



# Rheological Characterization of Biological Hydrogels in Aqueous State

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## Abstract

**Introduction:** Biological hydrogels provide a conducive extracellular environment for encapsulating and growing cells and play an important role in regulating cell behavior. Mechanical and rheological properties of hydrogels can influence cell function, mechanotransduction and cellular behaviors such as growth, migration, adhesion, self-renewal, differentiation, morphology and fate. Determination of rheological properties of biogels is important for printing tissues by controlling physical properties and developing efficient drug delivery systems. The main purpose of the current study was to determine some important rheological properties of two well-known hydrogels (agarose and gelatin methacryloyl [GelMA]).

**Materials and Methods:** Rheological properties of gel solutions with different concentrations were measured using oscillatory rheometry. Agarose gels of 1% and 2% (w/v) concentration were prepared in 100 mL de-ionized water. The GelMA solutions of 10% and 15% concentrations were prepared by dissolving dry GelMA in deionized water. Rheological measurements were performed using a rheometer with cone-plate geometry.

**Results:** Both storage modulus ( $G'$ ) and loss modulus ( $G''$ ) increased with an increase in frequency. Rheological properties of both types of gel solutions were strongly influenced by the amount of concentration. The shear stress profiles demonstrated shear thinning in both types of gels. Viscosity of 1% agarose and 2% agarose was found comparable with 10% GelMA and 15% GelMA, respectively.

**Conclusions:** Results obtained from experiments revealed that rotational rheometry can be confidently used to determine viscous and elastic response of hydrogels in the aqueous state. The results will help to select the right type of gel and amount of concentration for the bio-printing of tissues.

**Keywords:** Biological Gels, Agarose, Gelatin Methacryloyl, Rheology, Viscosity

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## Introduction

Biological hydrogels are widely used in experiments pertinent to cell culture and tissue engineering. Hydrogel are prepared as a solution and solidified to form a viscous gel. Gel formation can be performed either using physical (non-covalent) or chemical (covalent) crosslinking. A variety of commercially available hydrogels are commonly used for encapsulating cells in three dimensional structure of gels to study cellular mechanobiology. Biological hydrogels closely resemble native extracellular matrices (ECMs) as well as mechanics similar to many soft biological tissues.<sup>1</sup> Biogels are characterized by viscoelastic properties with modulus not more than a few kPa, which is much smaller compared to other solid materials.<sup>2</sup> Hydrogels can be classified based on their biophysical properties, method of preparation for a particular application, biodegradation and sensitivity. Determinations of mechanical and rheological properties of hydrogels are important towards understanding cellular mechanotransduction. Since hydrogels contain water and have swollen networks, therefore, they have

emerged as the most suitable polymer for cell culture and several other biomedical applications. Despite the availability of sophisticated equipment in the market, inconsistent data on physical properties of hydrogels at micro- and nano-levels has been reported in the published literature.

Mechanical and rheological properties, swelling, mesh size, concentration, and degradation of hydrogels affect cellular behavior in the substrate such as spreading, migration and stem cell differentiation. Typical mechanical properties of hydrogels reported in the published literature are elastic modulus, shear modulus and Poisson's ratio. In addition to mechanical properties, rheological properties are also considered important with regard to cell culture and growth. Research is focused on developing hydrogels with physical properties closed to the tissue. Two well-known hydrogels used for electrophoresis or chromatography and variety of cell culture applications are agarose and gelatin methacryloyl (GelMA). The GelMA hydrogel possesses essential properties of native ECM which facilitate cells to proliferate in GelMA-

based scaffolds. One of the fundamental techniques to determine the viscoelastic properties of these hydrogels is using rheometer. Rheological properties of agarose gel and GelMA in aqueous and semi solid state have thoroughly been studied over the past few decades.<sup>3-5</sup> The majority of those studies were dedicated to find the relationship between molecular weight, gelation temperature and gelation time on physical properties of hydrogels. Rheological properties of pure agarose and agarose mixed with nanowhiskers were determined using pure oscillatory shear stress tests.<sup>6</sup> A significant alteration in reinforcement of gel was observed by mixing nanowhiskers with agarose. Similarly, stiffness and rheological properties of the GelMA hydrogels using dual crosslinking was found to be improved compared to single crosslinking with either ultraviolet (UV) exposure or mTGase treatment.<sup>7</sup>

Determination of rheological properties of hydrogels may be challenging due to their soft nature. In addition, there are limited studies related to the determination of viscoelastic behavior of gels in solution state. This study is a step forward to measure viscous and elastic properties of biogels in oscillatory mode in aqueous state. The viscous modulus and loss modulus of the prescribed gels were determined using frequency imposed on the plates containing gel solutions sandwiched between them. This study provides engineering based information on the relationship between the rheological properties and concentration of the prescribed gels for a specific application in cell culture and tissue engineering.

## Materials and Methods

### Gel Preparation

Agarose powder (1 g and 2 g) were dissolved in 100 mL de-ionized water to prepare 1% and 2% (w/v) agar solutions. The agarose powder (LE, Analytical Grade) was purchased from PROMEGA Corporation USA. The main characteristics of the agarose used in this study were: gel strength (1%):  $\geq 1000$  g/cm<sup>2</sup>, gelling point (1.5%): 36–39°C, melting point (1.5%): 87–89°C, sulfate:  $\leq 0.14\%$  and moisture:  $\leq 7.0\%$ . Agar powder was slowly dispersed in the water in beakers while stirring to prevent clumping. The solution was then boiled for approximately two minutes in the microwave. Hot gel solution was swirled to dissolve agarose particles and reheated until the homogeneous solution was formed. The GelMA (Sigma-Aldrich, USA) with 10% and 15% concentrations were fabricated. To form GelMA hydrogels, GelMA precursor solutions were prepared by dissolving freeze-dried GelMA in deionized water at 40°C. Aqueous solutions of 10% and 15% GelMA were prepared since this range has been found to have stiffness suitable for cell viability.<sup>8,9</sup> Gel solutions were immediately shifted to an oven and were kept at 65°C to postpone gelation before dynamic rheological measurements.

### Equipment

Rheological measurements were performed using rheometer Gemini 200 (Molvern Instruments, UK). Cone-plate geometry with 40 mm disk diameter was used in the experiments. The upper plate was having cone angle of 4 degrees and there was 150  $\mu\text{m}$  gap between the upper and lower plate. The hot

gel solution was poured on the lower plate pre-heated to the required temperature (40°C and 25°C). Temperature was kept constant at 40°C and 25°C in all experiments. Experimental set up for rheological measurements and schematic of cone plate geometry are shown in Figure 1 and Figure 2.

## Results

In the linear viscoelastic regime, where deformation is small, the applied stress is proportional to the strain. The complex shear modulus ( $G^*$ ) can be calculated by:

$$G^* = G' + iG'' \quad (1)$$

Where,  $G'$  represents the elastic part while the imaginary part  $G''$  represents the viscous part. The elastic and viscous response is 90° apart. Materials with the elastic and viscous response between 0° and 90° are called viscoelastic. The ratio of viscous part and elastic part is known as loss factor.

### Frequency Sweep Tests

The frequency sweep and strain sweeps are well-known rheology measurements and are considered critical to the full characterization of fluid and soft biological gels. These tests provide fundamental understanding of the physical strength and cohesiveness of the fluid and gels. The tests typically determine storage modulus, loss modulus and complex viscosity as a function of frequency expressed in radians or hertz. Frequency sweep plots are typically plotted on log-log scale. Experiments are performed with progressively varying frequency and keeping amplitude constant. One of the unique characteristics of the frequency sweep test is the determination of viscoelastic properties of soft materials as a function of timescale. The tests allow measurements of several physical properties such melting point, rubbery behavior and processability measuring at different temperatures.

The effect of oscillatory frequency range (0.01 Hz-10 Hz) on the dynamic rheological properties ( $G'$ ,  $G''$ ) of both types of gel solutions was measured at a fixed temperature of 40°C. Amplitude sweep tests were conducted at a fixed frequency of



Figure 1. Left: Gel Preparation, Right: Experimental Set up for Rheological Measurements.

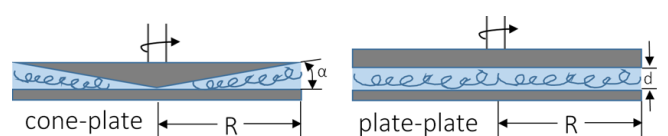


Figure 2. Schematic of Plates Used in Rheological Experiments.

1 Hz followed by frequency sweep tests. Mechanical spectra of the gel samples measured across the prescribed frequency range were slightly higher for gels with higher concentration. Variation of  $G'$  and  $G''$  with oscillatory frequency for two types of gels is shown in Figure 3 and Figure 4. The values of  $G'$  and  $G''$  for all levels of agarose and GelMA concentrations were increased with increasing frequency. No apparent transition of solution to gel was noticed in GelMA at the prescribed temperature (40°C). The gelation can be seen by the overlap of the  $G'$  and  $G''$  curves of 1% agarose as shown in Figure 3a. Both  $G'$  and  $G''$  crossover were found to be dependent on concentration. Overall, the values of  $G'$  and  $G''$  for GelMA were slightly higher than those obtained for agarose gels at higher frequencies. Apparently, both gels exhibited liquid-like behavior at 40°C.

**Flow Behavior**

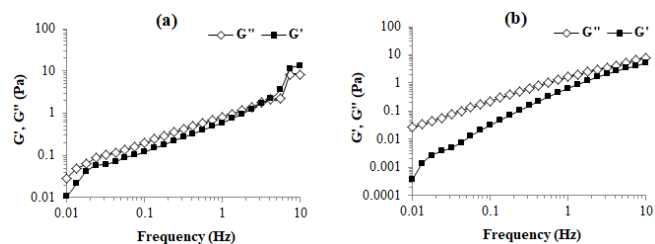
The flow curves (shear rate versus shear stress) obtained for both types of gels and concentrations are shown in Figure 5. The shear stress profiles of both types of gels demonstrated shear thinning of gel solutions. The slope of the shear stress versus shear rate curve, which indicate viscosity, was observed to decrease with increasing the shear rate. Apparently, viscosity of 1% agarose and 2% agarose was comparable with 10% GelMA and 15% GelMA, respectively.

The effect of varying shear rates on the viscosity of the gel samples with two different concentrations and two different temperatures was studied. The gel samples between the two plates were allowed to acquire the required temperature before the shear load was applied. The acquired rheological data at 40°C and 25°C is shown in Figure 6 and Figure 7. The viscosity of both types of gels at prescribed temperatures were found to decrease with increasing the shear rate. This confirmed shear thinning behavior of gels regardless of the temperature used.

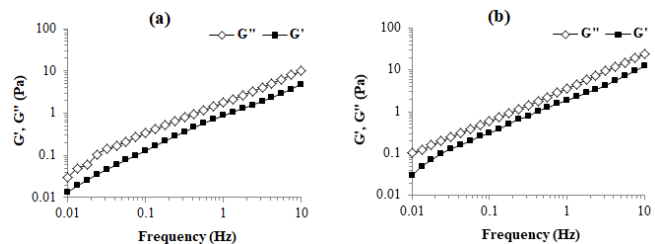
The viscosity of gels was found to significantly increase with an increase in the additive amount (concentration) in the gels and dropped with an increase in temperature. A pronounced drop in viscosity was found at lower shear rates and was gradual at higher shear rates.

**Discussion**

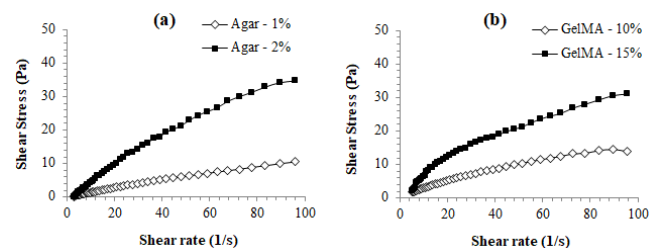
The elastic properties of gel can be modified by varying the amount of concentration. The results indicate that the rheological properties of the gelatin-based hydrogels can be controlled by the degree of substitution, polymer concentration, initiator concentration, and UV irradiation conditions. Mechanical, biochemical and rheological properties of hydrogels are strongly linked to their chemical composition, the way hydrogel is polymerized and density of cross linkers.<sup>10,11</sup> Detailed information on the types of bio gels, preparation, physical properties and their role in tissue fabrication and medicine therapy can be found in recent studies.<sup>2</sup> In rheology experiments, the frequency at which the shear force is applied determines the flow condition by calculating elastic and viscous behavior. Increase in frequency of oscillation leads to an increase of the elastic and viscous moduli which indicate the strengthening of hydrogels.



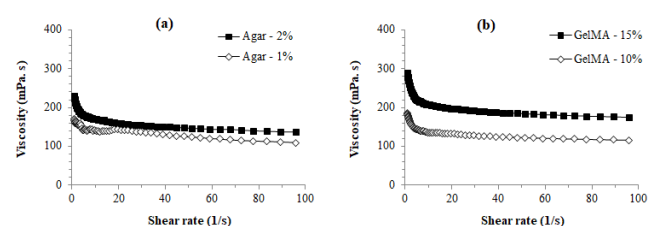
**Figure 3.** Variation of  $G'$  and  $G''$  With Frequency, (a) 1% Agarose, (b) 2% Agarose..



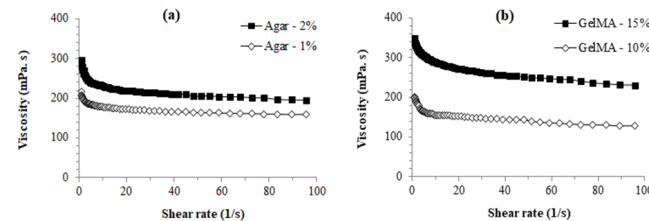
**Figure 4.** Variation of  $G'$  and  $G''$  with frequency, (a) 10% GelMA, (b) 15% GelMA



**Figure 5.** Variation of Shear Stress Profile With Shear Strain Rate, (a) Agarose, (b) GelMA



**Figure 6.** Flow Behavior of Gels at 40°C, (a) Agarose, (b) GelMA.



**Figure 7.** Flow Behavior of Gels at Room Temperature of 25°C, (a) Agarose, (b) GelMA.

Generally, a polymer solution or a melt has a complex shear modulus, both components of  $G'$  and  $G''$  depending on frequency.

The finding of this study is in consistent with other studies published earlier.<sup>12</sup> The frequency range (0.1 to 10 Hz) used in this study was consistent with those reported earlier.<sup>12,13</sup> The ratio  $G'/G''$  determines the condition for weak and strong gels. The adjacent zones between helices of the hydrogels are stronger at lower temperature and weaker at higher temperature.<sup>14</sup> The results obtained from the current study is also consistent with a recently published study.<sup>5</sup> The mechanical properties such as elastic modulus and elastic strain of agarose and GelMA for the concentration used in this study (1%-2% agarose and 10-20 % of GelMA) are comparable. The average elastic modulus for 2% agarose gel and 15% GelMA samples were found to be 65 kPa and 70 kPa, respectively, using uniaxial compression tests.<sup>15,16</sup> It is hypothesized that two different types of gels and levels of concentrations in aqueous state and the same types of gels and levels of concentrations in solid state will show similar trend with regard to viscoelastic properties. At higher temperatures, the number of junction zones should decrease leading to a weaker gel (the elastic moduli decrease). Rheological measurement usually does not provide gelation when tested at higher temperatures. The rheological characterization of the gels should be performed using frequency ranges above what has been used in this study. Increase concentration in gels produce stronger gel network with decreasing deformability.

The stiffness and concentration of the gel structure plays an important role in dictating cell behavior and fate.<sup>17,18</sup> The elastic properties of agarose gel are strongly linked with terminal temperature at the end of the cooling process and are nor dependent on the cooling rate or the thermal path.<sup>19</sup> The decrease in viscosity of polymers with a rise in temperature has well established phenomena. Decrease in viscosity with increase in temperature has been reported for hydrogels and was attributed to an increase in kinetic energy of gel.<sup>20,21</sup> The adjacent zones between helices of the hydrogels are stronger at lower temperature and weaker at higher temperature.<sup>14</sup> At higher temperatures the number of junction zones should decrease leading to a weaker gel (the elastic moduli decrease). Shear-thinning, also known as pseudoplastic behavior is one of the prime requirements for bioprinting. It is expected hydrogel flow in printing needles during extrusion will facilitate lead easier filament deposition.

## Conclusions

Rheology can be successfully used to characterize the hydrogels of different concentrations. Dynamic oscillatory measurements using a range of frequency can be used to quantify gel strength. The technique can be used to determine the condition for gelation for a range of temperatures. The increase in oscillatory frequency and level of concentration in gels caused a monotonic increase in both elastic and viscous moduli. Within the range of concentrations used in this study, both types of gel solutions demonstrated shear thinning flow behavior. Further studies are required to see the effect of gel

concentration and operating temperature other than those used in this study. This will allow studying the relationship of gel concentrations with rheological properties. Results obtained in this study may serve as a benchmark for future investigations pertinent to the effect of substrate properties on the characteristics of cells.

## Authors' Contributions

All authors equally contributed according to the order of authorship.

## Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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