Rheological Properties Of Bioinks For Printing Optimisation

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INTRODUCTION

- Rheological analytics can be applied to biomaterials to develop bioinks for 3D bioprinting applications, via determining their printability and structural fidelity.

- Ideal bioinks should possess non-Newtonian characteristics such as shear-thinning properties during printing, followed by quick structural recovery, with the later corresponding to the viability of live cells during the printing process and structure retention after printing.

- Viscosity is a characteristic defined as a flow resistance caused by internal friction under the application of stress and can be quantified using rotational rheological tests.

- Here quantitative rheological assessments of 2 materials, Alginate and Gelatin were explored to predict 3D bioprinting parameters via determination of the flow behaviour and deformation of the materials studied.

METHODS

- ***Rheometer*** - Using Anton Par Rheometer MCR 72, data was recorded through RheoCompass 1.30 software. A cone plate of 50mm and an angle of 1 degree was used.

- ***Temperature Ramp*** - The sample was subjected to a constant pre-shearing for 3 minutes at 10 s^{-1} and at 20°C, after which the temperature ramp begins, with the sample now being subjected to a constant shear rate of 50 s^{-1}. The initial temperature was 10-20°C and increased by 1°C/min until reaching 40°C. The inverse temperature ramp is subject to the same parameters as above but with a starting temperature of 40°C.

- ***Flow Curve*** - The flow behaviour of the samples was analysed using a linear flow curve with controlled shear. The samples were subjected to a linear shear rate of 1 s^{-1} to 100 s^{-1} at 25°C. The output was subject to the Herschel-Bulkley regression which describes the flow curve of the sample with a yield stress and shear-thinning or shear-thickening behaviour at stresses above the yield stress.

- ***Statistical Analysis*** - All data sets statistics were analysed using Minitab® Statistical Software. Graphs were generated using Microsoft Excel®. Design of Experiment (DOE) was performed as a Factorial 2-factor 2-level DOE.

RESULTS

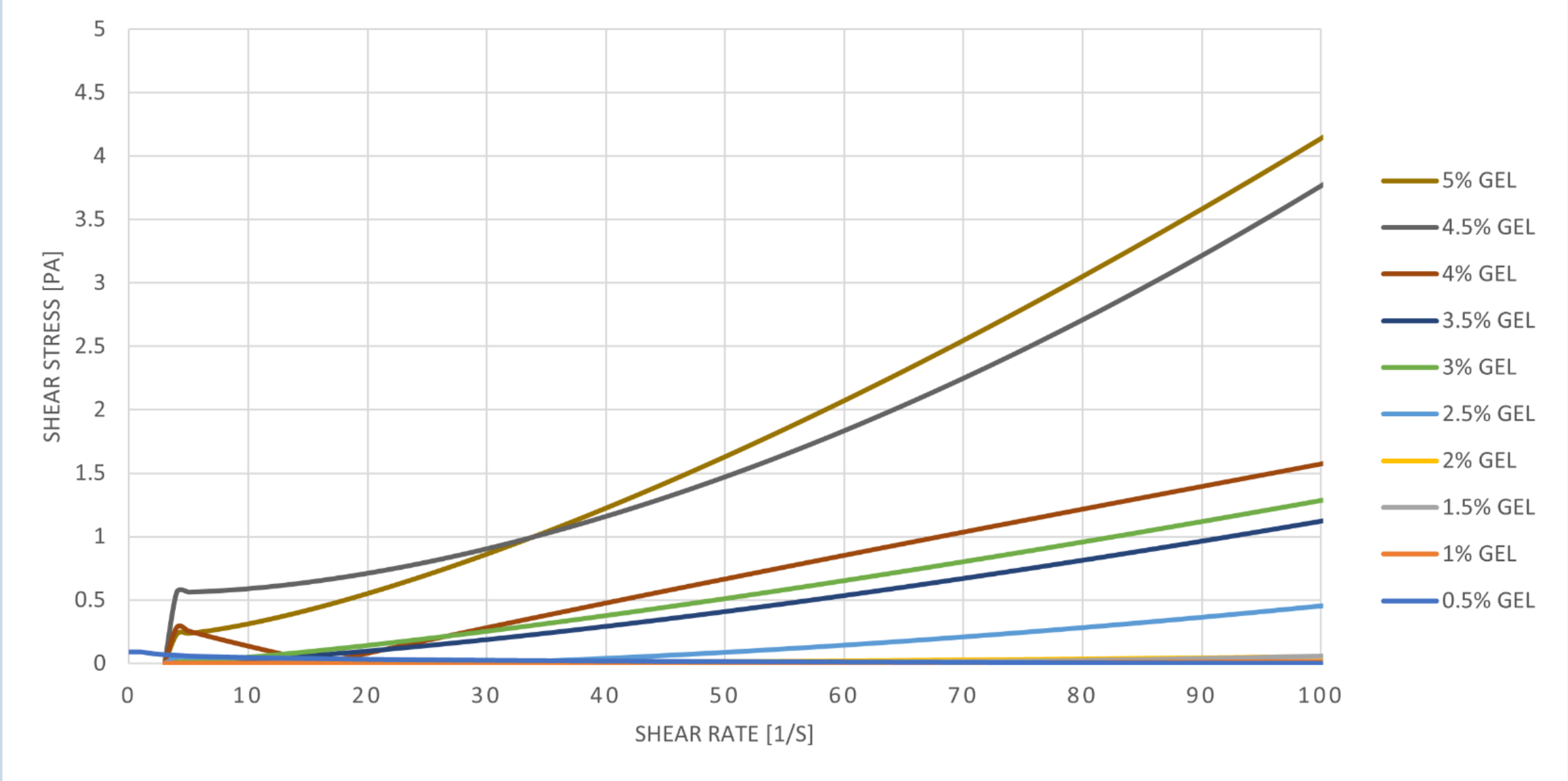

***Figure 1*: Gelatin Linear Flow Curve -** Observations of Gelatin display shear-thickening properties, and also indicates the presence of yield stress. Lower concentrations <2w/v% exhibit Newtonian behaviour. Output data was analysed using the Herschel-Bulkley regression model.

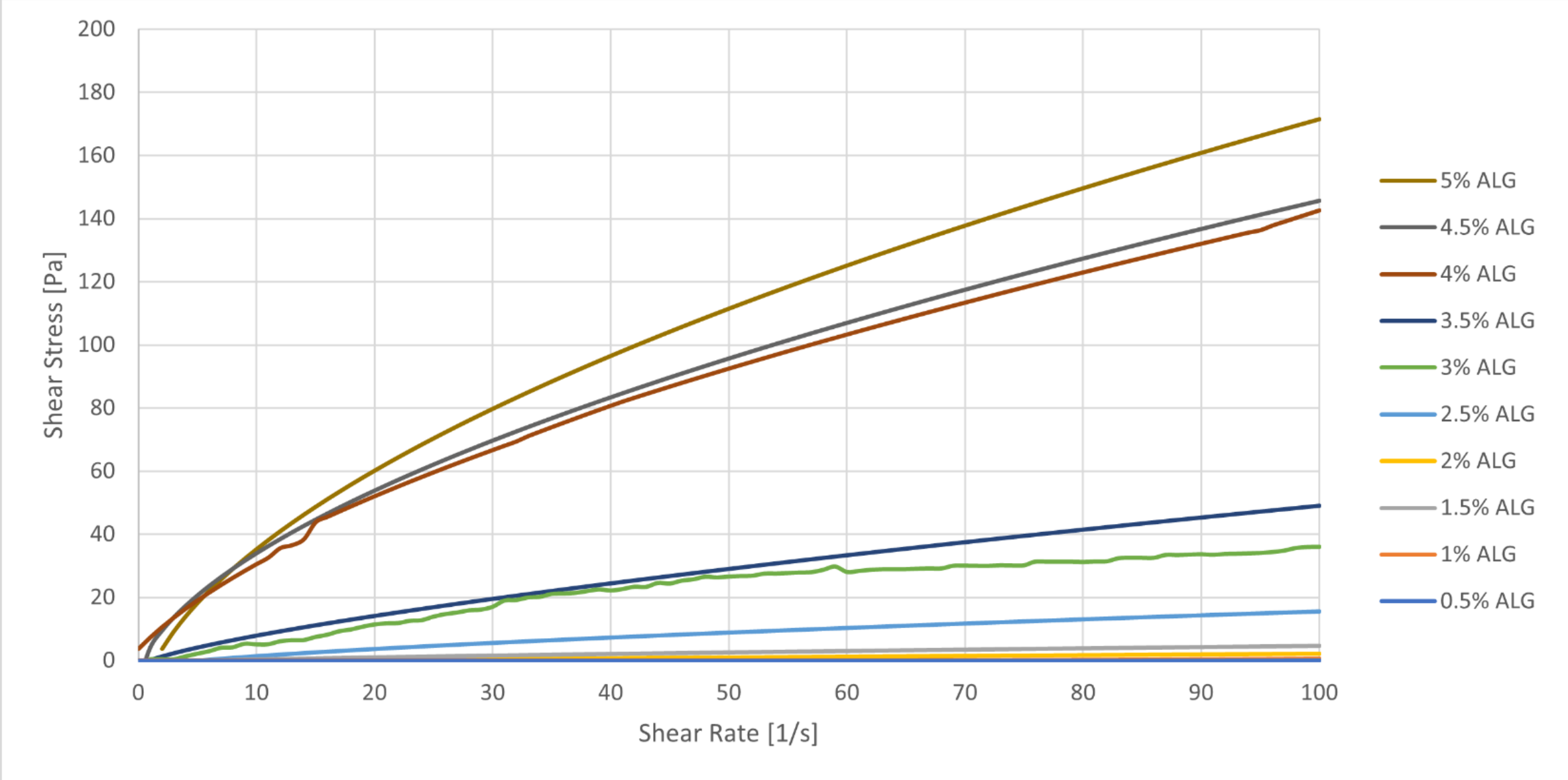

***Figure 2*: Alginate Linear Flow Curve -** Observations of Alginate display shear-thinning properties, without yield stress. Lower concentrations of < 1.5% display Newtonian fluid behaviour. Output data was analysed using the Herschel-Bulkley regression model.

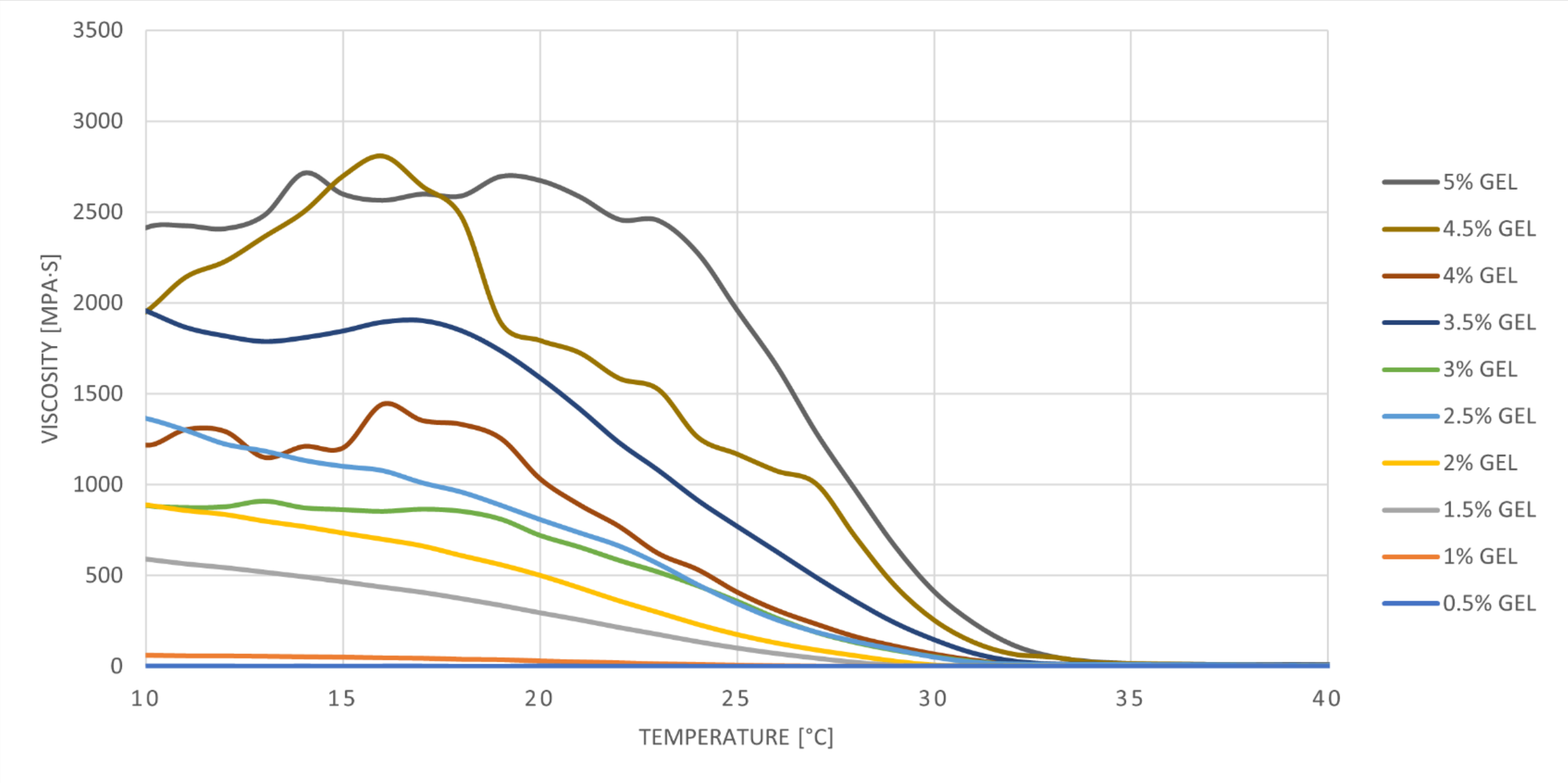

***Figure 3*: Increase Temperature Ramp (Gelatin)–** This figure displays the effect of temperature (10°C - 40°C) on the viscosity of 0.5% - 5 w/v% Gelatin concentrations. As the temperature starts to increase the initial viscosity is high and begins to decrease at the temperature increases. Instability in the viscosity is observed in the initial temperature between 10°C – 25°C.

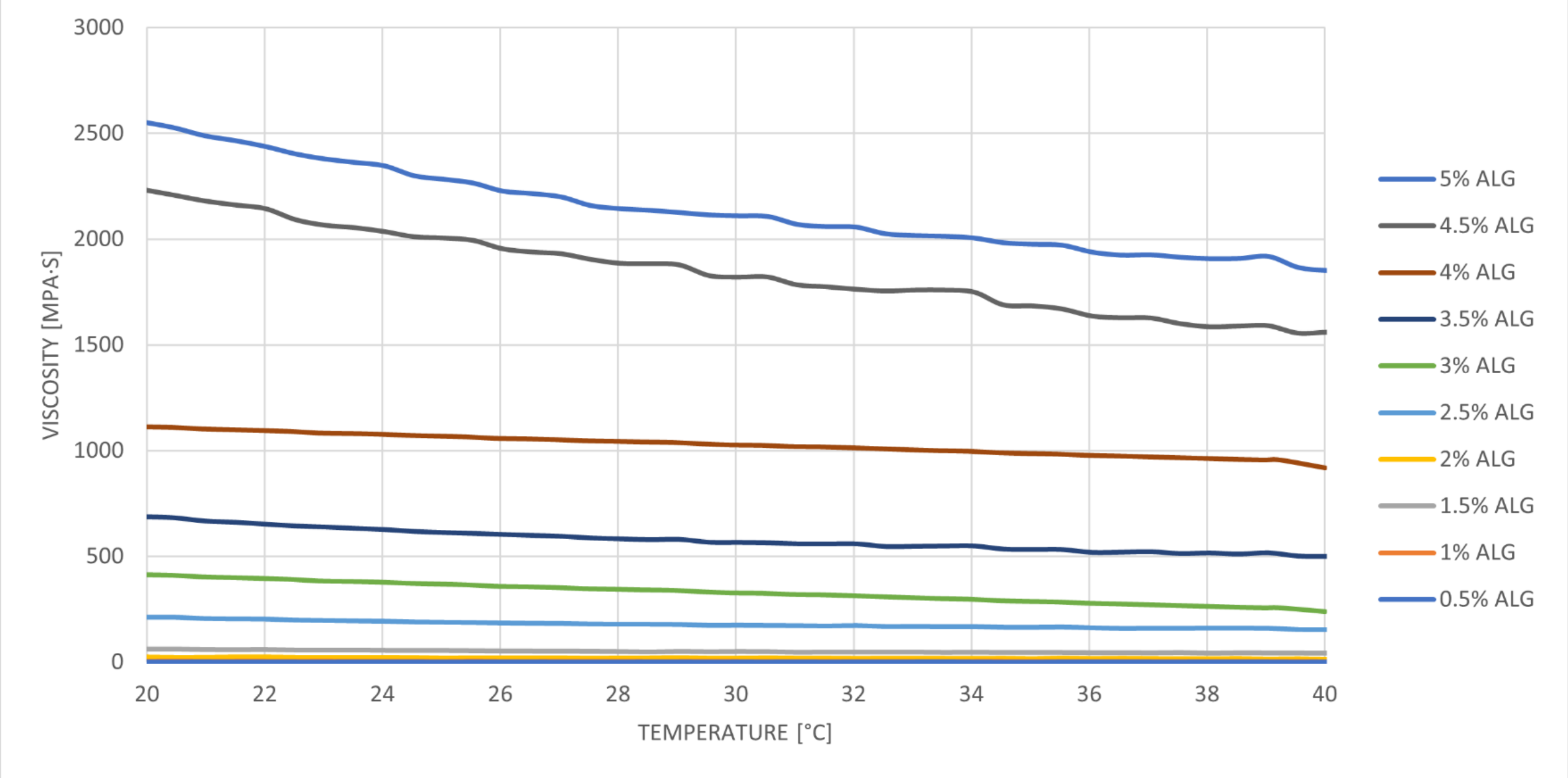

***Figure 4:* Increase Temperature Ramp (Alginate) –** This figure displays the effect of temperature (20°C - 40°C) on the viscosity of 0.5% - 5 w/v% Alginate samples. Temperature has little effect towards each concentration of Alginate < 3 w/v%. Concentrations of Alginate > 3w/v% show a larger difference between the initial and final viscosities, with viscosities decreasing as the temperature increases.

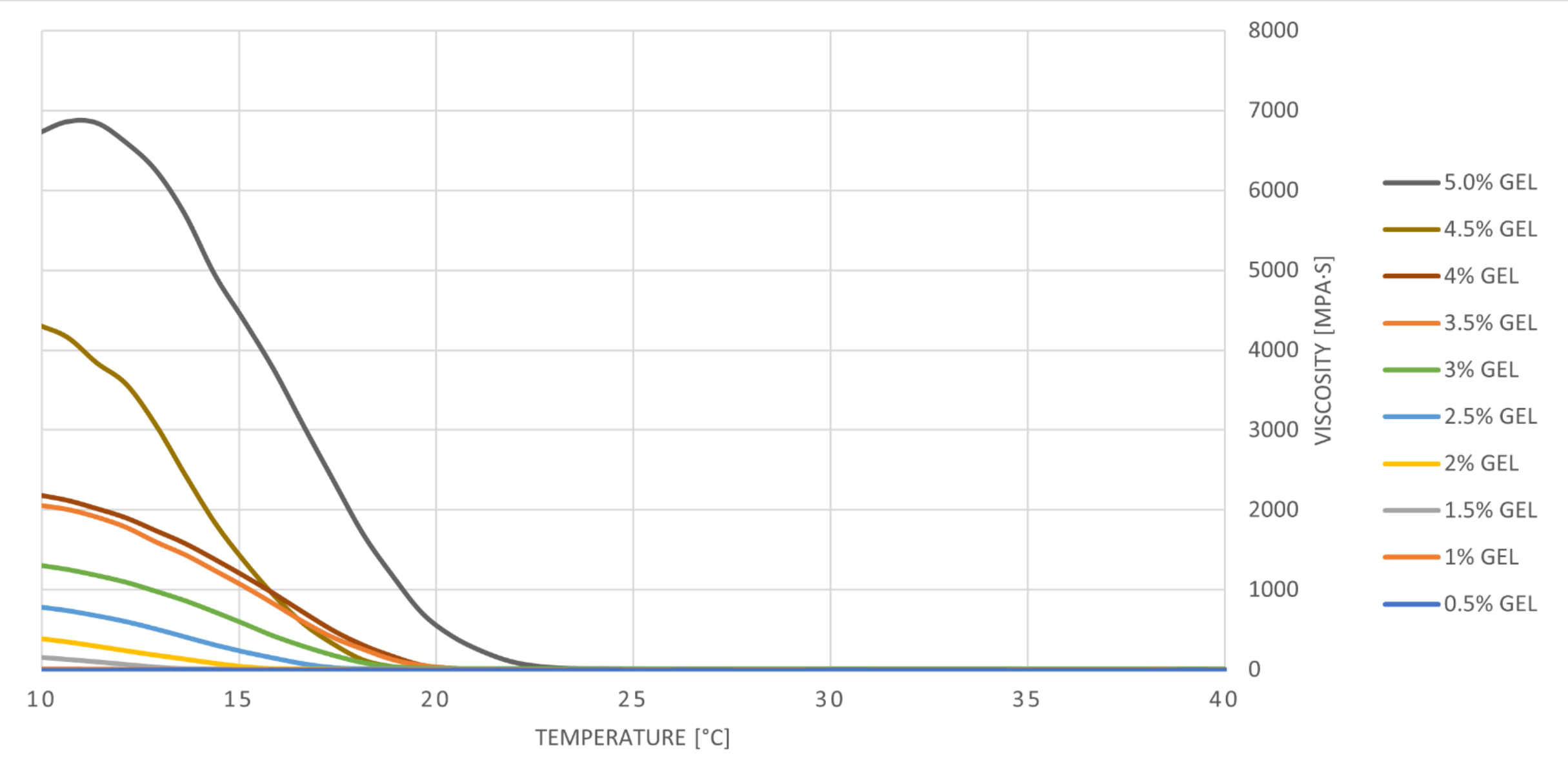

***Figure 5*: Inverse Temperature- Ramp (Gelatin) –** This figure displays the effects of temperature (40°C - 10°C) on the viscosity of 0.5% - 5 w/v% Gelatin concentrations. As the temperature decreases the viscosity remains significantly low until it reaches approximately 22°C after which the viscosity increases as the temperature continues to decrease.

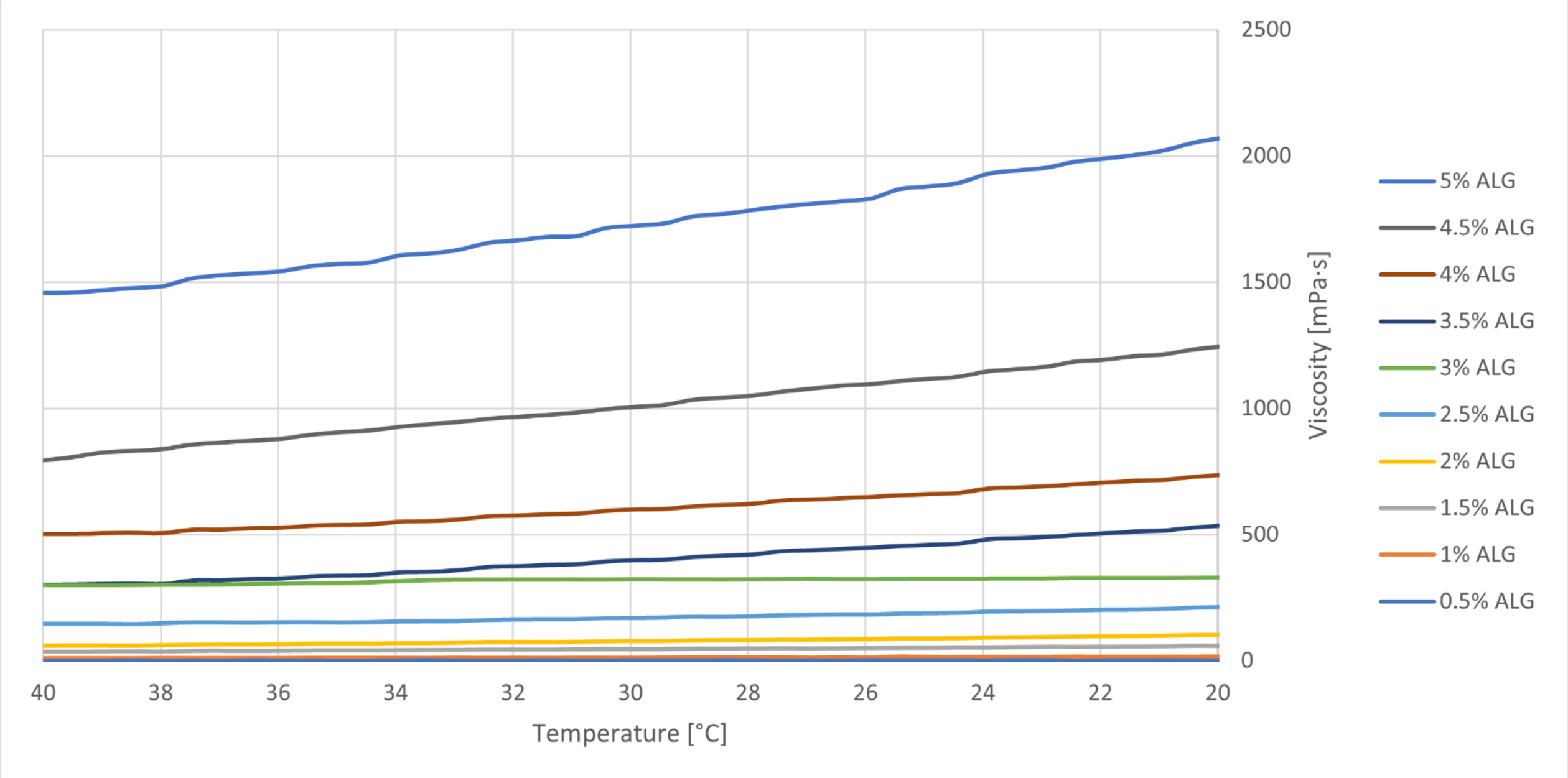

***Figure 6:* Inverse Temperature- Ramp (Alginate) –** This figure displays the effect of temperature (40°C – 20°C) on the viscosity of 0.5% - 5 w/v% Alginate concentrations. Temperature has little effect towards each concentration of Alginate < 3%. Concentrations of Alginate > 3% show a larger difference between the initial and final viscosities, with viscosities decreasing at the temperature decreases.

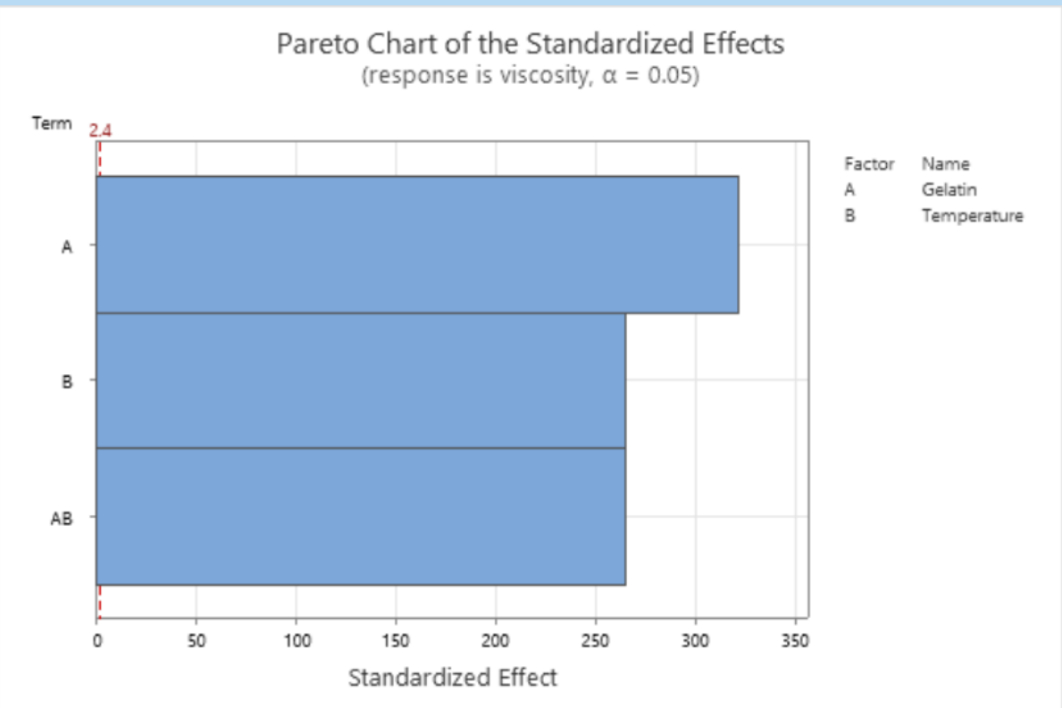

***Figure 7:* Gelatin DOE – Pareto Chart –** Factor A represents Gelatin concentrations and B represents temperature. Factors that cross the reference line (2.4) are statistically significance. Factors A, B and the interaction between A and B show statistically significant effects towards the viscosity output of Gelatin. Factor A has a greater effect towards the viscosity output of Gelatin.

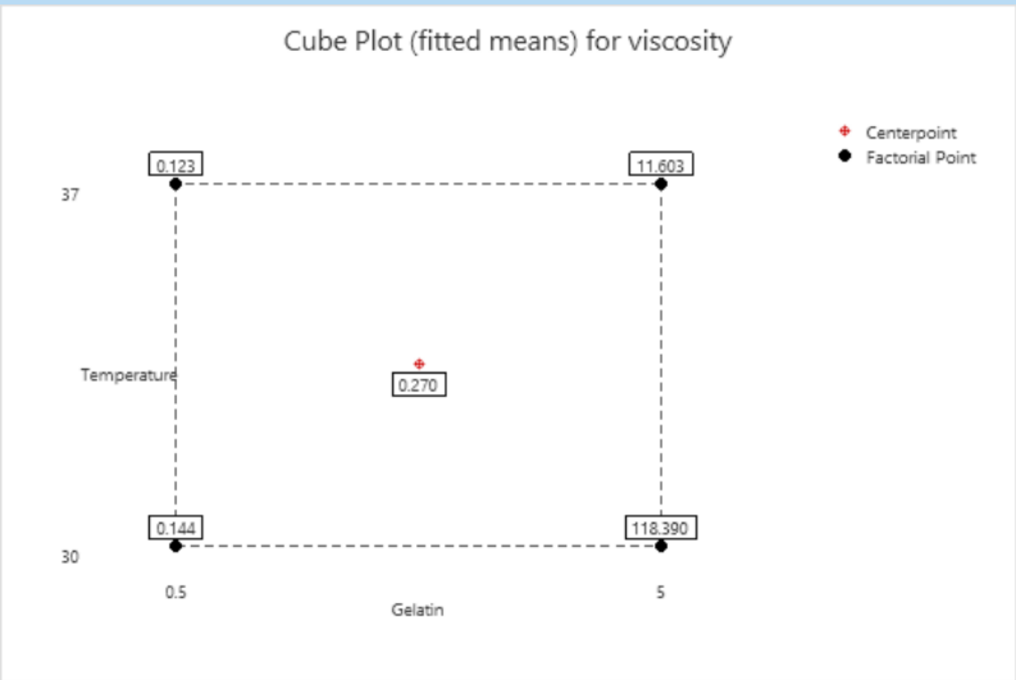

***Figure 8:* Gelatin DOE – Cube Plot -** Factorial Points represent the average viscosity (mPa·s) at the minimum and maximum boundaries of temperature (°C) (y-axis) and concentration of Gelatin (%) (x-axis). The centre point represents the viscosity halfway point (0.270 mPa·s) between the concentration of Gelatin (2.75%) and temperature (33.5 °C).

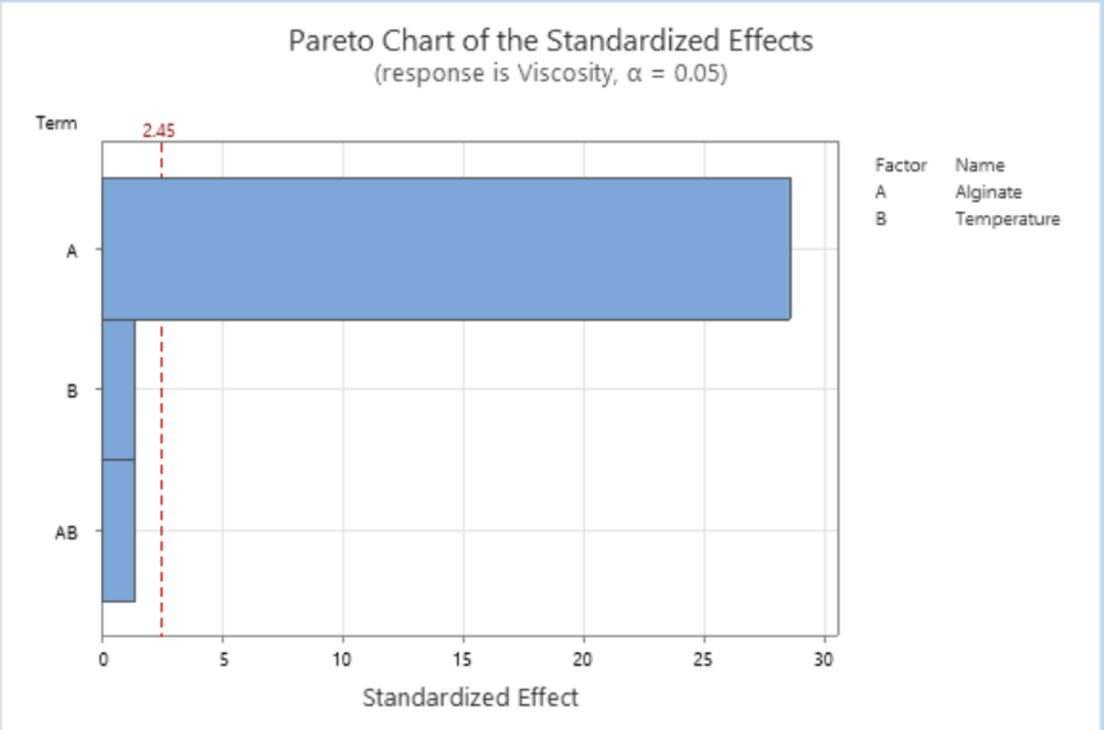

***Figure 9:* Alginate DOE – Pareto Chart –** Factor A represents Alginate concentrations and B represents temperature. Factors that cross the reference line (2.45) are statistically significance. Factor B shows no significant effect towards the output response to viscosity and the interaction between A and B shows no significant effect towards the output response to viscosity. Factor A displays a significant effect towards the viscosity output.

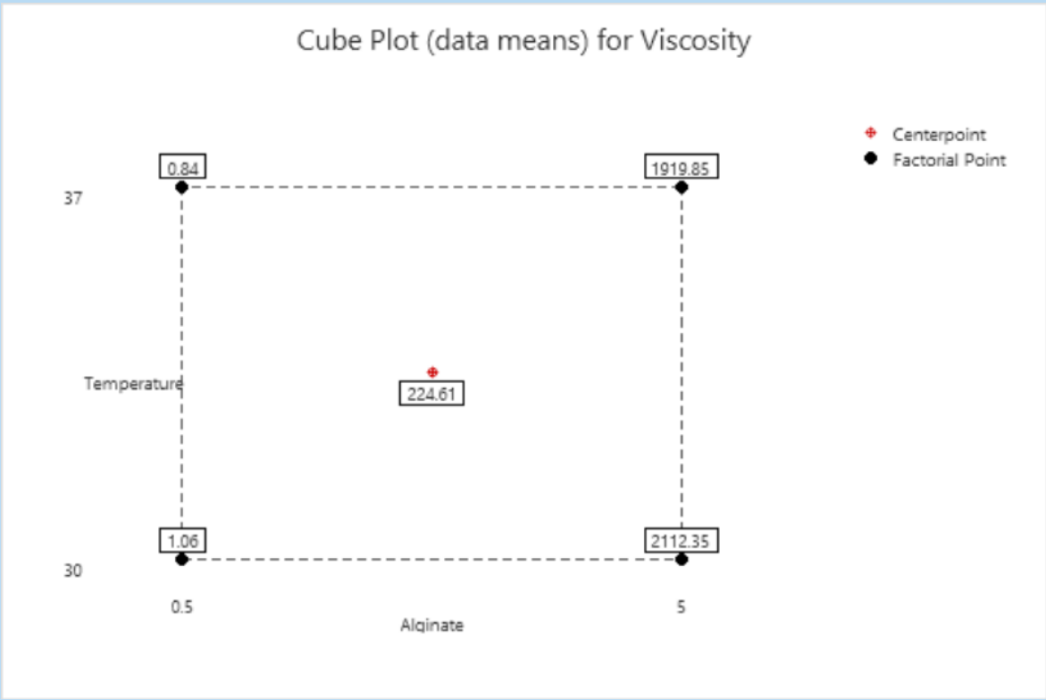

***Figure 10:* Alginate DOE – Cube Plot -** Factorial Points represent the average viscosity (mPa·s) at the minimum and maximum boundaries of temperature (° C) (y-axis) and concentration of Alginate (%) (x-axis). The centre point represents the viscosity halfway point (224.61 mPa·s) between the concentration of Alginate (2.75%) and temperature (33.5 °C).

DISCUSSION

- Rheological assessments of materials are an important tool in bioink development to establish the behaviour of the material; however, it does not provide a predictive tool on which material or concentration to select and how to relate this to the application of 3D bioprinting.

- In current literature (2022) there are no standardised guidelines on how to overcome the challenges of bioink development. What we are proposing here is the use of Design of Experiment (DOE). This allows for parameters to be easily predicted while reducing experimental time and cost in optimisation and development.

- Temperature-controlled DOE was performed on solutions of Alginate or Gelatin to quickly and reliably identify that the viscosity output was significantly impacted by the concentration of the aforementioned materials. Figures 7 & 9 shows the relationship between viscosity and the variables of temperature of Gelatin (Figure 7) or Alginate (Figure 9).

- Figure 7 shows that the greater contributor to viscosity can be attributed to the Gelatin concentration, followed by temperature and finally the interactions between concentration and temperature. Figure 9 shows the concentration of alginate alone has a greater effect on viscosity with no contribution attributed to temperature or the interactions of temperature and concentration. These findings allow the user to control Gelatin viscosity by manipulation of Gelatin concentration and temperature. Conversely, Alginate viscosity is manipulated by the concentration of Alginate alone.

- Rheological analysis of either Alginate or Gelatin solutions under increasing shear rate displayed shear-thinning behaviour across all concentrations examined, which is desired in extrusion-based 3D bioprinting. This data allows the user to evaluate cellular response to the sheer stress exposure during the print process.

- Performing temperature ramp experiments revealed reversible changes in viscosity with increasing/decreasing temperature ramps. Alginate showed little change in viscosity in both temperature ramps indicating structural stability compared to Gelatin which showed a significant change in viscosity (P=<0.05).

- Additional rheological studies can further establish the behaviours of the materials such as thixotropy which is a time-dependent test that describes the properties of the material, at rest, during application and recovery. Oscillation tests can be performed to characterise the viscoelastic properties of the materials such as identifying the Linear-viscoelastic (LVE) range and performing a frequency sweep to look at the short term and long-term stability.

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