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## **WhitePaper: Thermogels mixed with biocomponents for 3D cell scaffold**

December 10, 2013

### **Purpose**

To determine materials which may be utilized to form 3D gels at body temperature when combined with cell-growth promoting gelatin.

### **Materials and Methods**

The following reagents/polymers were used for this study

Gelatin Type A from porcine skin lot # 059K0019 (Sigma)

Gelatin Type B from bovine skin lot # 089K0167 (Sigma)

Phosphate buffer saline (Aldrich)

Poloxamer 407 lot #061M0242V (Sigma)

PolyVivo AO25 Stearate-Modified Methyl Cellulose (Lot# 20929JSG)

PolyVivo AO26 Poly(poloxamer 407)-Methylene-diphenyl-di-isocyanate linked (Lot# 21008JLB)

### **Methods**

Gelatin dissolved in PBS at 1%, 5%, 10% or 50% w/v solutions by adding gelatin to PBS and keeping in 60C oven for approximately 1 hour to dissolve. AO26 and AO25 dissolved at 10% w/v in PBS by putting it on a shaker with ice packs for 2 days. Poloxamer 407 dissolved at 20% w/v in PBS by putting it on a shaker with ice packs for 1 day. Solutions were stored cold and for gelatin solutions were warmed prior to mixing. Solutions were mixed together at room temperature at the ratios indicated in Table 1.

### **Rheology**

Rheology performed on AR550 (TA instruments) with 60mm 2degree cone on indicated polymer solutions. Viscosity of solution at 0.1 (sec-1) at temperature indicated in Table 1 was measured (1 minute peak hold 5 second test intervals). Rheology performed by oscillating at constant 6.283 rad/s, 0.1% strain, in increments of 2.5C ranging between 5-45C with 3 minutes of temperature equilibration at each point. Note solutions which were notably solidified at cool temperatures were tested with initial temperature at 45C and then cooling at 2.5C increments.

### **Rheology Charts**

Table 1 below displays data obtained from the viscosity test and the rheology temperature profiles. Note that solutions of interest have been bold highlighted.

Table 1. Rheology data

<b>Graph #</b>	<b>Solution</b>	<b>Viscosity solution</b>	<b>Max G'</b>	<b>Temp max G'</b>	<b>Max G''</b>	<b>Temp max G''</b>
1	AO26-10%	0.2333 Pa.s at 5C	1123	45.0	113.1	37.5
2	Poloxamer 407-20%	0.004593 Pa.s at 5C	14300	35.0	1537	27.5

3	AO25-10%	1.798 Pa.s at 5C	892100	45.0	219300	45.0
4	Gelatin Type A (10%) in PBS	1.256 Pa.s at 45C	39810	5.0	4101	5.0
5	Gelatin Type B (10%) in PBS	Below detectable limit at 45C	4847	5.0	45.39	5.0
6	<b>(1:3) Gelatin Type A (10%):Poloxamer 407</b>	<b>19.85 Pa.s at 5C</b>	<b>5282</b>	<b>37.5</b>	<b>1049</b>	<b>45.0</b>
7	Gelatin Type B(50%) in PBS	Below detectable limit at 45C	8541	5.0	142.7	5.0
8	<b>(1:3) Gelatin Type A (10%):AO26</b>	<b>22.10 Pa.s at 5C</b>	<b>1326</b>	<b>45.0</b>	<b>382</b>	<b>45.0</b>
9	<b>(3:1)Gelatin Type B (10%): AO26</b>	<b>57.97 Pa.s at 5C</b>	<b>611</b>	<b>45.0</b>	<b>237.1</b>	<b>32.5</b>
10	Gelatin type A (1%) in PBS	0.3661 Pa.s at 45C	33.31	5.0	0.3835	5.0
11	Gelatin type B (1%) in PBS	Below detectable limit at 45C	2.066	5.0	0.2921	5.0
12	Gelatin type A (5%) in PBS	0.5013 Pa.s at 45C	2744	5.0	32.00	5.0
13	Gelatin type B (5%) in PBS	0.00566 Pa.s at 45C	1555	5.0	16.86	5.0
14	<b>(1:3) Gelatin (1% type A): AO26 in PBS</b>	<b>42.90 Pa.s at 5C</b>	<b>1208</b>	<b>45.0</b>	<b>132.3</b>	<b>45.0</b>
15	(3:1) Gelatin (1% type A): AO26 in PBS	0.08107 Pa.s at 45C	1.552	5.0	0.2710	5.0
16	(3:1) Gelatin (5% typeA): AO26 in PBS	0.2423 Pa.s at 45C	1608	5.0	17.64	7.5
17	(3:1) Gelatin (1% type A): AO25 in PBS	23.97 Pa.s at 45C	48300	5.0	10650	5.0
18	(3:1) Gelatin (5% type A): AO25 in PBS	10.72 Pa.s at 45C	2637	5.0	580.2	5.0
19	(3:1) Gelatin (5% typeA): Poloxamer in PBS	3.658 Pa.s at 45C	260.7	5.0	15.77	37.5
20	<b>(1:3) Gelatin(1% type A): AO25 in PBS</b>	<b>0.6774 Pa.s at 5C</b>	<b>2906</b>	<b>45.0</b>	<b>234.7</b>	<b>45.0</b>
21	<b>(1:3) Gelatin (5% type A): AO25 in PBS</b>	<b>12.38 Pa.s at 5C</b>	<b>60180</b>	<b>45.0</b>	<b>10820</b>	<b>45.0</b>
22	(3:1) Gelatin(1% type A): Poloxamer 407 in PBS	0.2696 Pa.s at 45C	0.5191	5.0	0.1534	12.5
23	<b>(1:3) Gelatin (5% type A): Poloxamer 407 in PBS</b>	<b>0.1083 Pa.s at 5C</b>	<b>2073</b>	<b>45.0</b>	<b>106.2</b>	<b>45.0</b>
24	<b>(1:3) Gelatin (1% type A): Poloxamer 407 in PBS</b>	<b>0.1490 Pa.s at 5C</b>	<b>1770</b>	<b>45.0</b>	<b>85.38</b>	<b>45.0</b>

Figures 1-24 display the rheology curves for each solution respectively. Note that  $G'$  is red,  $G''$  is blue, and delta is black. The X axis indicates temperature and the color-coded Y axis indicates  $G'$ ,  $G''$ , and delta. Gelation is generally indicated by a rise in the  $G''$  and  $G'$  with a corresponding decrease in delta.

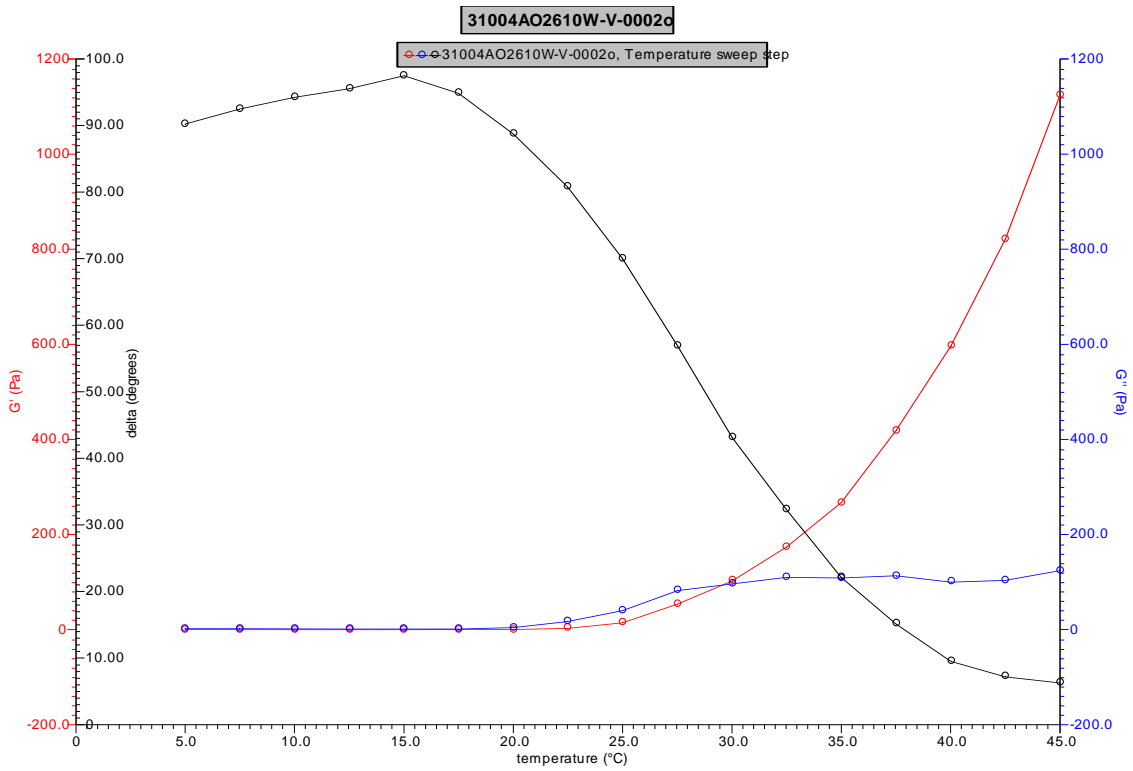


Figure 1. Rheology curve solution AO26 10% in PBS

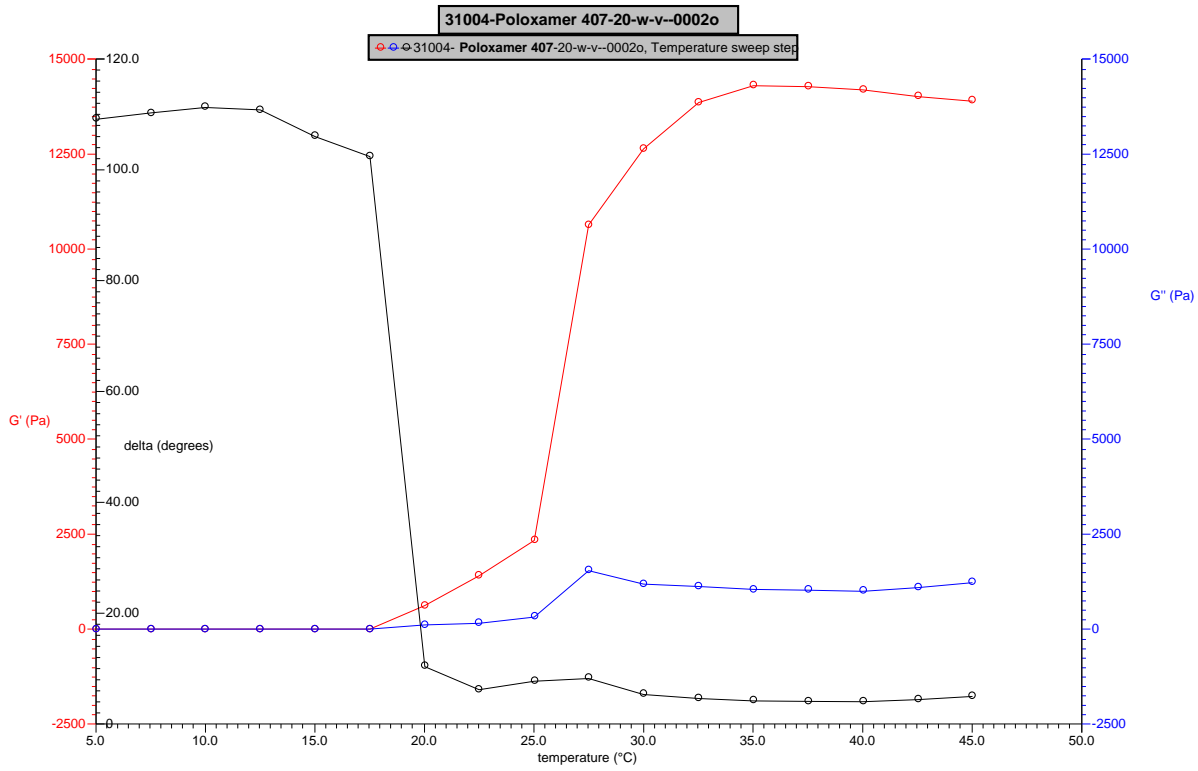


Figure 2. Rheology curve solution- Poloxamer 407 20% in PBS

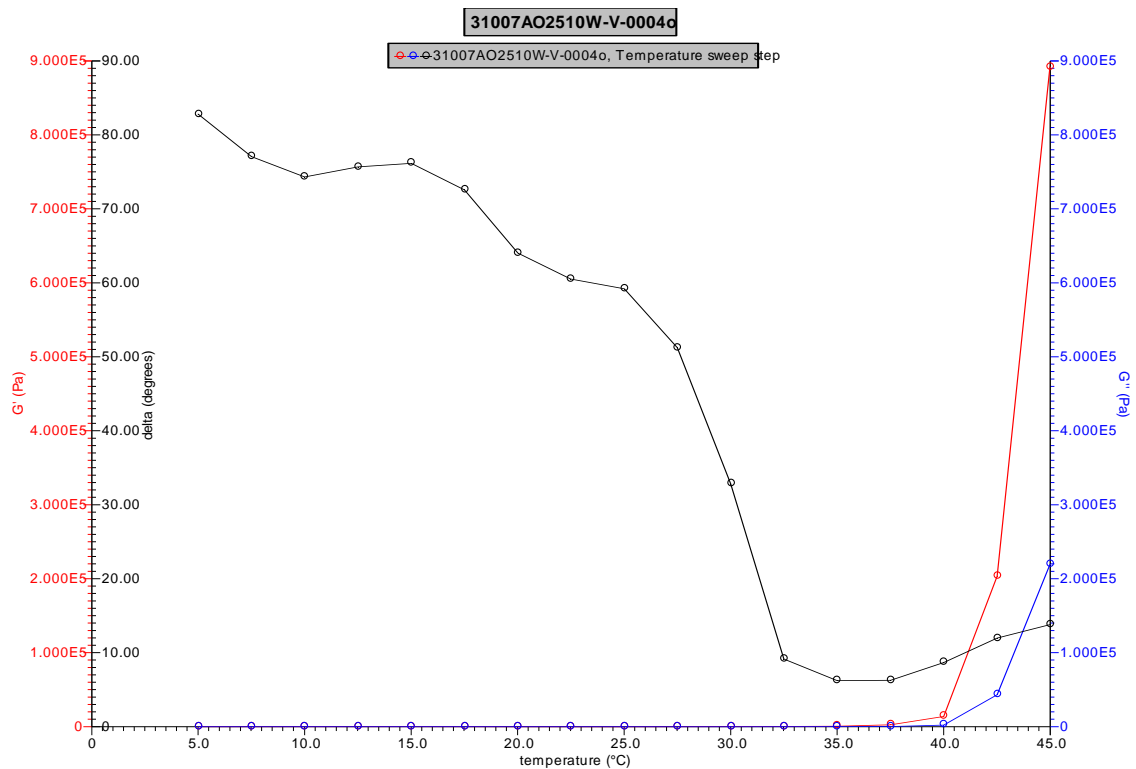


Figure 3. Rheology curve solution AO25-10% in PBS

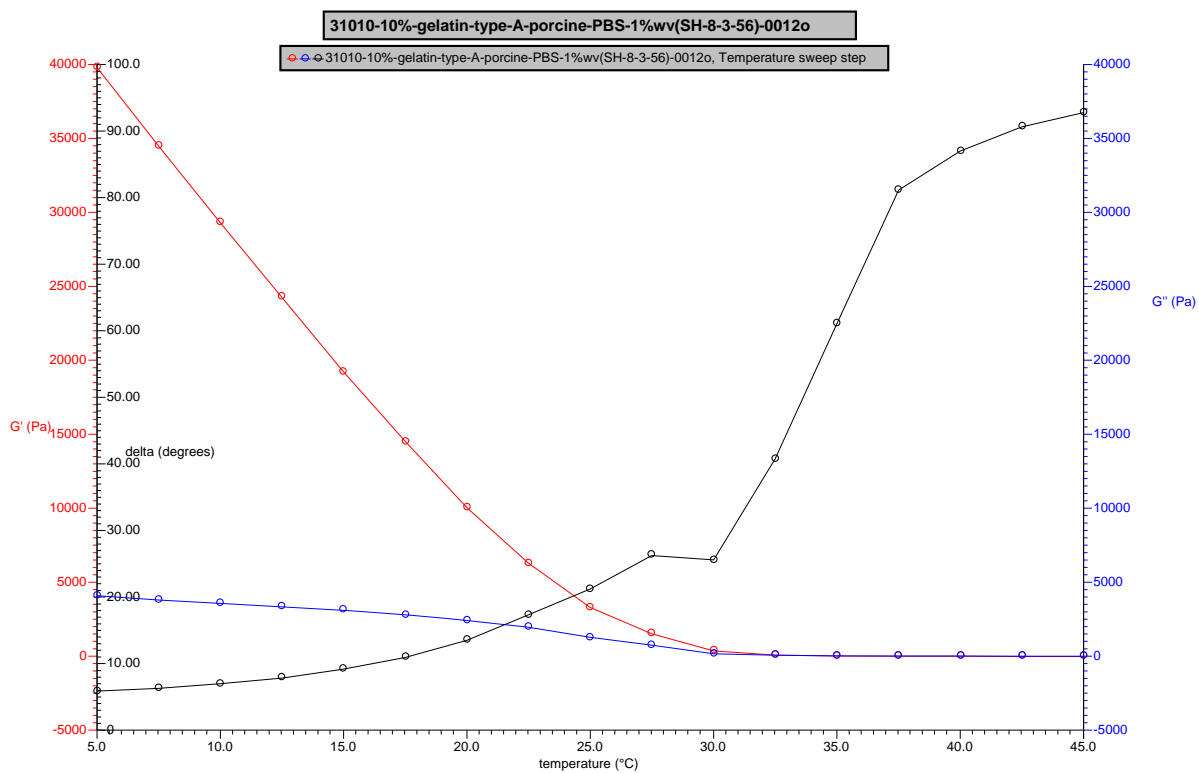


Figure 4. Rheology curve solution 10% Gelatin type A in PBS

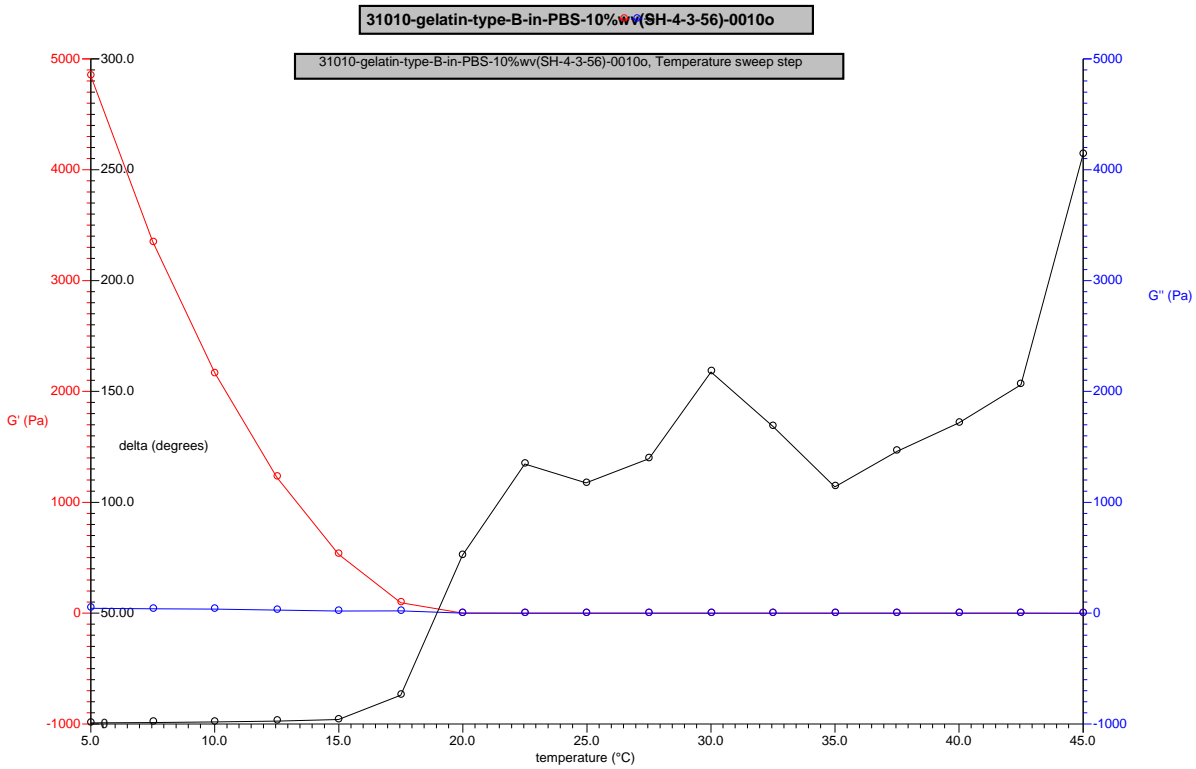


Figure 5. Rheology curve solution 10% Gelatin type B in PBS

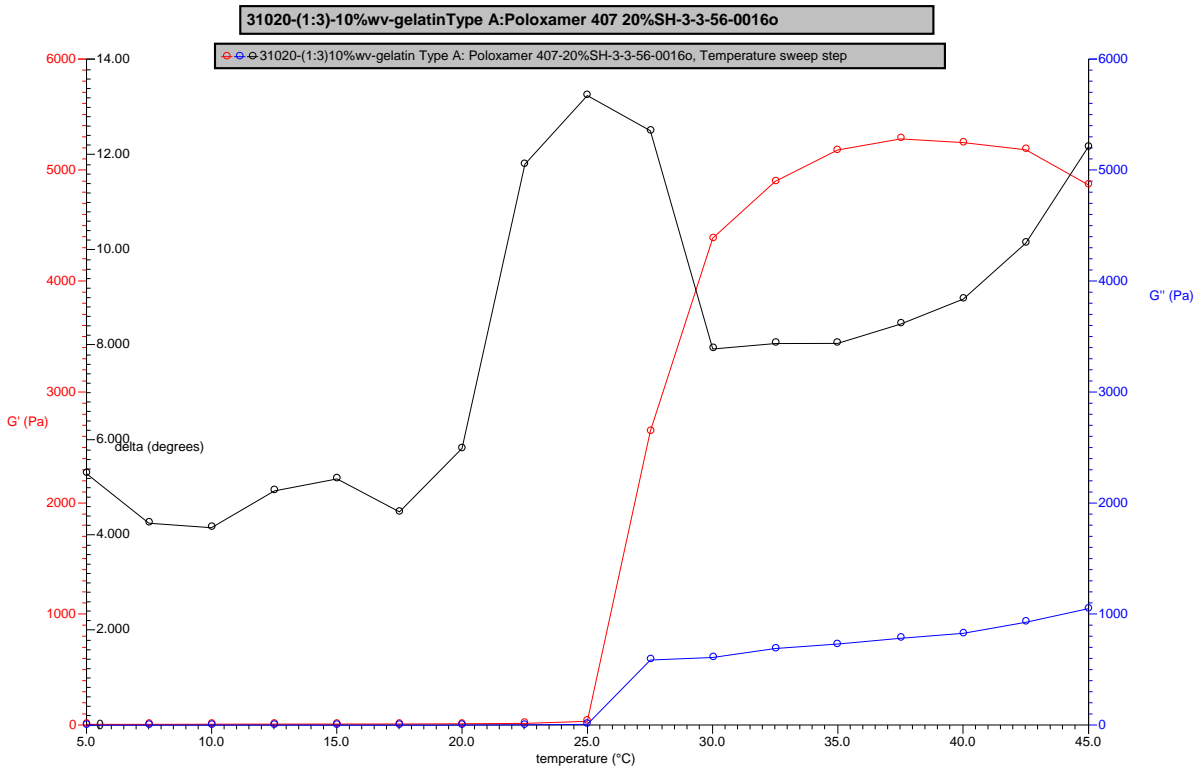


Figure 6. Rheology curve solution (1:3) Gelatin type A (10%):F127

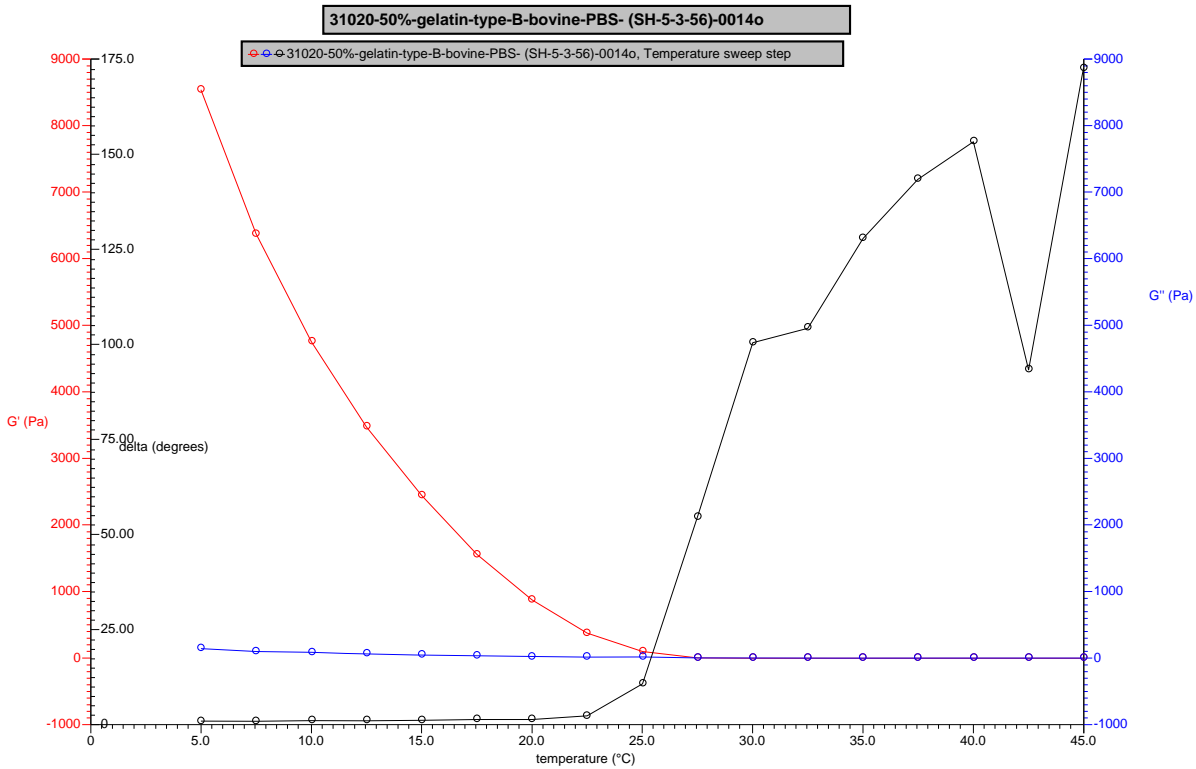


Figure 7. Rheology curve solution Gelatin type B (50%) in PBS

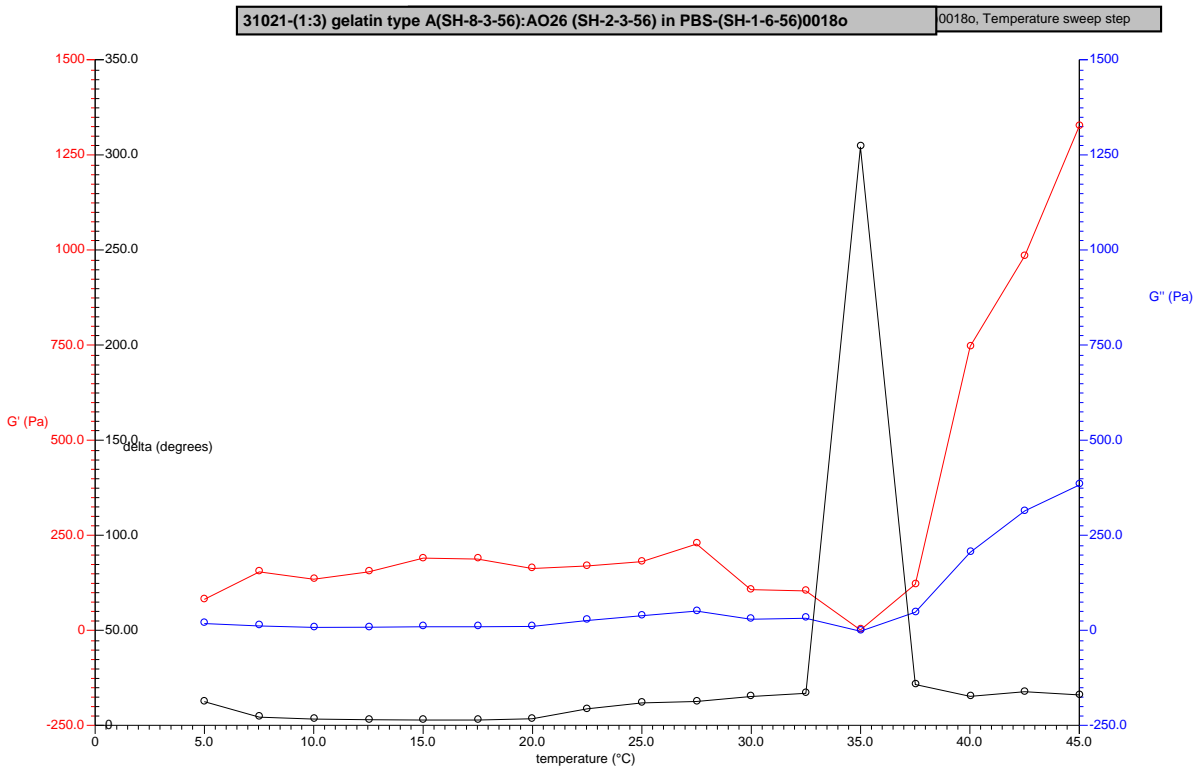


Figure 8. Rheology curve solution (1:3) Gelatin type A (10%):AO26 in PBS

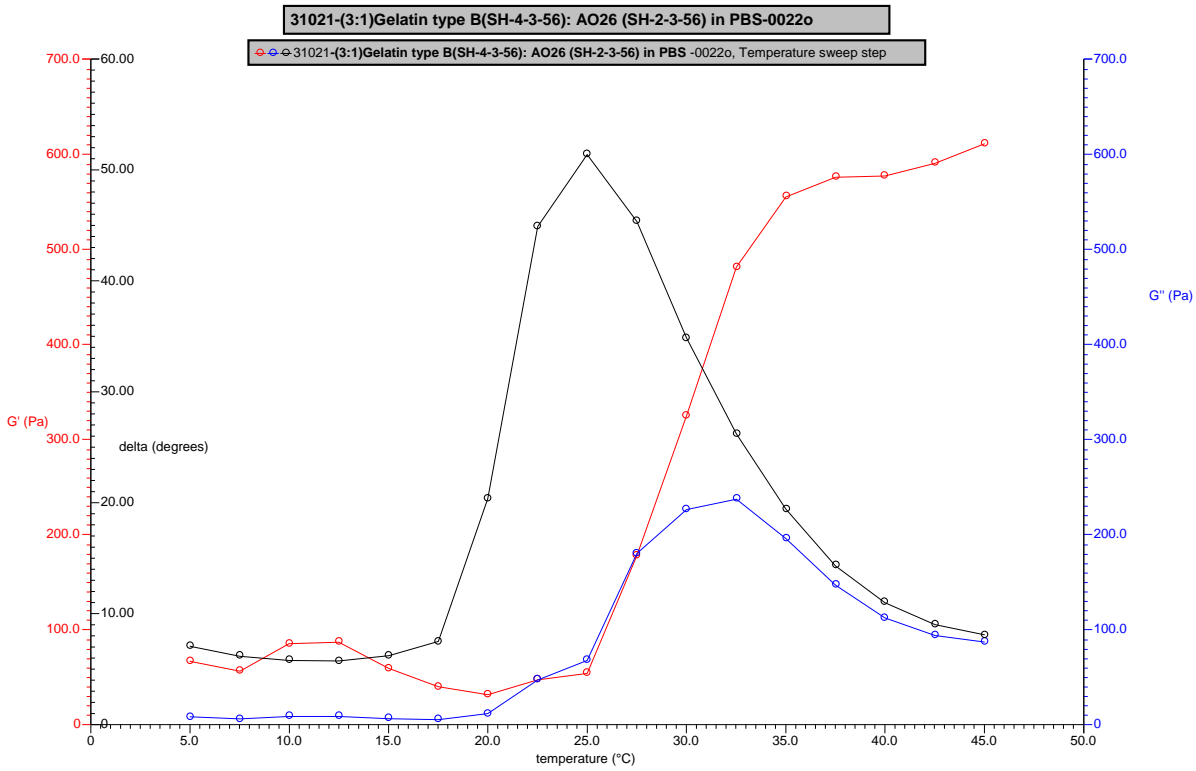


Figure 9. Rheology curve solution (3:1) Gelatin type B(10%):AO26

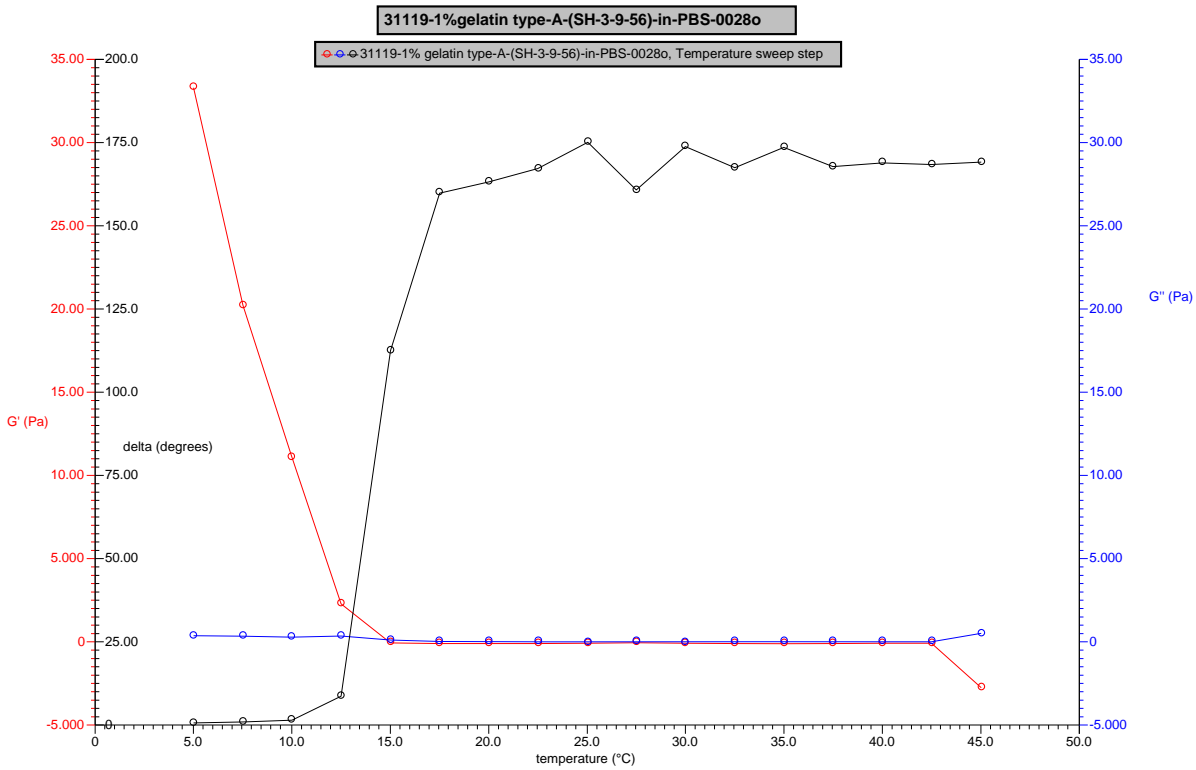


Figure 10. Rheology curve solution gelatin type A (1%)

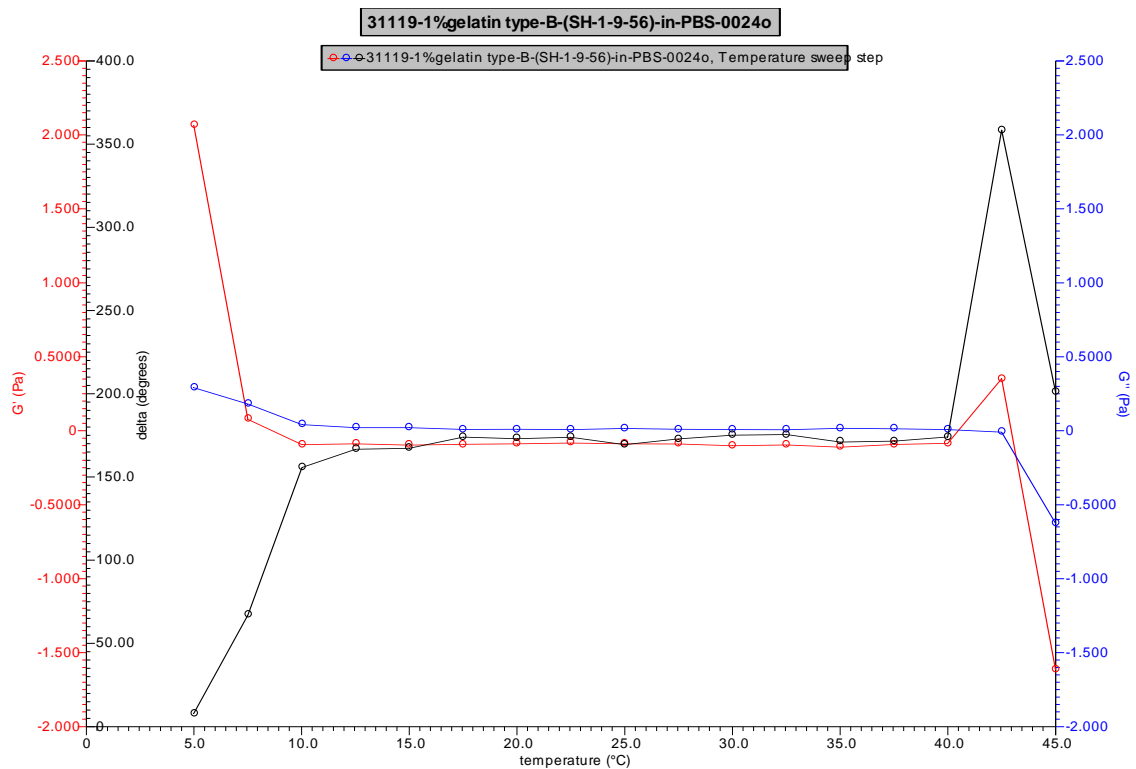


Figure 11. Rheology curve solution 1% Gelatin type B

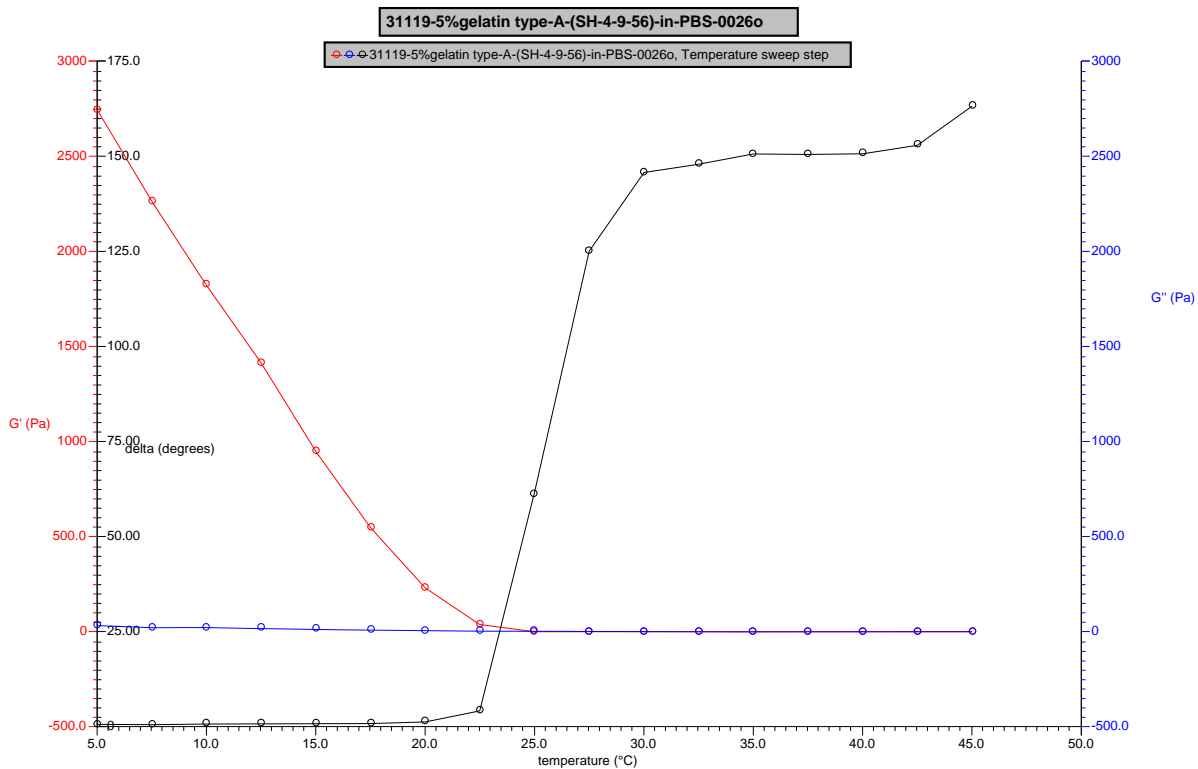


Figure 12. Rheology curve solution 5% Gelatin type A



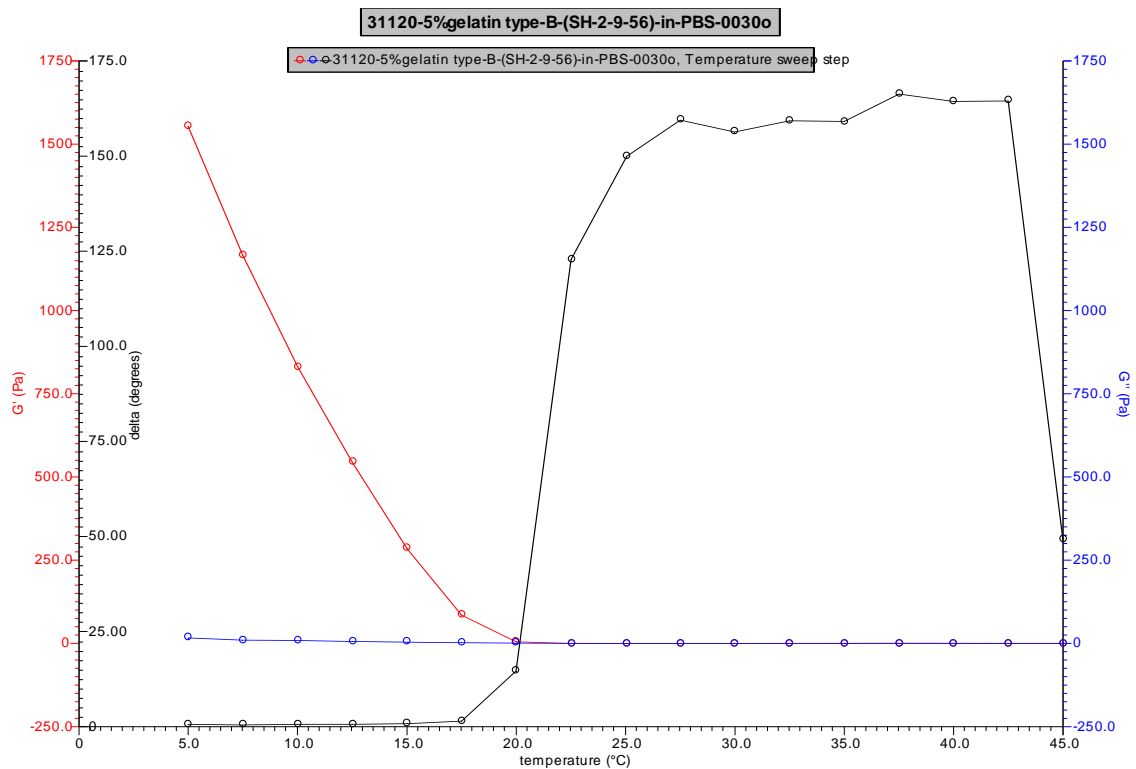


Figure 13. Rheology curve solution 5% Gelatin type B

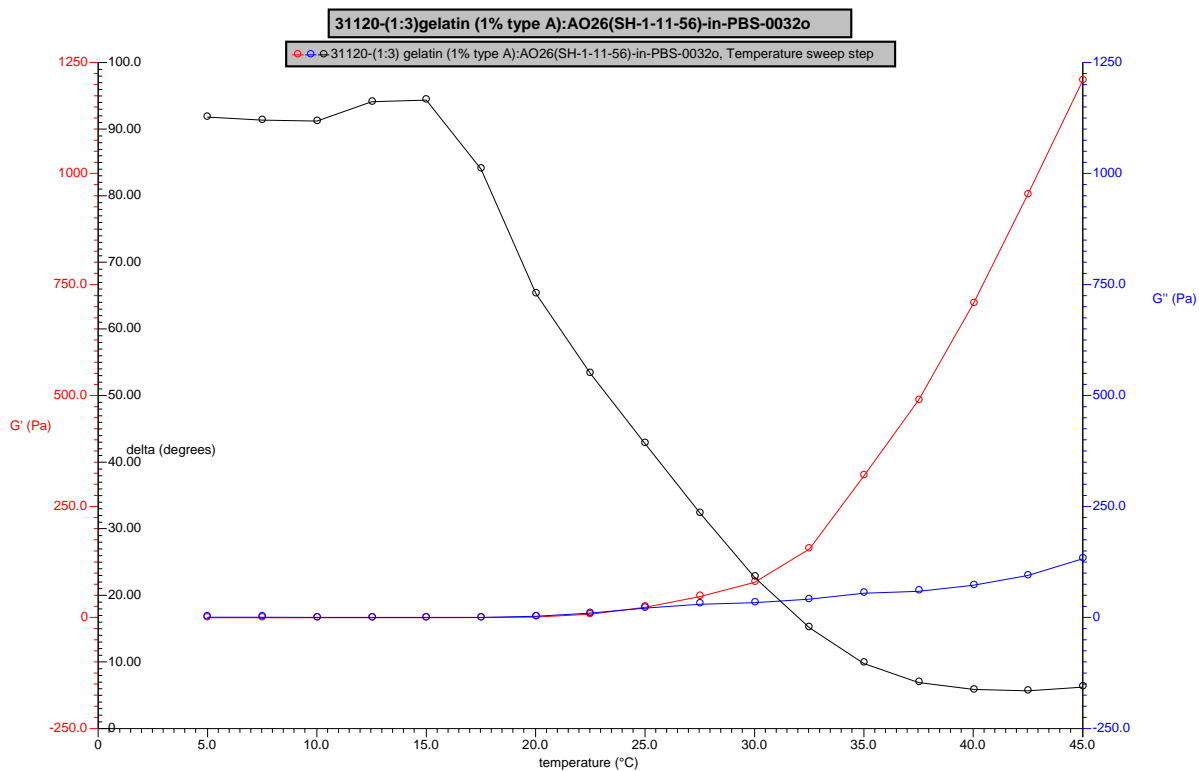


Figure 14. Rheology curve solution (1:3) Gelatin (1% type A):AO26 in PBS

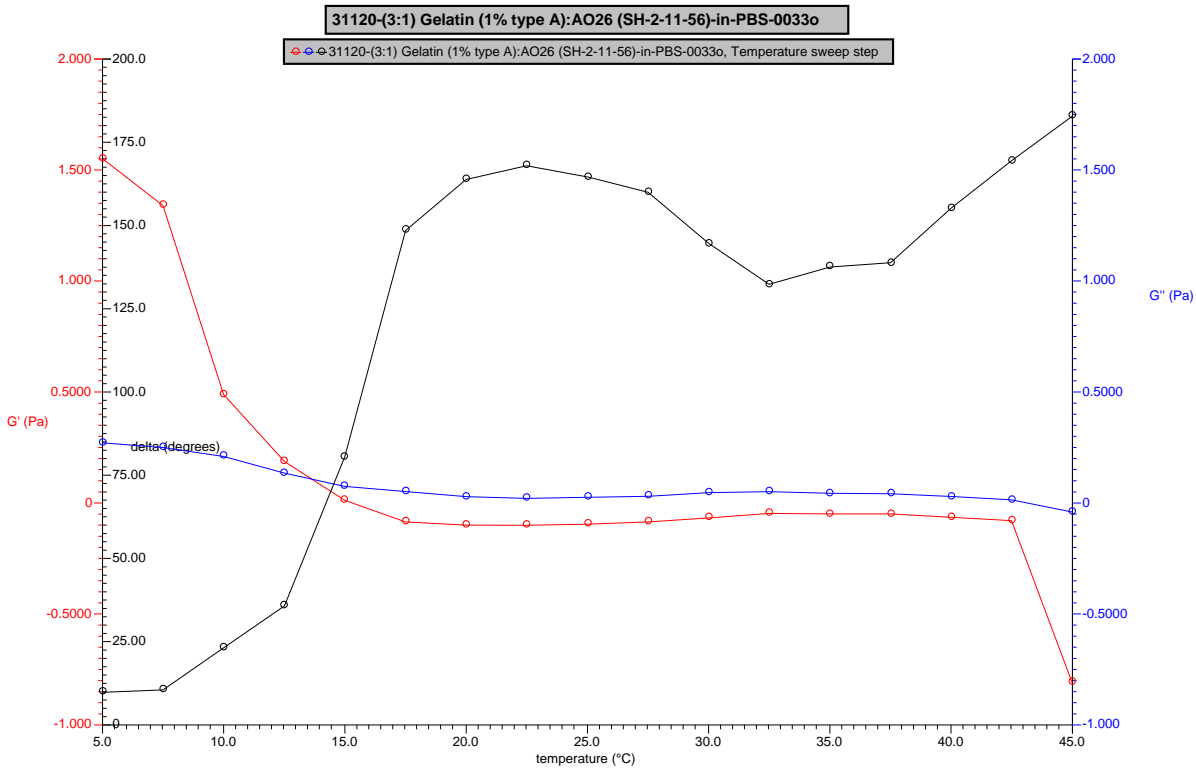


Figure 15. Rheology curve solution (3:1) Gelatin (1% type A):AO26 in PBS

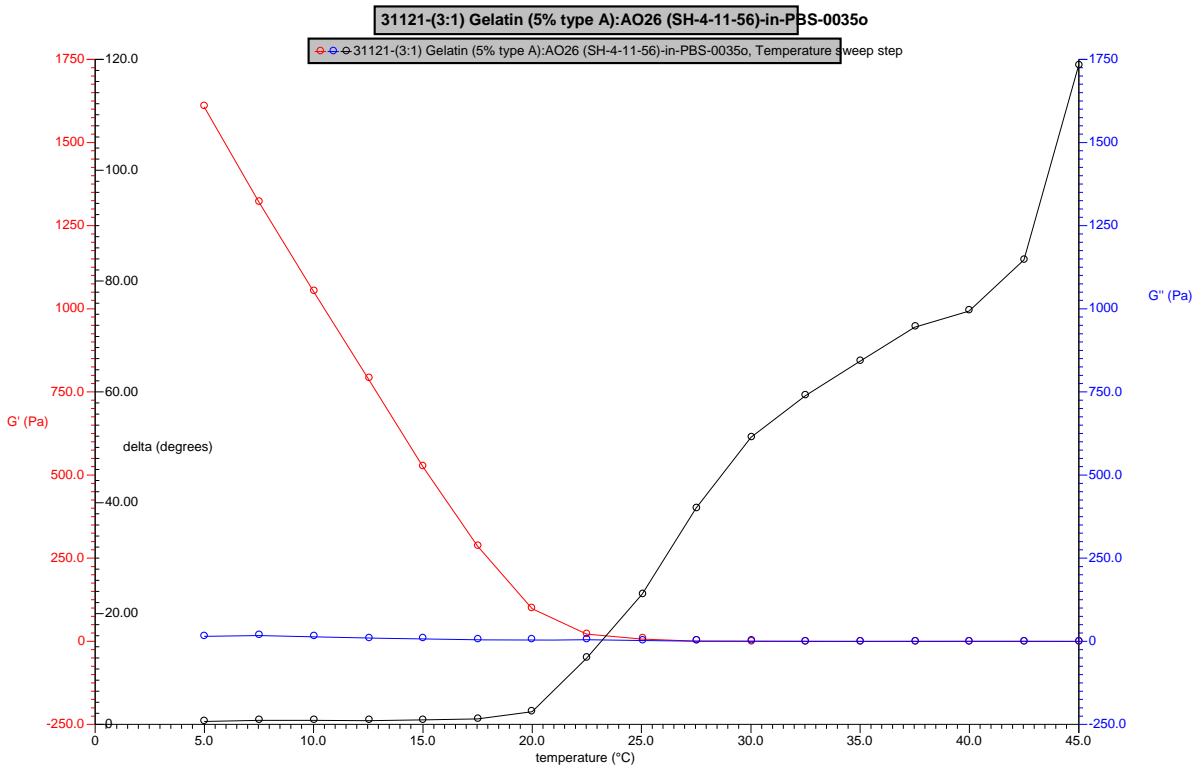


Figure 16. Rheology curve solution (3:1) Gelatin (5% type A):AO26 in PBS

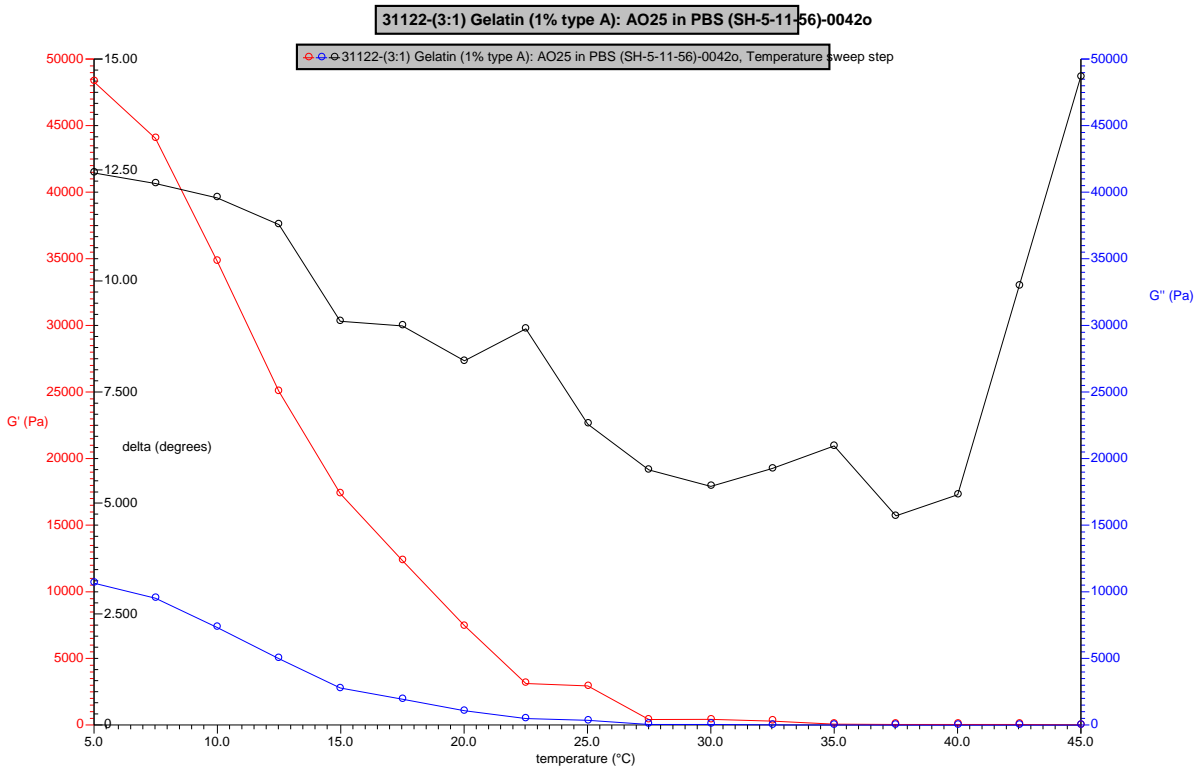


Figure 17. Rheology curve solution (3:1) Gelatin (1% type A): AO25 in PBS

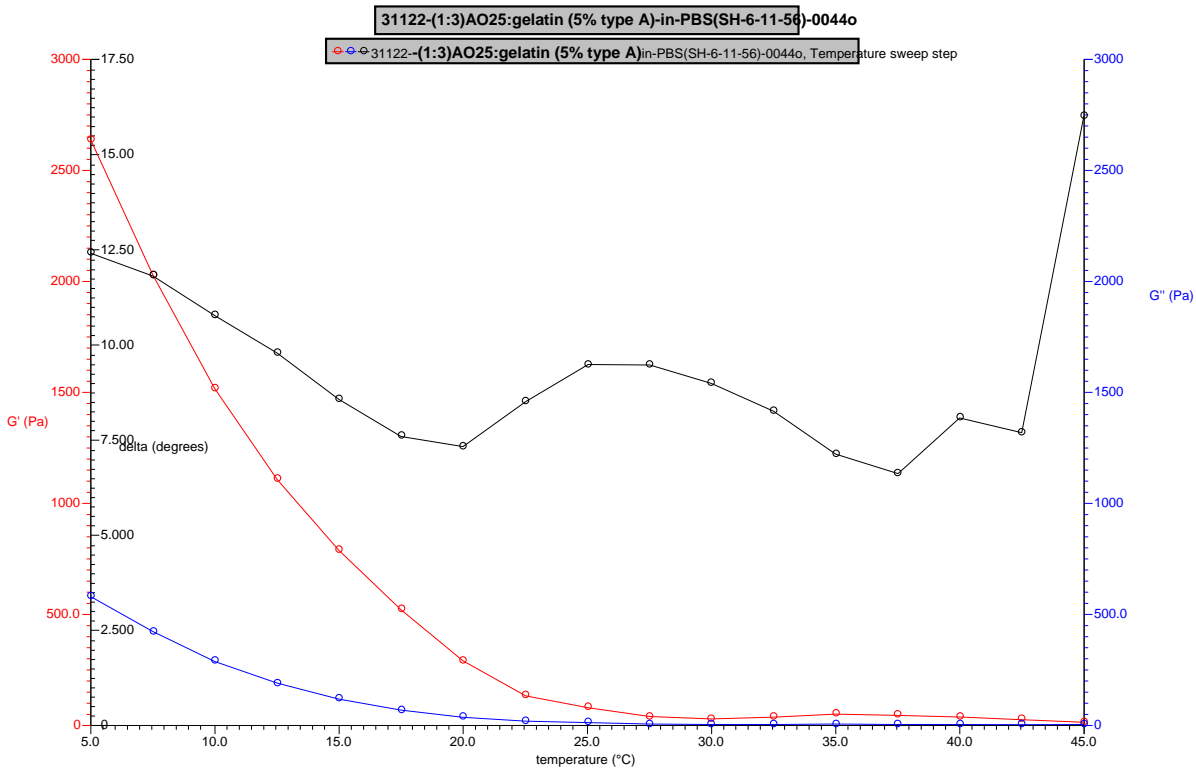


Figure 18. Rheology curve solution (3:1) Gelatin (5% type A): AO25 in PBS

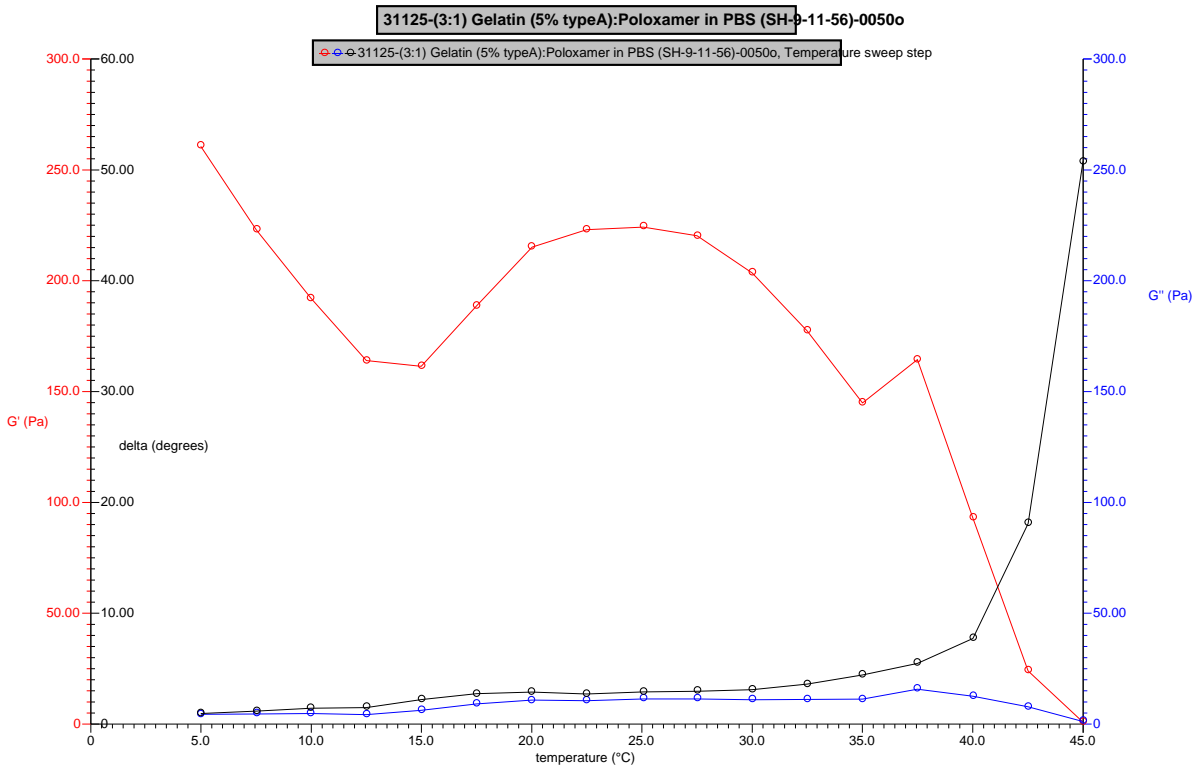


Figure 19. Rheology curve solution (3:1) Gelatin (5% typeA):Poloxamer in PBS

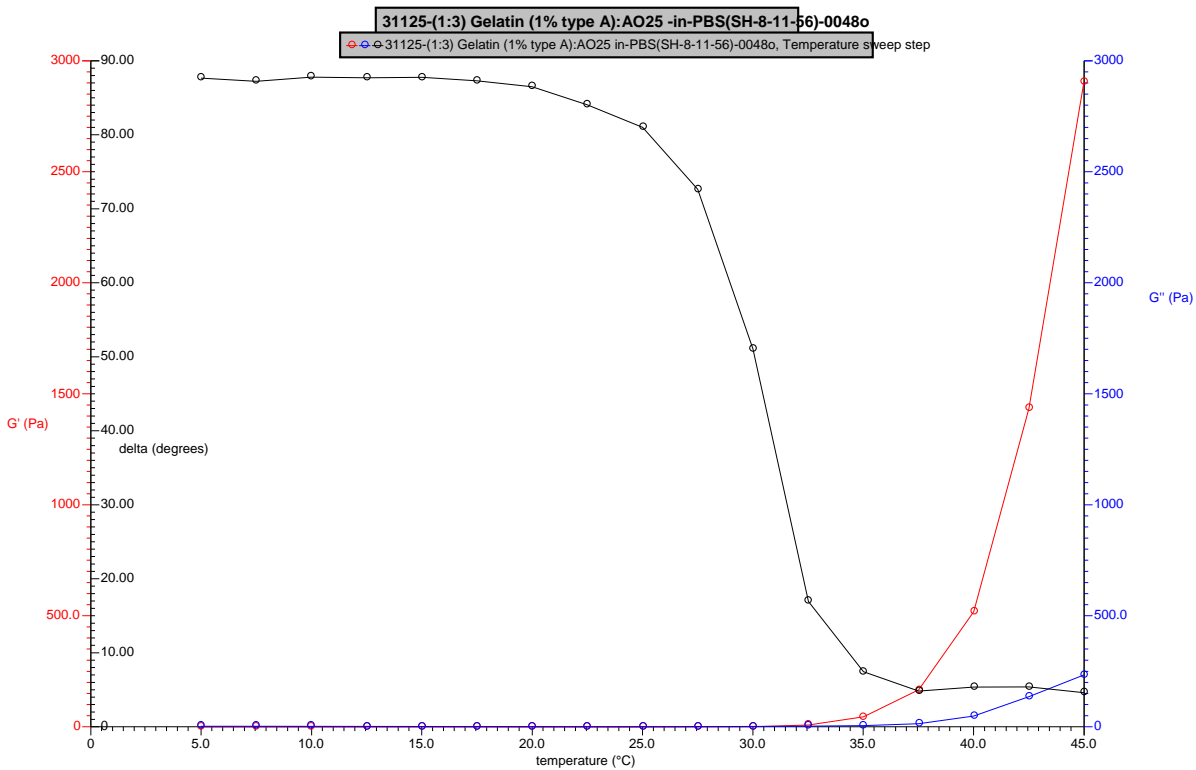


Figure 20. Rheology curve solution (1:3) Gelatin (1% type A):AO25 in PBS

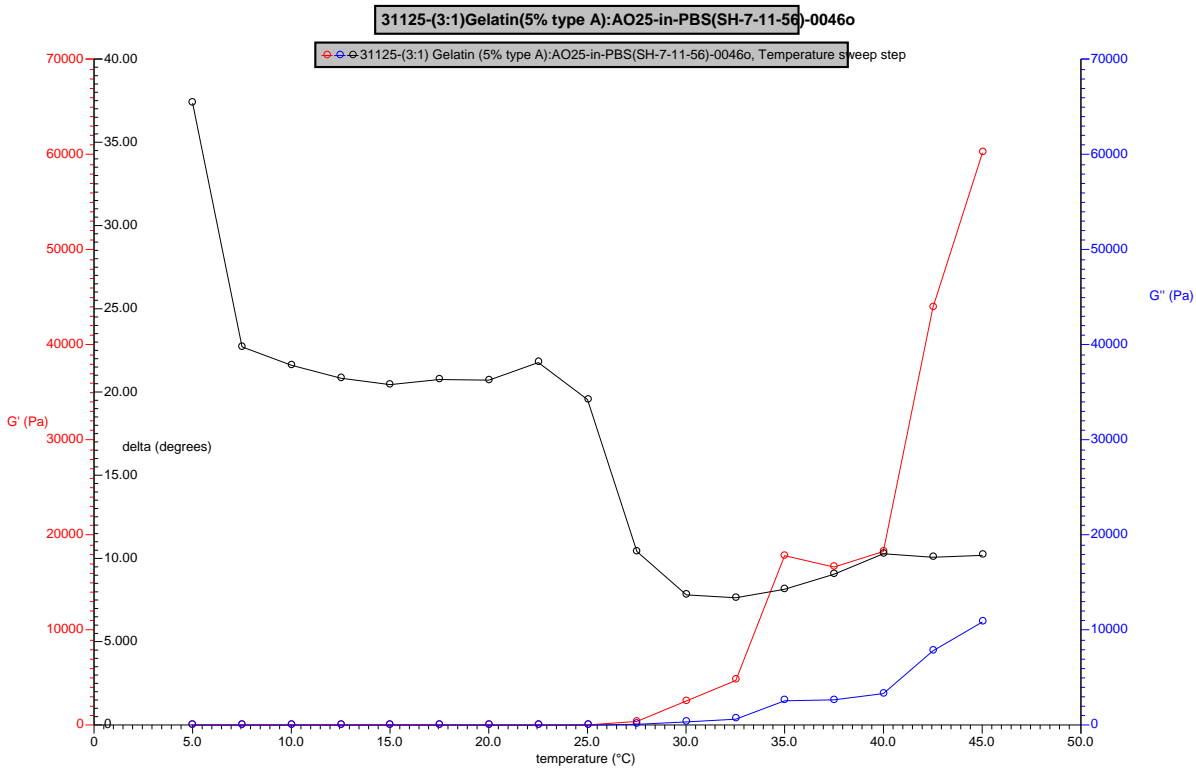


Figure 21. Rheology curve solution (1:3) Gelatin (5% type A):AO25 in PBS

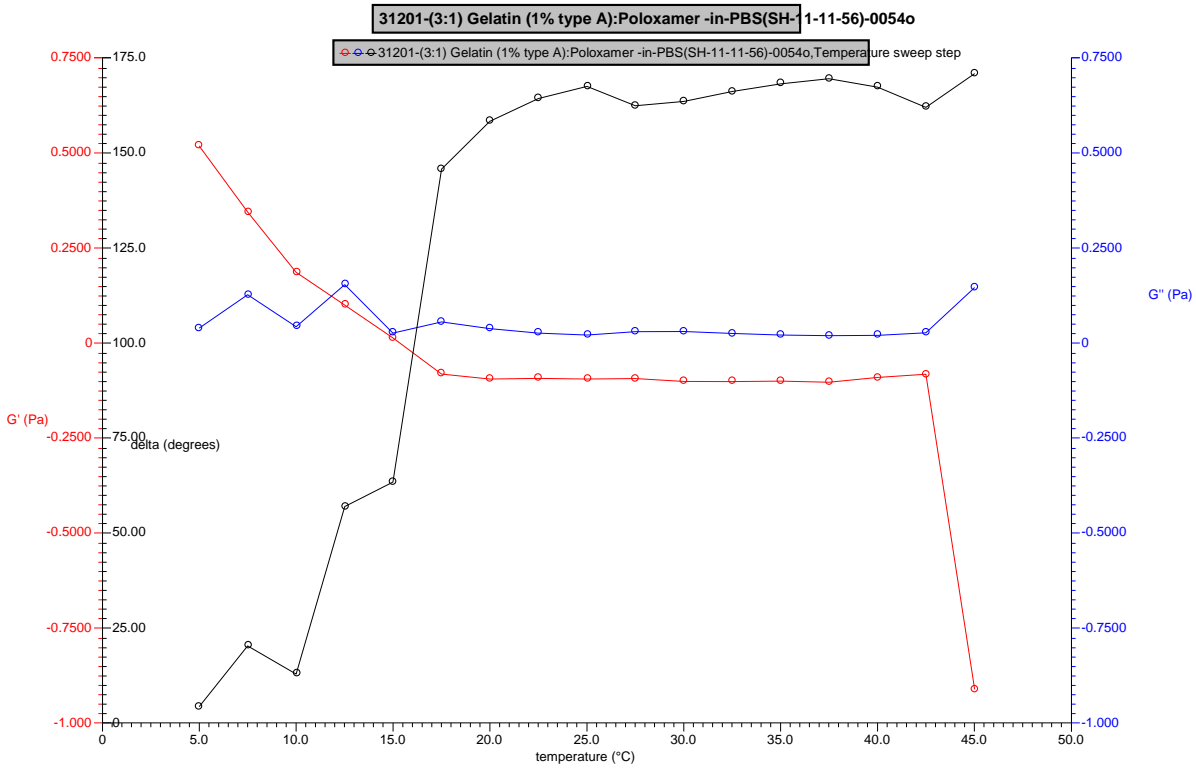


Figure 22. Rheology curve solution (3:1) Gelatin (1% type A):Poloxamer in PBS

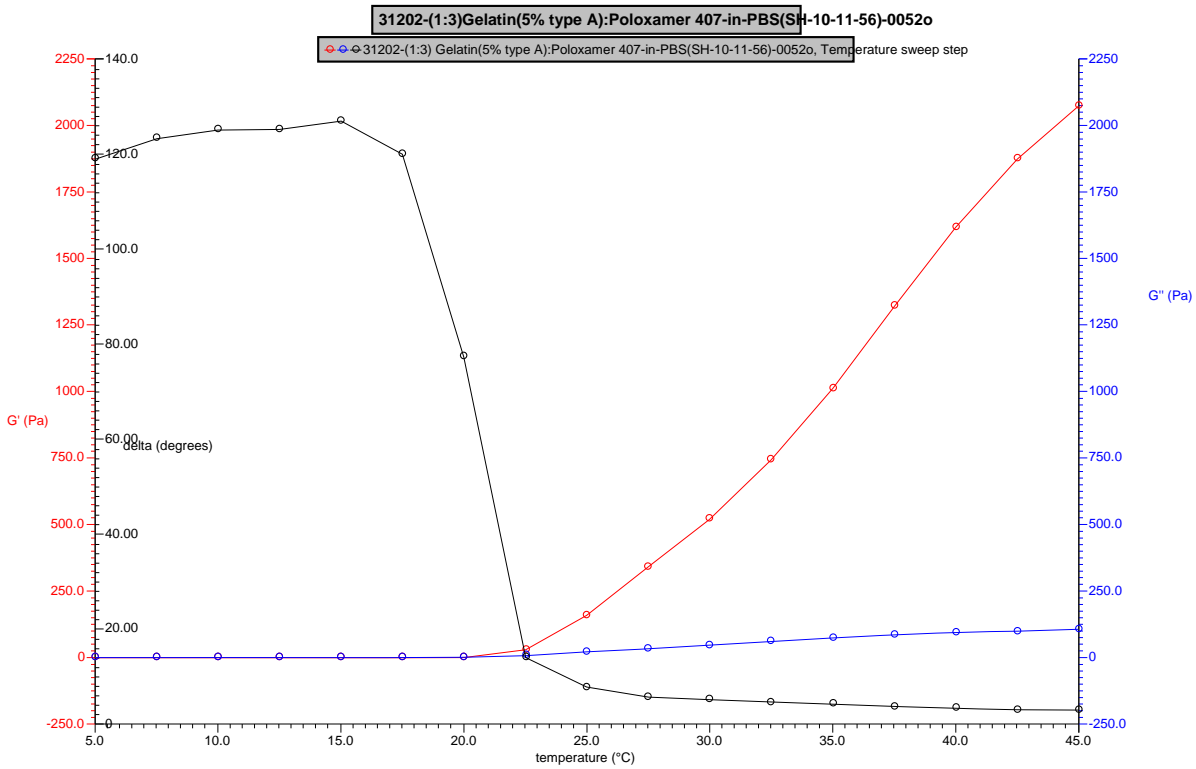


Figure 23. Rheology curve solution (1:3) Gelatin (5% type A):Poloxamer 407 in PBS

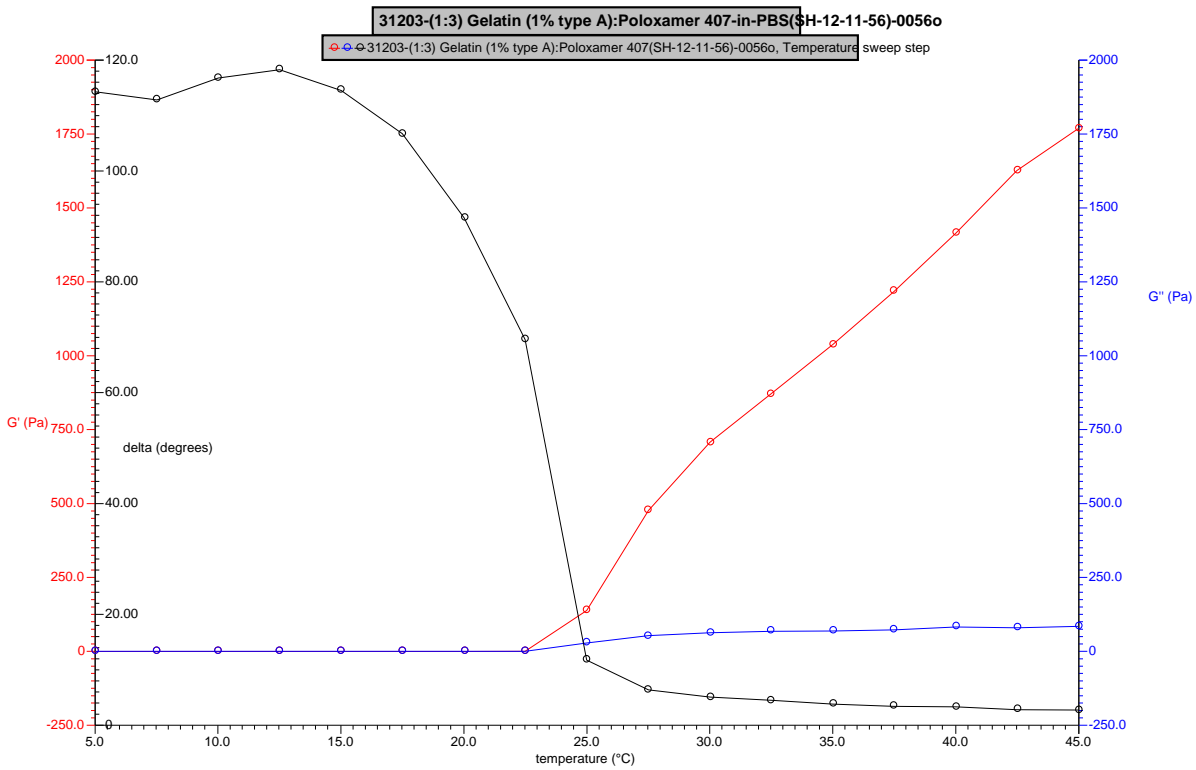


Figure 24. Rheology curve solution (1:3) Gelatin (1% type A):Poloxamer 407 in PBS

## Conclusion

Ideally for 3D cell constructs a material should allow for cells to be mixed with the polymer solution at cold temperature and then upon warming form into a gel around the cells giving them physical support. In order to

replicate a typical living system the material should incorporate a biomaterial or polypeptide, such as gelatin, to allow for the cells to easily anchor onto the construct. This research indicates that when solutions of poloxamer 407, polyvivo AO25, or polyvivo AO26 are mixed with gelatin in the ratio's and at the solution concentrations shown in Table 2 their thermogel properties are retained.

Table 2. Mixtures which retain thermogelation properties.

Gelatin	Thermogel	Mixture ratio (Gelatin:Thermogel)
Gelatin (5% type A)	Poloxamer 407 (20% w/v)	1:3
Gelatin (1% type A)	Poloxamer 407 (20% w/v)	1:3
Gelatin (1% type A)	PolyVivo AO25 (10% w/v)	1:3
Gelatin (5% type A)	PolyVivo AO25 (10% w/v)	1:3
Gelatin (10% type B)	PolyVivo AO26 (10% w/v)	3:1
Gelatin (10% type A)	PolyVivo AO26 (10% w/v)	1:3
Gelatin (1% type A)	PolyVivo AO26 (10% w/v)	1:3
Gelatin (10% type A)	Poloxamer 407 (20% w/v)	1:3

These mixtures may prove useful for designing 3D cell systems as well as potentially being used as part of 3D printing and other additive machining techniques.