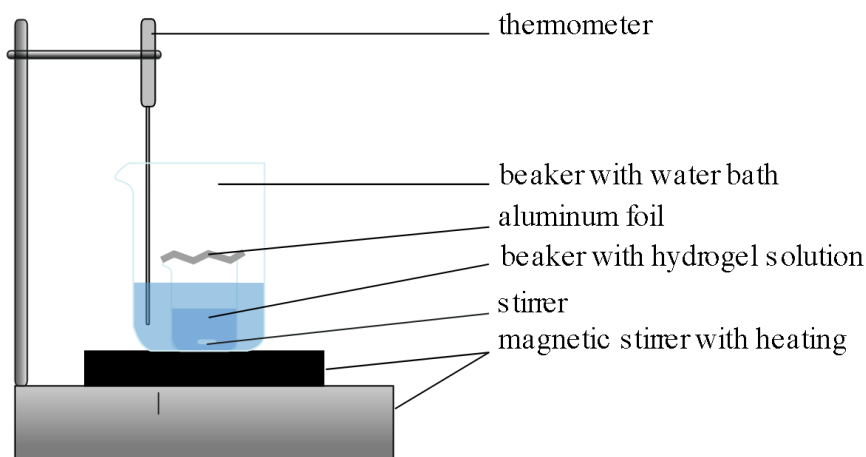
### 4.3 Sample preparation

#### 4.3.1. Physically crosslinked hydrogels

##### *Agarose*

A hydrogel made of polysaccharide agarose (Sigma-Aldrich, Type I, low EEO, Art. No. A6013-50G) was prepared in four different concentrations (0.5%, 1%, 2%, and 4% wt.). The preparation involved dissolving the required weight of agarose in deionized water with constant stirring and heating the solution to 90°C until the agarose was completely dissolved. The solution was then poured into a Petri dish and allowed to cool to room temperature. To prevent water evaporation from the hydrogel, the Petri dish was covered with two layers of Parafilm® and placed in container with 100 % humidity. for 24 hours.

The hydrogel was then ready for use in subsequent experiments. Figure 8 displays the equipment utilized for the preparation of agarose.



*Fig. 8 apparatus for the preparation of agarose*

### *Alginate*

Alginate is categorized as an ion hydrogel that falls under the classification of physically cross-linked hydrogels. To prepare alginate, a 2 % (w/v) alginate solution was first created. A 0.1 M  $\text{CaCl}_2$  solution was also prepared separately. The alginate solution was poured into a Petri dish, followed by the addition of the  $\text{CaCl}_2$  solution using a spraying bottle to evenly distribute calcium ions in a 2:1 ratio. The resulting hydrogel was sealed with Parafilm® and left in a desiccator for 24 hours.

### **4.3.2. Chemically crosslinked hydrogels**

#### *Poly(ethylene-glycol) thiol*

The PEG hydrogel was prepared according to the instructions in the article “PEG-thiol based hydrogels with controllable properties” [36]. PEG-Thiol based hydrogels were produced by blending solutions of PEG-4SH (molecular weight 10 kDa, Laysan Bio) with solutions of PEGDA (molecular weight 10 kDa) to achieve a molar ratio of 1:1 between thiol and acrylate functional groups. The PEG-4SH powder was accurately weighed in a glove box to prevent moisture from affecting the samples and then added to phosphate buffer saline pH 7.5 (PBS), which was stirred for different periods of time. PEGDA powder was then added to PBS and stirred until complete dissolution was achieved. The PEG-4SH and PEGDA solutions were subsequently combined to create a mixture with a final concentration of 10 % (w/v) PEG-4SH and 5 % (w/v) PEGDA.

Table 1: Chemicals used for PEG.

	Molecular weight [kDa]	Concentration wt. [%]
PEG-4SH	10	20
PEGDA	10	10

Table 2: Chemicals used for phosphate buffer.

	Molecular weight [kDa]	Producer	CAS
NaCl	58.44	Lach-Ner, s.r.o.	7647-14-5
KCl	74.56	PENTA s.r.o.	7447-40-7
Na <sub>2</sub> HPO <sub>4</sub> • 2H <sub>2</sub> O	177.99	PENTA s.r.o.	10028-24-7
KH <sub>2</sub> PO <sub>4</sub>	136.09	PENTA s.r.o.	7778-77-0

Table 3: phosphate buffer composition

	Weight [g]	Concentration [M]
NaCl	8	0.14
KCl	0.2	0.003
Na <sub>2</sub> HPO <sub>4</sub> • 2H <sub>2</sub> O	1.8	0.01
KH <sub>2</sub> PO <sub>4</sub>	0.24	0.002

The phosphate buffer solution was generated by accurately measuring and combining the listed chemicals in Table 3. Deionized water was added to achieve a final volume of 1 liter, and the resulting mixture was thoroughly mixed.

#### *DIY Ultrasound Phantom Gel (EPDM)*

This chemically crosslinked gel was purchased from a medical company named Huminic, and no information is available regarding the preparation of this hydrogel. Based on the properties of the gel measured by the Huminic company, it can be inferred that the Young's modulus of the gel is 0.53 MPa, and the gel has an elastic ratio of 0.89.

#### **4.3.3. Semi interpenetrated hydrogels**

Semi-interpenetrating gels were produced using 0.01 wt. % solutions of interpenetrating Alginate, Polystyrene sulfonate, and Chitosan samples. To prepare these gels, the dissolved samples were added to 1 % agarose and the same procedure was followed as in the agarose preparation. Alginate and polystyrene sulfonate were dissolved in water, and no further adjustments were necessary. Chitosan was dissolved in 5 % acetic acid and then its pH was brought to 7 with 1M NaOH. The stock solutions were prepared at a concentration of 1 gram per liter. The preparation process involved stirring the solutions on a magnetic stirrer for a duration of 24 hours under controlled laboratory temperature with a stirring speed of 250 revolutions per second. Following this, a 25 millilitre sample

of the overall hydrogel was prepared, comprising of 22.5 millilitres of 1% agarose and 2.5 millilitres of the prepared hydrogel solution provided.

#### **4.4 Optimization of the DMA method**

The rheometer has several functions. This chapter will deal with the preparation of the sample for the measurement itself, the selection of a suitable geometry for the measurement and finally the measurement itself.

##### **4.4.1. Sample preparation**

The sample's preparation is a crucial component of the measurement. This part will describe how particular samples were prepared. The proper use of the sample for measurement is covered in this section. There are numerous traps in the measurement itself. It is dependent on the sample's height due to the dependence on the measurement gap, normal force, and of course the sample itself. To be able to obtain a comparable results with others, whether it is a test of the geometry or the samples themselves, it is best to try to keep the same conditions during all preparations. It is ideal to use identical Petri dishes and to use the same amount of prepared, semi-solid hydrogels in each dish. By pouring the same volume of samples into the same sized Petri dishes, we obtain hydrogels that are very similar in shape especially height. As it was already said, each hydrogel behaves differently, so it cannot be guaranteed that they will all be the same. The next step is to place the sample in the desiccator after covering the Petri dishes with parafilm to maintain a constant humidity level in the sample. After allowing sufficient time for the gel to solidify, the sample is ready for measurement. Since the rheometer can use a variety of geometries, it is essential to choose the geometry first. This will allow to use a corkscrew in accordance with the geometry's diameter to achieve a regular shape. The smooth shape on the top and bottom surfaces is due to solidification in the Petri dish. Once the shape of the hydrogel has been cut out with a corkscrew, it is enough to carefully remove the prepared sample using tweezers and a spatula and place it on the rheometer sensor. If it is more watery hydrogels, it is advisable to lightly dry the surface of the hydrogel with pulp.

##### **4.4.2. Optimized system**

###### *The influence of geometry*

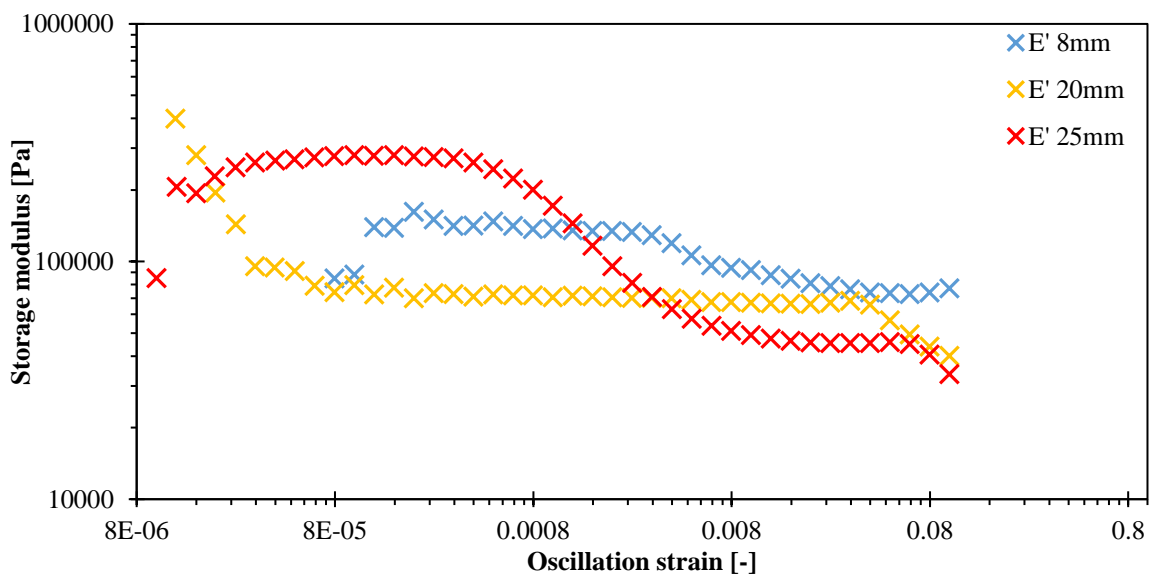
One significant issue encountered when using DMA is that repeated measurements of the same sample can lead to sample compression, requiring adjustment of the gap. However, altering the gap can impact the obtained results, requiring recalculation to correct for this effect:

$$strain = \frac{Displacement}{gap} \quad (9)$$

As previously mentioned, the choice of geometry significantly impacts the measured mechanical properties. Thus, it was imperative to evaluate the three types of geometries. The tests were conducted on different hydrogels, including agarose, alginate (the preparation procedure can be found in the sample preparation chapter), DIY ultrasound phantom gel, and semi interpenetrated physically crosslinked hydrogels based on agarose (alginate, chitosan, poly (sodium 4-styrenesulfonate)), each exhibiting distinct viscoelastic properties, rendering them suitable for comparing the influence of geometry on measurement outcomes. Notably, during the measurement process, specific trends were observed for each geometry, which are vital for comparing the geometries.

*8 mm (Peltier plate Sandblasted 109411)*

The 8 mm sandblasted geometry is optimal for use when working with limited sample amounts. It consistently displays the highest storage and loss modulus values, as well as the highest Young's modulus in most cases. The only exception to this trend is when testing the semi-interpenetrated hydrogel based on agarose with alginate sample. In all other tests, this geometry yields the largest values. The second trend pertains to the 25 mm geometry. Both the 8 mm and 25 mm smooth geometries tend to exhibit two linear viscoelastic regions, albeit to a greater extent with the 25 mm geometry. Graph 1 illustrates the storage modulus of each geometry when tested on the Semi-interpenetrated hydrogel based on agarose with poly (sodium 4-styrenesulfonate) sample.

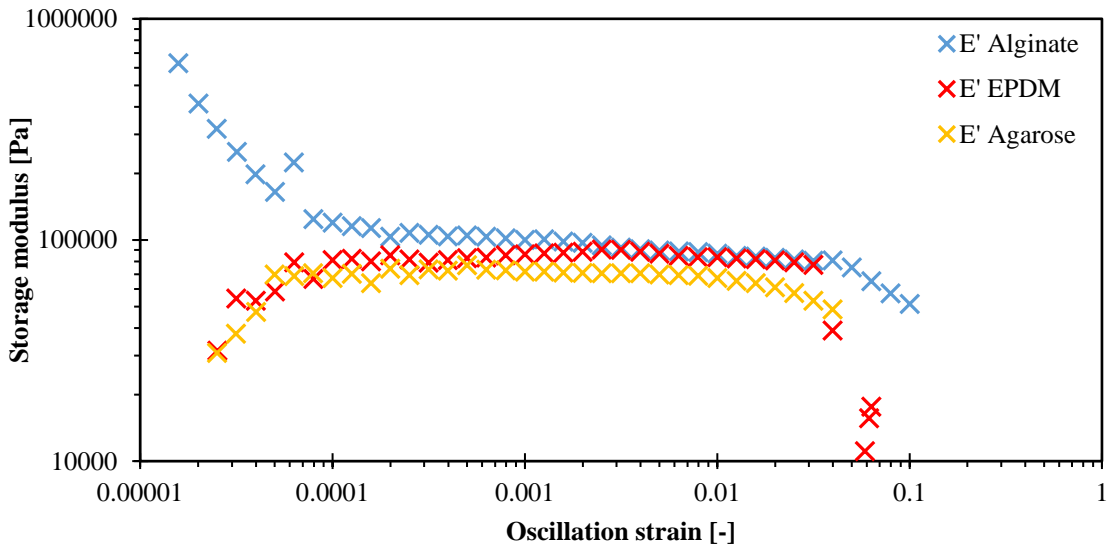


*Graph 1: Storage modulus for Semi interpenetrated poly (sodium 4-styrenesulfonate) with different geometries*

*20 mm (Peltier plate CrossHatched 115802)*

The geometry with a diameter of 20 mm is the only geometry among those tested to possess a serrated surface. This crosshatched surface prevents the gel against slipping on the wall. Out of all the data collected, this geometry yielded the most favourable results. As depicted in graph 1, this geometry boasts the longest linear viscoelastic region, and is

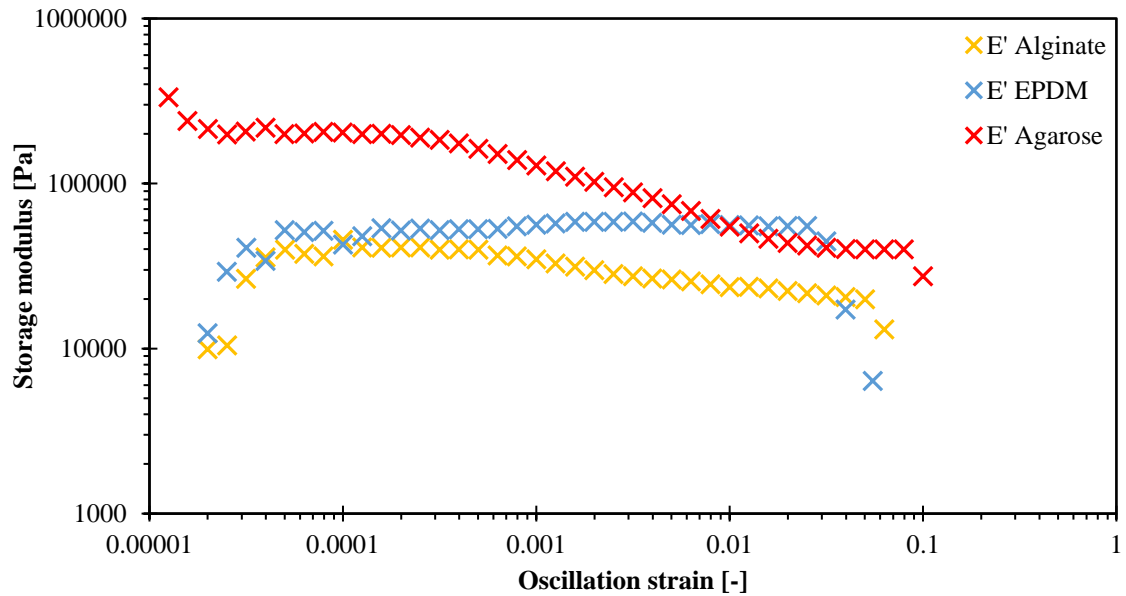
situated between the other two geometries. The first trend observed in this geometry is similar to that of the other geometries, whereby the storage and loss moduli decrease as the geometry's diameter increases. The second trend is the stability of the samples, which manifests as a prolonged viscoelastic region. This trend is illustrated in graph 2, where three samples of varying elasticity are compared.



Graph 2: Storage modulus for different hydrogels with 20 mm crosshatched geometry

25 mm (Peltier plate – smooth)

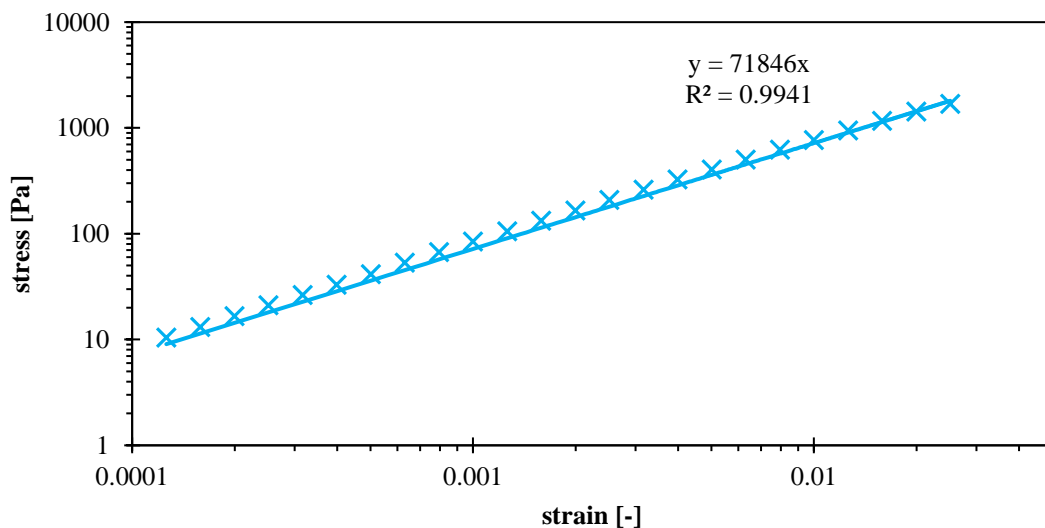
The 25 mm diameter smooth geometry is appropriate for samples with higher elasticity. EPDM exhibited the most precise measurement results, as it displayed a linear viscoelastic region. In contrast, the remaining hydrogels displayed either two linear viscoelastic regions or none at all. These measurements indicate that the more viscous the sample, the more challenging it is to process with this particular geometry. Additionally, as discussed in the subsection regarding the 8 mm geometry, this geometry follows the trend of decreasing storage and loss modules. Graph 3 depicts disparities in the storage modulus among EPDM, alginate, and agarose. In graph 3, at the end of the curves an anomalous point can be observed that does not align with the rest of the data points throughout all measurements. This deviation arises from a significant gap, leading to the sample no longer being in contact with the sensors during the final phase of measurement.



Graph 3: Storage modulus for different hydrogels with 25 mm geometry

#### Young modulus

An essential aspect of processing the results is the calculation of Young's modulus. This value is crucial for comparing results obtained through different methods. Young's modulus is defined as the ratio of stress to strain within the elastic deformation region of a material. In this work, Young's modulus was determined by using the linear regression equation from the stress-strain graph. The values for the graph were selected from the linear region to ensure a reliable determination of R. To obtain an accurate value for Young's modulus, it is necessary to determine the value of y as zero in the linear regression equation. The determination of Young's modulus is illustrated in graph 4.



Graph 4: dependence of stress on strain for calculation of Young's modulus



### *Optimized system*

In this section, the precise steps for powering on the rheometer and configuring the experiment in the Trios system will be described.

#### *Rheometer*

1. Turn on the compressor.
2. Release the air supply.
3. Turn on the device with the cooling liquid.
4. Turn on the rheometer.
5. Turn on the computer.
6. Remove the shaft cover.
7. Screw in the suitable geometry

#### *Trios*

1. Turn on the software and connect to the rheometer.
2. Set temperature conditions: Environmental > set temperature.
3. Disable toggle smart swap.
4. Select geometry in compression mode.
5. Calibration:
  - a. Gap temperature compensation is not required. (Needed only for temperature tests)
  - b. Axial mapping: Read alignment position > Motor: Home position > Axial mapping > calibrate.
6. Zero gap: Manually slide the sensor 2 mm above the bottom sensor > Gap: Zero gap > when zero gap is finished proceeding to the loading position.
7. Motor: Move to the alignment position

#### *Experiment setup*

1. Sample: Fill in the name of the experiment, the operator and determine where the results will be saved (Name, Operator, File Name)
2. Geometry: There is no need to change anything
3. Procedure:
  - a) Conditioning Options:
    - Mode > active (compression)
    - Axial Force: 1 N, if set initial value is chosen, it could cause an increase in the time before the experiment starts.
    - Proportional force mode: Force tracking
    - Axial Force > Dynamic Force: 1,0e-9 %
    - Minimum Axial Force: 0.1 N

b) Oscillation Amplitude:

- Soak time: Choose a time suitable for tempering the sample to the desired temperature. Recommended is 180 s but it depends on amount of sample.
- Frequency: 1 Hz
- Logarithmic sweep
- Axial strain: The rheometer is not capable of measuring more than 10 N in compression mode. For this reason, the experiment can be set up within this range 1.0e-3 % to 10 %
- Points per decade: The number of data points desired has a direct impact on the duration of the experiment. In this study, a uniform set of 10 data points was utilized across all measurements.

## 5 Discussion

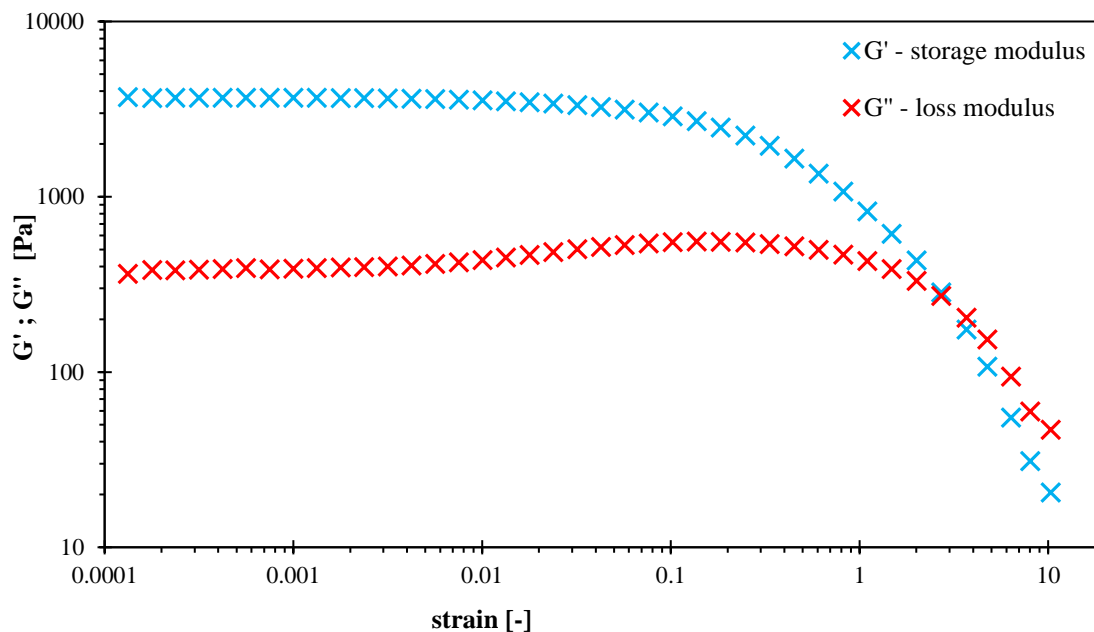
The primary objective of this study was to determine the applicability of dynamic mechanical analysis (DMA) in assessing the mechanical properties of hydrogels with distinct cross-linking types or with semi-interpenetrated polymer networks. To achieve this objective, physical and chemical cross-linked hydrogels were subjected to experiments, and the results will be presented in the subsequent sections.

### 5.1 Physically cross-linked hydrogel

In this experimental section, the focus was on examining the concentration dependence of agarose hydrogel at concentrations of 0.5 %, 1 %, 2 %, and 4 % by weight. Agarose was selected as the primary hydrogel to optimize the DMA method due to its physical type of cross-linking, due to its simple preparation process and its good reproducibility. It was also chosen as it has been extensively studied using oscillatory shear rheometry, making it ideal for comparing and contrasting with the results of dynamic mechanical analysis. The measurements were conducted using two distinct techniques, namely oscillatory shear rheometry and dynamic mechanical analysis, which are elaborated in section 2.2. A comprehensive amplitude strain sweep experiment was conducted for both methodologies. The sensor with a crosshatched surface and a diameter of 20 mm, which was found to be the most appropriate in prior experiments, was employed for all measurements in both techniques. The results obtained from oscillatory shear rheometry will be presented first, followed by the results obtained from dynamical mechanical analysis. Subsequently, the results from both methods will be compared and analysed.

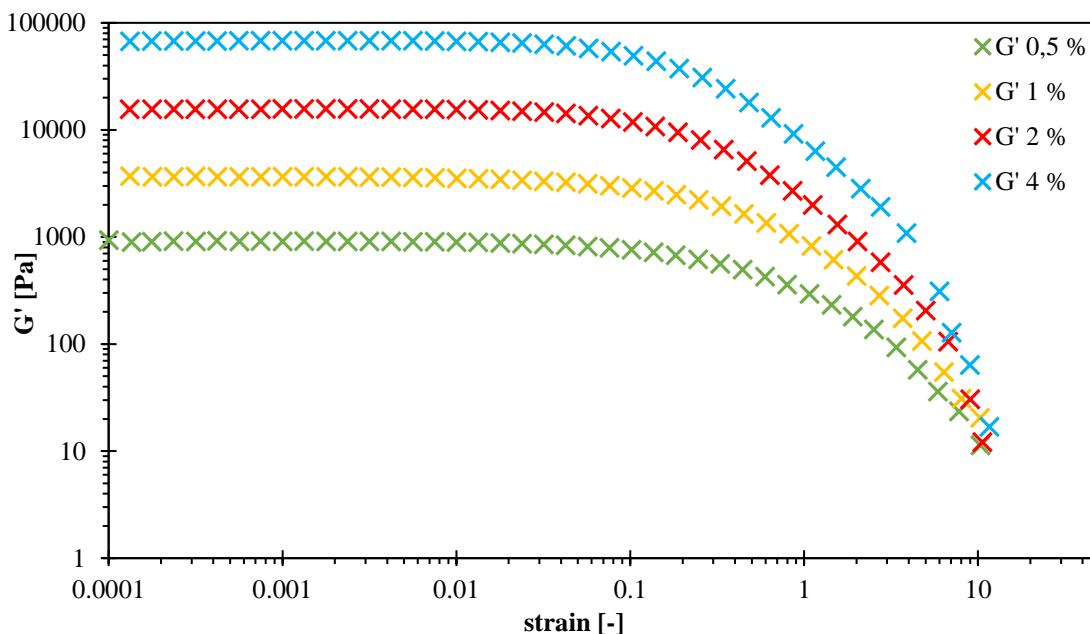
#### *Oscillatory shear rheometry*

During the oscillatory shear rheometry measurement of agarose hydrogel, the Trios system generated graph 5, which displays the relationship between the viscoelastic moduli and applied oscillation strain. The graph comprises two curves, the storage modulus, and the loss modulus. The storage modulus, as discussed in the theoretical section, represents the sample's capacity to absorb energy during deformation, while the loss modulus indicates its ability to release energy. Since the hydrogel in this study was fully cross-linked, the storage modulus was greater than the loss modulus. These moduli values demonstrate that the sample was predominantly solid rather than liquid. Both moduli curves had a linear viscoelastic region (LVR), which was established by averaging six values within the LVR. The end of the viscoelastic region was determined as a 5 % deviation from the average LVR value. When the moduli values exceeded this deviation, deformation of the sample commenced, and the proof was storage and loss moduli crosspoint. The intersection of these two curves indicated permanent deformation and the breakdown of the sample's structure.



Graph 5: Dependence of viscoelastic moduli on strain in 1 % agarose

Throughout the experiments, all hydrogels were entirely cross-linked, and hence there was no requirement to specify the loss modulus. The viscoelastic characteristics of the samples are typically defined by the end of the linear viscoelastic region (LVR). However, in this study, the data obtained from the ends of the LVR were not utilized, and the results presented in the tables and graphs were derived from the average LVR values. As a result, the endpoints of the LVR were not exhibited in the results.

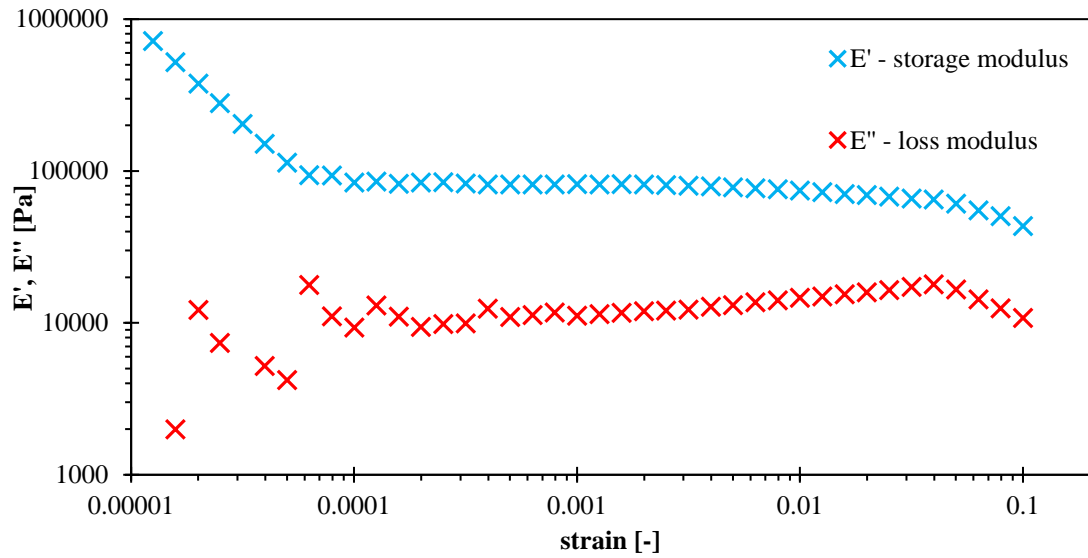


Graph 6: Comparison of storage modulus for different concentrations of agarose (oscillatory shear rheometry)

Graph 6 displays the elastic moduli of all measured agarose concentrations. The data suggest that the mechanical properties of the hydrogel are influenced by the concentration of agarose. As the concentration of agarose increases, the elastic moduli also increase, indicating that samples with higher concentrations are stiffer. This phenomenon is also noticeable during the preparation of the samples for measurement. A higher concentration of agarose leads to a larger number of polymer chains that physically cross-link and form a more robust structure. Table 4 presents the precise values of both elastic modulus and Young's modulus for all concentrations. Further discussion of this graph is deemed unnecessary as the oscillatory shear rheometry of agarose has already been extensively studied and discussed in various literature [37].

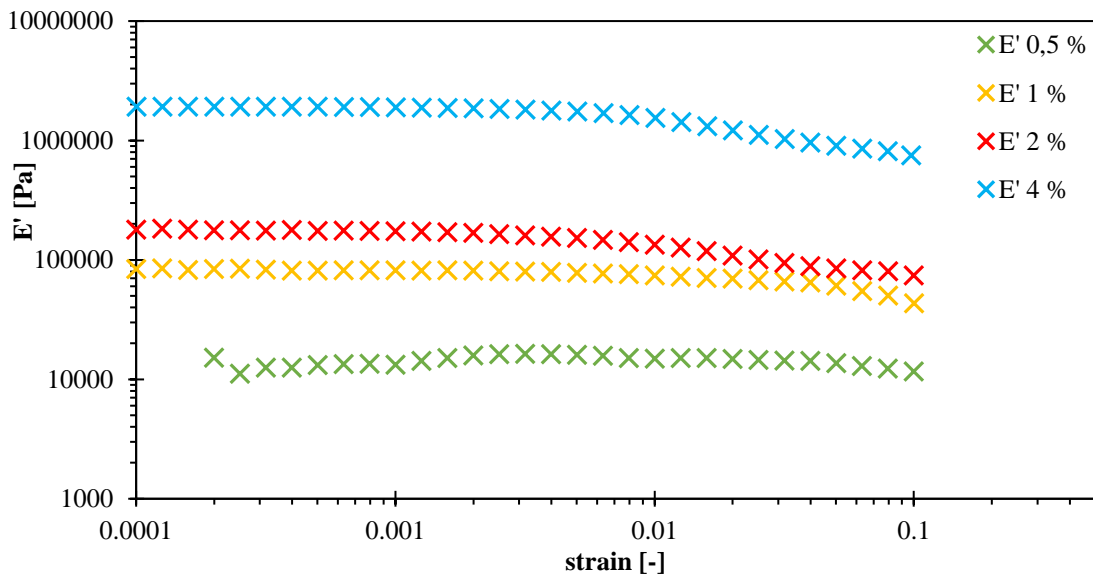
#### *Dynamic mechanical analysis*

Graph 7 represents the output of the Trios software obtained through dynamic mechanical analysis. Similar to the previous case, a detailed explanation of the method's principle is available in the theoretical section 2.2.3. The graph illustrates a reduction in the storage modulus, followed by the onset of LVR as the oscillation strain gradually increases, reaching a value of 0.0001. The observed decrease in storage modulus during the initial measurement, ranging from 0.00001 to 0.0001 strain, could potentially be attributed to inadequate responsiveness of the rheometer sensors. Another plausible hypothesis suggests that the sensors might need to overcome the forces exerted by the surface of the gel, as the surface may contain less evaporated water, rendering it drier and stronger compared to the rest of the sample. However, it is important to note that neither of these hypotheses has been experimentally tested or confirmed. The end of LVR is determined in the same way as in oscillatory shear rheometry, i.e., as a 5 % deviation from the average value of six points within the LVR. It is important to note that the Discovery HR-2 rheometer has a limitation of measuring a strain higher than 10 % in DMA mode. As a result, we are unable to determine the crosspoint and hence identify the conditions under which permanent deformation occurs. The hydrogel and measurement conditions in this experiment are identical to those in oscillatory shear rheometry.



Graph 7: Dependence of viscoelastic moduli on strain in 1 % agarose

Graph 8 illustrates the relationship between storage modulus and agarose concentration measured via DMA. The values on the graph were obtained at a strain value of 0.0001, as it was observed in graph 6 that the values settle into the linear viscoelastic region (LVR) only at these points. The storage modulus of 0.5 % agarose begins at 0.0002, which is because DMA sensors are better suited for measuring stiffer samples, resulting in earlier detection of values.



Graph 8: Comparison of storage modulus for different concentrations of agarose (DMA)

### Comparison of measured results

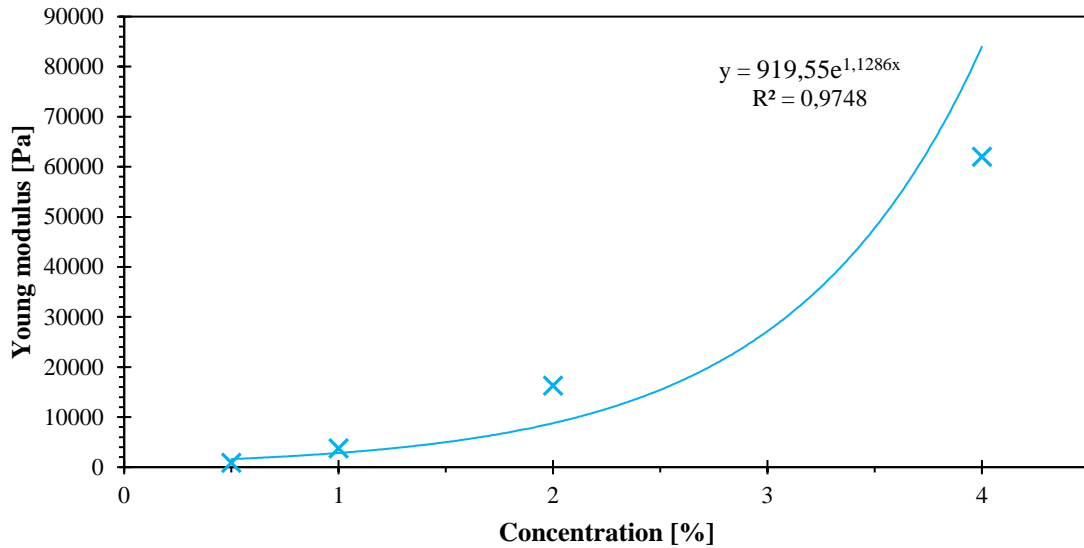
Table 4: Comparison of results from DMA and oscillatory shear rheometry

Concentration	Elastic modulus		Viscoelastic modulus	Young modulus
	G' [Pa]	E' [Pa]	G [Pa]	E [Pa]
0.5	923	23073	896	19283
1	3846	83674	3754	63338
2	16567	271746	16261	213872
4	60929	1816197	61989	1682164

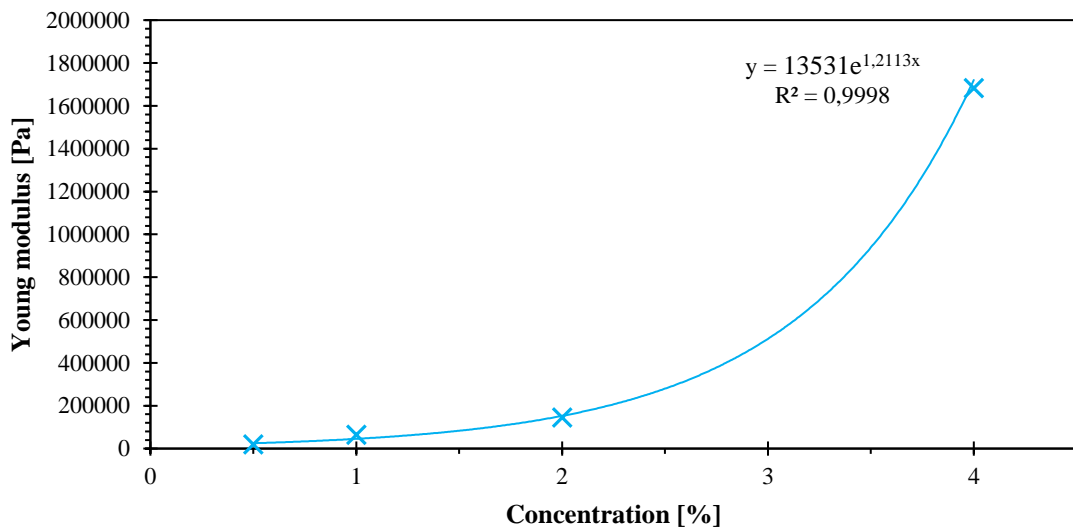
The obtained measurement results for the shear and Young's moduli were compared between oscillatory shear rheometry and dynamic mechanical analysis methods. The comparison showed that the values of G' obtained from oscillatory shear rheometry were at least 94 % smaller than the values of E' obtained from dynamic mechanical analysis for all concentrations of agarose. This significant difference may be due to the anisotropic nature of agarose, which can have different properties when subjected to different directions of force. In addition to the structural characteristics of the sample, it is plausible that the observed problem is associated with the relationship between Young's modulus and the elastic modulus, which can be expressed through the Poisson's ratio. This relationship is represented by equation 10.

$$E = 2 \cdot G \cdot (1 + \nu) \quad (10)$$

An additional potential explanation for the decrease in storage modulus observed during the initial measurement could be attributed to measurement errors resulting from sensor slippage over the sample during the oscillatory shear test. Sliding or slippage of the sensor can lead to a decrease in the modulus values. To minimize this error, it is recommended to utilize the CrossHatched 20mm geometry, as it can provide better grip and reduce the likelihood of sensor slippage during testing. In addition, Table 4 also shows the differences in Young's moduli values for the various agarose concentrations. When measured using oscillatory shear rheometry (G), the Young's modulus values are very similar to the storage modulus values. However, when measured using DMA (E), the differences between the two values are larger. The Young's modulus and storage modulus values obtained from DMA are much larger, at least by 92 %.



Graph 9: Exponential dependence of young modulus on concentration (oscillatory shear rheometry)



Graph 10: Exponential dependence of young modulus on concentration (DMA)

A further comparison of the results can be achieved by analysing the concentration-dependent graphs of Young's modulus obtained by measuring with oscillatory shear rheometry and DMA. Graph 9 illustrates the exponential dependence of Young's modulus on concentration when determined by oscillatory shear rheometry, while graph 10 shows the corresponding concentration dependence when using DMA. Comparison of the two graphs reveals that the results from DMA follow the exponential dependency better than those obtained from oscillatory shear rheometry. The observed discrepancy could be due to the occurrence of material slippage between the sensors during oscillatory shear rheometry measurements. The obtained results collectively



indicate that Dynamic Mechanical Analysis (DMA) is highly suitable for measuring physically cross-linked hydrogels. Unlike oscillatory shear rheometry, DMA does not suffer from slip-related issues, thereby offering more accurate results. However, it is worth noting that the DMA instrument at the Faculty of Chemistry has a limited range compared to oscillatory shear rheometry. Consequently, it may not be possible to conduct a comprehensive analysis of most samples, including complete deformation measurements, using the available DMA setup.

## **5.2 Chemically cross-linked hydrogel**

A sample of PEG thiol was chosen for the investigation of chemically cross-linked hydrogels, which is a newer material at the Faculty of Chemistry. An additional reason for selecting this particular sample was to make the DMA method applicable to a wide range of systems, emphasizing its versatility and broad applicability. To thoroughly analyse the material, multiple methods were employed. Both the DMA and oscillatory shear rheometry methods were used to measure the time-dependent gelation and mechanical properties of the sample. The gelation process was monitored over a period of 72 hours with measurements taken at approximately 24 hour intervals, starting 3 hours from the beginning of gelation. This experiment was repeated twice due to differences in sample storage.

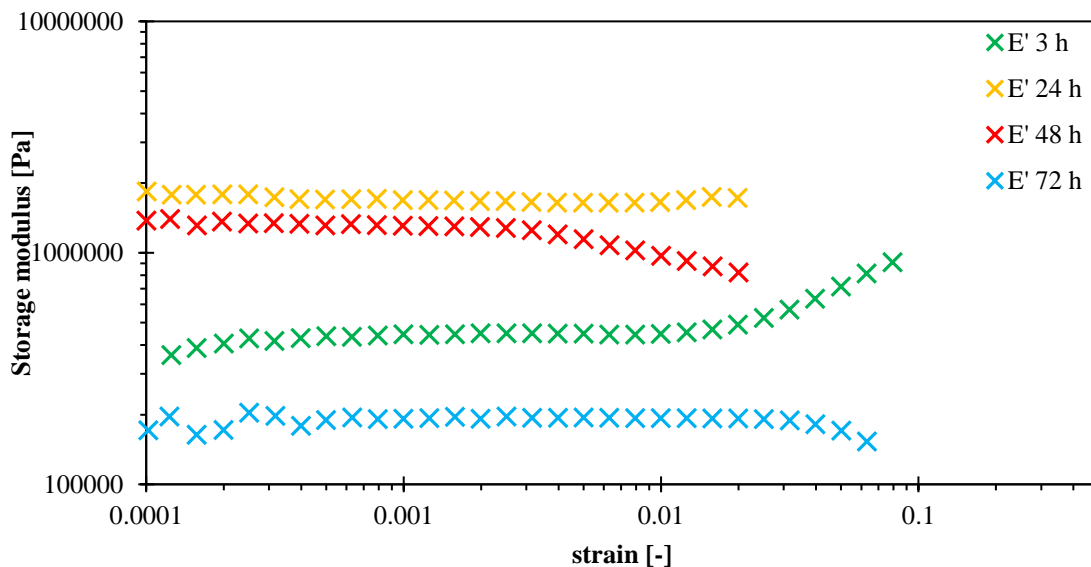
### *Swelling test*

During the first measurement, the PEG thiol sample was hydrated in water, while in the second measurement, it was hydrated in the buffer solution from which it was prepared. In both the DMA and oscillatory shear rheometry experiments, it was observed that the sample underwent significant swelling during the measurements. Consequently, a separate swelling test was conducted to assess the extent of changes in the viscoelastic properties of the sample under different swelling conditions. This test was crucial in determining the influence of swelling on the viscoelastic behaviour of the sample. The test was performed for 5 minutes, during which the sample was placed in the respective liquid solution, and its weight and size were measured at regular intervals. The results in Table 5 indicate that the storage in both liquids led to swelling of the hydrogel. The highest increase in weight occurred within the first 60 seconds in both cases. These findings suggest that the gel swells similarly in both liquids. Nonetheless, for long-term storage, it is better to keep the sample in the buffer as the bonds in the hydrogel are more stable, as will be further explained in subsequent parts of the study.

Table 5: swelling test

Time [s]	Distilled water				Phosphate buffer solution (PBS)			
	m [g]	%	l [mm]	%	m [g]	%	l [mm]	%
0	0.044	0 %	8	0 %	0.047	0 %	8	0 %
30	0.067	34 %	9	11 %	0.065	28 %	9	11 %
60	0.074	41 %	10.5	24 %	0.076	38 %	9.5	16 %
120	0.081	46 %	11	27 %	0.083	43 %	10.5	24 %
180	0.085	48 %	11	27 %	0.091	48 %	11	27 %
240	0.089	51 %	12	33 %	0.096	51 %	12	33 %
300	0.091	52 %	12	33 %	0.102	54 %	12	33 %

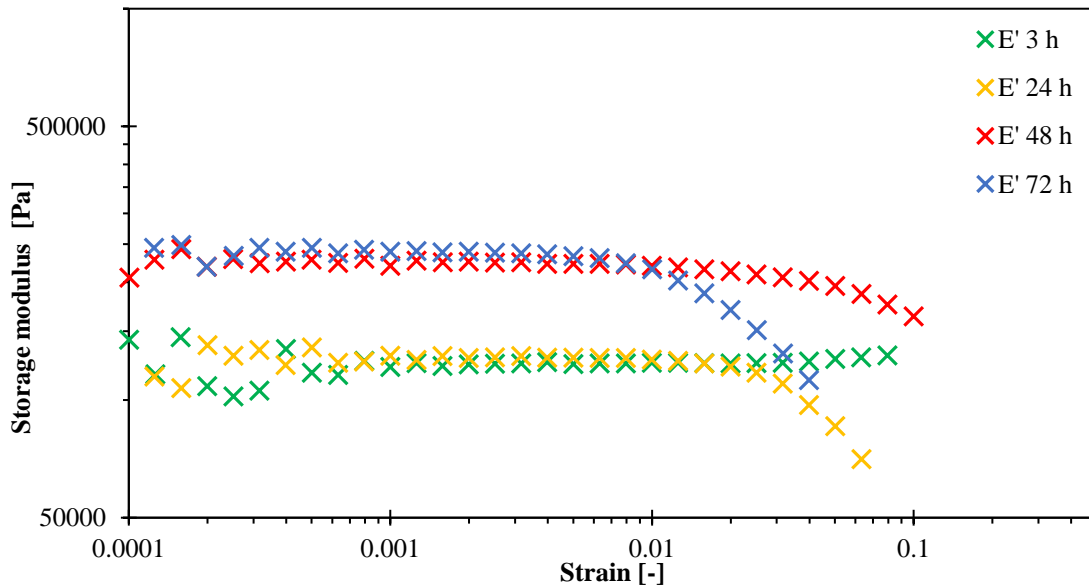
Time dependence of PEG thiol gelation



Graph 11: Time dependence of PEG thiol stored in water (DMA)

Graph 11 displays the temporal evolution of gelation for a sample stored in water, while Graph 12 illustrates the same for a sample stored in buffer. In both cases, the average storage modulus remains smallest after the initial measurement (taken 3 hours into the process) due to continued gelation over several hours. Graph 11 shows an increase in storage modulus within 48 hours of the start of gelation. Following a duration of 72 hours, the gel underwent disintegration, leading to a subsequent decrease in the modulus values. The sample stored in water absorbed a significant amount of water, leading to weakened bonds and reduced storage modulus in the last measurement. The swelling of the gel was visibly apparent without the need for any magnification or specialized equipment. The initial sample, weighing 0.017 g, has undergone a significant increase in weight to reach 0.176 g. This corresponds to a percentage increase of 935%. These observations suggest changes in the gel's structure and disruption of the gel cross-linking.

Graph 12, which depicts the buffered sample, provides more accurate results. However, a measurement problem occurred between 24 h and 48 h, causing the sample to crack due to an incorrect rheometer setting. Therefore, a replacement sample was used. These different conditions explain the large difference in modulus between 24 h and 48 h. Despite the storage of the sample in a buffer solution, there were no significant differences observed in its mechanical properties compared to the sample stored in water. The buffer solution did not have a substantial impact on the mechanical behaviour of the sample in comparison to water storage, as evidenced by the small difference in modulus between 48 h and 72 h.



Graph 12: Time dependence of PEG thiol stored in buffer (DMA)

Table 6 presents the moduli results for the time dependence of PEG thiol stored in water, comparing measurements obtained using DMA and oscillatory shear rheometry. The trend observed in both methods is identical, with the gel undergoing gelation within 24 hours, followed by stabilization of the moduli. In both cases, there is a rapid reduction in moduli after 72 hours, attributed to the gel absorbing a significant amount of water and disrupting the hydrogel bonds. Similar to physically cross-linked agarose, the percentage of modulus measured by DMA ( $E'$ ,  $E$ ) is at least 98 % higher than that obtained using oscillatory shear rheometry ( $G'$ ,  $G$ ).

Table 6: Time dependence of PEG thiol stored in water.

Time [h]	Storage modulus		Viscoelastic modulus	Young modulus
	G' [Pa]	E' [Pa]	G [Pa]	E [Pa]
3	6528	428947	6785	440820
24	9178	1638103	8674	1513245
48	9237	1153663	8548	1025992
72	1684	196752	1659	195860

Table 7 displays the viscoelastic moduli values for PEG thiol stored in phosphate buffer. As previously noted, a measurement error occurred between 24 h and 48 h, leading to the replacement of the sample with another from the original material. Despite this, it is apparent that the sample stored in the buffer is more stable, as evidenced by the moduli remaining similar even after 72 h. Therefore, it is recommended to store the sample in the buffer from which it was created.

Table 7: Time dependence of PEG thiol stored in buffer.

Time [h]	Storage modulus		Viscoelastic modulus	Young modulus
	G' [Pa]	E' [Pa]	G [Pa]	E [Pa]
3	4227	138897	4233	140213
24	2443	136903	2415	132793
48	6518	221869	6396	211254
72	5197	238280	5090	232412

Dynamic Mechanical Analysis (DMA) has demonstrated its efficacy in the measurement of chemically cross-linked hydrogels. In this study, the focus was on investigating the time-dependent gelation of PEG thiol and the consequential significance of sample storage. The findings revealed that the choice of storage medium played a vital role in maintaining the viscoelastic properties of the PEG thiol gel over time. Storing the sample in a phosphate buffer was identified as the optimal method, resulting in minimal changes in its viscoelastic behaviour.

## 6 Conclusion

The aim of this work was to find out whether dynamic mechanical analysis (DMA) can be used as a complementary for investigation of mechanical properties of hydrogels with different types of cross-linking and then comparing the results of DMA with oscillatory shear rheometry. Another goal was to create an optimized procedure for working with DMA. Dynamic Mechanical Analysis (DMA) offers a notable advantage by mitigating issues associated with sample slippage during measurements, thus providing more accurate and reliable results compared to other techniques such as oscillatory shear rheometry. However, it is important to acknowledge that the primary drawback of DMA at the Faculty of Chemistry lies in its limited scope. The available DMA instrument has a restricted operational range, which can impose limitations on the comprehensive analysis of certain samples, particularly in terms of complete deformation measurements.

To optimize the procedure, a precise guide was summarized, starting with the correct preparation of the sample, and ending with the exact setup of the experiment in the Trios software (TA Instruments). For a correct comparison of the results, the basis is that all the measured hydrogel samples are as similar as possible in terms of dimensions. DMA compresses the sample, and the gap needs to be adjusted for re-measurement. For correct results, a recalculation using Equation 9 is appropriate. Another aspect for optimizing the results was determining the correct geometry. The geometries were tested on hydrogel samples with different types of cross-linking. Of the selected geometries, the 20 mm CrossHatched geometry seems to be most suitable for hydrogels. The serrated surface of this geometry partially prevents the hydrogel from slipping between the sensors and gives the most accurate results. If there is not enough sample, then it is advisable to use a geometry with a diameter of 8 mm. This geometry has shown to give the highest values of viscoelastic moduli compared to the others in most cases. This geometry is not completely smooth, and therefore its surface also partially prevents the sample from slipping. The latter geometry is smooth and has a diameter of 25 mm, and the results showed that it is the least suitable for measuring hydrogels, likely due to the large sample size that can lead to slippage. Using DMA and oscillatory shear rheometry, hydrogels with different types of cross-linking were measured. Hydrogel with a physical type of cross-linking (agarose) was analysed by both methods as a concentration series. The results showed that DMA is a more accurate method than oscillatory shear rheometry. The confirmation of this statement can be observed by comparing the Young's modulus values in graphs 9 and 10. During oscillatory shear rheometry, the sample slips between the sensors, and this may not give as accurate information as DMA, where slippage almost does not occur. The same results also apply to the time dependence of poly(ethylene-glycol) thiol gelation, which is a hydrogel with a chemical type of cross-linking. The effect of PEG thiol hydration of the hydrogel using water and the phosphate buffer from which the hydrogel was prepared was observed. The hydrogel was found to be more stable

when hydrated with phosphate buffer solution, as it retained similar viscoelastic properties even after 72 h of preparation, which was not the case when hydrated with water. It is nearly impossible to directly compare the two techniques in this work as they employ different methods for measuring mechanical properties, rendering them almost incomparable for anisotropic materials. To obtain a comprehensive understanding of the mechanical properties of a material, it is recommended to utilize both techniques.

*"In the end, it's only a passing thing, this shadow. Even darkness must pass. A new day will come. And when the sun shines, it'll shine out the clearer." These powerful words, spoken by Samwise Gamgee in J.R.R. Tolkien's "The Lord of the Rings" series, beautifully encapsulate the essence of our quest. As we approach the culmination of our efforts in developing an optimized procedure for utilizing DMA, we must remember that challenges are transient, and light will usually triumph over darkness. This procedure holds the promise of a brighter future, where a comprehensive understanding of materials and their accurate characterization awaits us. Let us persevere, for just as the sun rises to dispel the shadows, our endeavours shall yield illuminating insights and pave the way for progress.*

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