ADVANCED BIOMATRIX
A BICO COMPANY

LAP Dosing Map Rheology Study with PhotoGel® 50% Degree of Methacrylation

Abstract: *This study was conducted to assess the effect of different LAP concentrations on the rheological properties of PhotoGel® 50% degree of methacrylation. PhotoGel® at 10% concentration was mixed with LAP to obtain the following concentrations: 0.01%, 0.034%, 0.1%, 0.25%. 0.5% and 1% of LAP. Rheology was tested using the Elastosens to generate stiffness curves, measuring shear storage modulus against time. Higher concentrations of LAP lead to faster polymerization and stronger hydrogels, though there is a clear upper limit, or even detrimental effect at the extremes.*

Materials

Name/Description	Part Number	Lot Number
PhotoGel® 50% DoM	5361	9530
LAP	5269	9761
DPBS	21600-044 1x50 L	2814720

Results

Figure 1 below depicts the stiffness curves of PhotoGel® with different concentrations of LAP photoinitiator. The vertical dotted lines depict the start and end of UV light exposure. The Control group depicts the thermal gelation curve of PhotoGel® without any LAP or photostimulation. The stiffness of PhotoGel® increases with increasing LAP content at concentrations of 0.01% to 0.1%. At concentrations of 0.25% to 1%, the opposite trend is seen, where the stiffness decreases slightly.

The rate at which the stiffness increases as photocrosslinking occurs, i.e. the slope of the linear portion of the curves, increases with increased LAP content for all groups. LAP concentrations of 0.25%, 0.5%, and 1% show comparable rates of crosslinking.

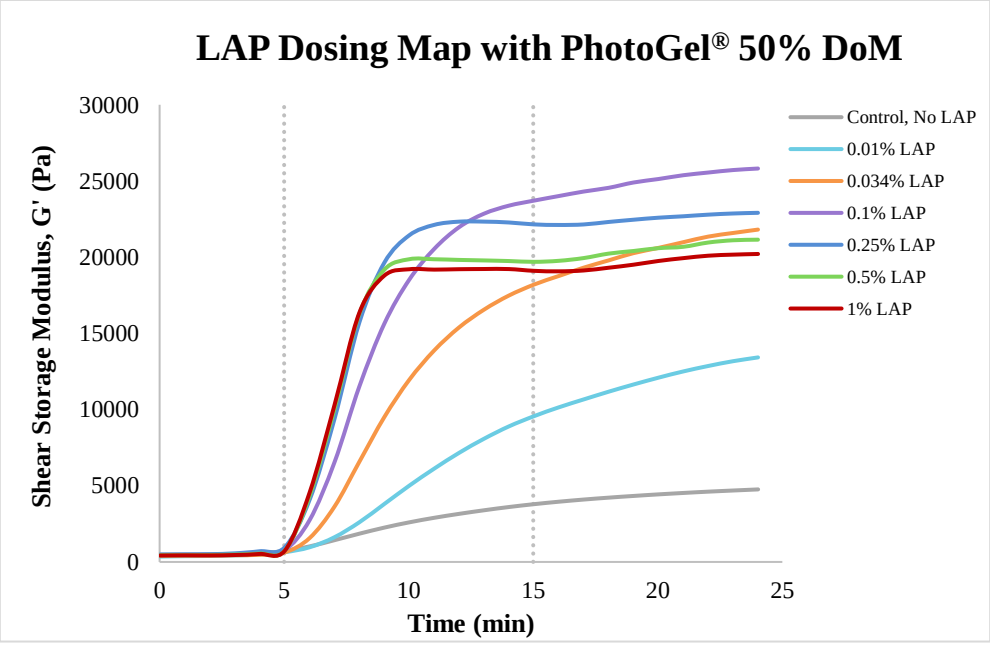

Figure 1. Resulting Rheology Curves for PhotoGel® and LAP at different concentrations.

Figure 2 below shows the trend of the maximum stiffness obtained for each experimental group. The decrease in maximum stiffness corresponding to higher concentrations of LAP may be attributed to solution/hydrogel opacity. The higher concentrations of LAP changed the solution from transparent to slightly opaque, which may have acted as a photoabsorber.

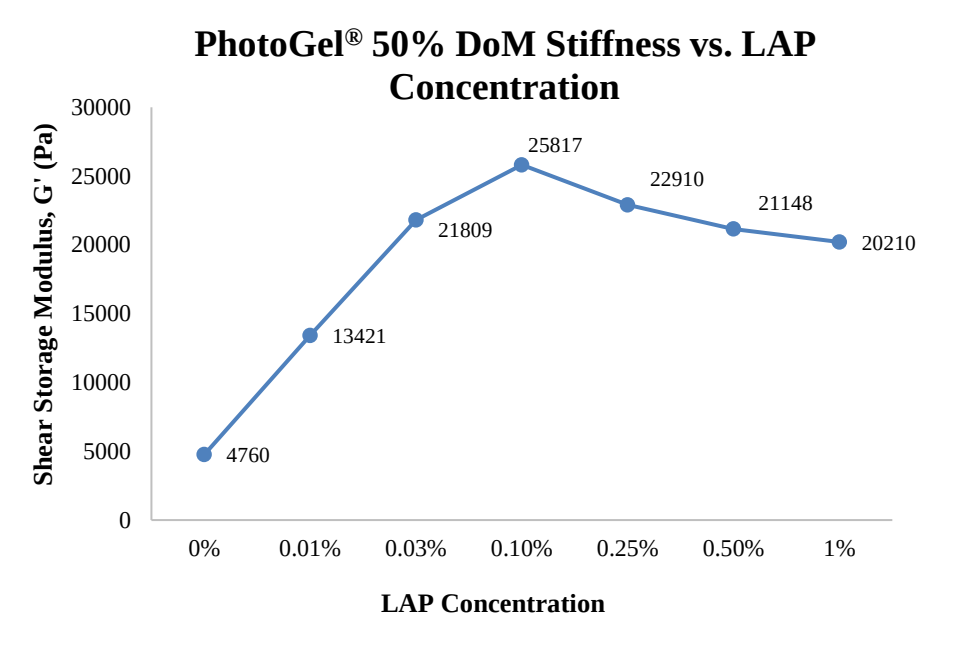

Figure 2. Maximum Stiffness of PhotoGel® with LAP at different concentrations.

Conclusion

In conclusion, the concentration of LAP plays a significant role in the rheological properties of PhotoGel®. As the photoinitiator content increases, the maximum stiffness and rate of crosslinking increase up to a concentration of 0.1%. At concentrations higher than 0.1%, the rate of crosslinking no longer increases, and the maximum stiffness decreases.

This may be important when fine tuning PhotoGel® hydrogels with a desired stiffness, as a higher LAP concentration may not translate to a stiffer gel. Choosing the right combination of gelatin and LAP concentration is key to optimizing photocrosslinking for 3D bioprinting, 3D hydrogels, and tissue engineering applications.

Procedure

The following procedures were performed to carry out the study. First, PhotoGel® samples were prepared with different LAP concentrations (see *"PhotoGel® Sample Preparations"* procedure below). Then, the Elastosens was calibrated, and the experimental parameters set (see *"Elastosens Experimental Set Up"* procedure below). Each PhotoGel® sample was then tested in duplicate sequentially and the test data collected and processed. The same bulk components, i.e. PhotoGel®, 1X PBS, LAP, were used for all test groups in the study.

PhotoGel® Sample Preparations

1. A highly concentrated solution of PhotoGel® was incubated at 40°C to liquefy and was diluted down from 12.9% (129 mg/mL) to 10% (100 mg/mL) using 1X PBS.
2. The bulk solution was aliquoted into individual tubes and kept at 40°C.

3. Individual LAP solutions were prepared by dissolving LAP in 1X PBS in the following concentrations:
    a. 5 mg/mL for 0.01% final LAP concentration.
    b. 17 mg/mL for 0.034% final LAP concentration.
    c. 50 mg/mL for 0.1% final LAP concentration.
    d. 125 mg/mL for 0.25% final LAP concentration.
    e. 250 mg/mL for 0.5% final LAP concentration.
    f. 500 mg/mL for 1% final LAP concentration.

4. The LAP solutions were incubated at 40°C to prevent the PhotoGel® from gelling when mixed.
5. Following ABM's directions for use, 100 µL of the corresponding LAP solution was added to one 5 mL aliquot of PhotoGel® and mixed gently.
6. For the Control group, 100 µL of 1X PBS with no LAP was added.
7. The mixed solutions were covered in aluminum foil to minimize light exposure and incubated at 40°C prior to testing.

Elastosens Experimental Set Up

1. The Elastosens was turned on and calibrated once via vibration calibration.
2. The temperature was set to 20°C using the manual temperature control.
3. Once the temperature was equilibrated, a large empty sample cup was inserted in the testing chamber and secured. The same sample cup was used for all test groups in this study.
4. A new sample file was created in the following folder: LAP Dosing Map.
    a. File format: product name, test condition.
        i. E.g.: PhotoGel-50 0.01% LAP.
        ii. E.g.: PhotoGel-50 0.1% LAP.

5. The following test parameters were set for all test conditions:
    a. Type – Stiff.
    b. Sample File – Named as noted above.
    c. Test Name – Tester's initials followed by the number of the test run under that file name (each test was run in duplicate and the resulting curves averaged).
        i. Ex - the first test ts01, second test ts02.
    d. Custom Information:
        i. Volume – 2 g.
        ii. Oil – No.
        iii. Concentration – 100 mg/ml.
        iv. Photoinitiator – LAP.
        v. Photoinitiator concentration – Varies.
        vi. Cup size – Large.
        vii. Light intensity – 100%.
        viii. Exposure time – 10 minutes.
        ix. Temperature – 20°C.

6. The following test sequences were set in the "Measurement Sequences" window:
    a. Sequence 1: Thermal equilibration
        i. Duration: 5 min.
        ii. Step: 1 min.
        iii. Temperature configuration: manual.
    b. Sequence 2: Photocrosslinking
        i. Duration: 10 min.
        ii. Step: 1 min.

iii. Temperature configuration: manual.
iv. Photostimulation LED 405nm: 100%.

c. Sequence 3: Equilibration
i. Duration: 10 min.
ii. Step: 1 min.
iii. Temperature configuration: manual.

7. For the Control group, the photostimulation setting was not turned on. The test simply incubated the sample at 20°C for 25 minutes and the stiffness was recorded as gelation occurred.
8. The test was initialized, and the sample cup calibrated once prior to testing each group.
9. The sample cup was removed from the machine and placed on a scale to add 2 g ± 0.1g of sample.
10. The cup was placed into the machine and the test started.
11. After the first test was finished, a second test (following the procedure above) was run to obtain a duplicate test under the same file name.

The sample was removed from the cup after gelled, and the cup rinsed with milli-Q water and dried to be reused.