Preparation and Activity of Synthetic 'Antifreeze' Macromolecules: Structure-Property relationships

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Antifreeze proteins found in both plants and animals have many potential applications industrially and in healthcare. However these proteins are not readily available and their mechanism of action is poorly understood. Here we address this this challenge by a biomimetic approach using synthetic macromolecules, obtained by controlled radical polymerisation techniques. These polymers have unique properties previously only associated with antifreeze proteins and their peptide mimics.

Poly(Vinyl Alcohol) as an antifreeze agent

- Surprisingly little work has been carried out regarding antifreeze polymers.
- The first experiments demonstrating that poly(vinyl alcohol) (PVA) displayed Ice Recrystallization Inhibition (IRI) were made by Knight *et al.* in 1995.[1]
- PVA has a molecular weight dependant ice recrystallization inhibition (IRI) activity comparable to antifreeze proteins.[2]
- PVA is also nontoxic and readily available, however commercial PVA is impure, has varying levels of hydrolysis, and a high polydispersity index (PDI), which makes it difficult to examine the molecular weight dependence.

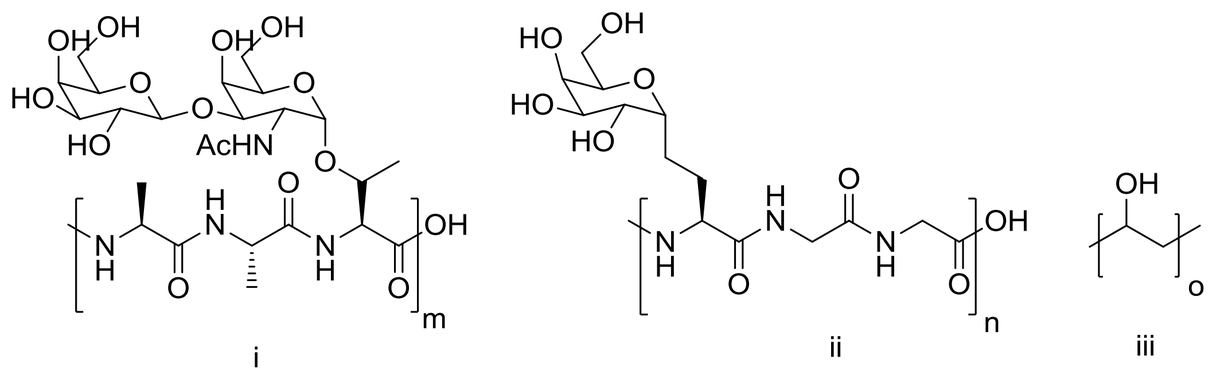


Figure 1. A Brief history of antifreeze protein mimics. *(i) Antifreeze glycoprotein (AFGP) (ii) Synthetic AFGP mimic by Ben* et al*, which shows little thermal hysteresis (TH) but similar IRI (iii) PVA the most active IRI polymer discovered.*

PVA synthesis

This RAFT agent affords the poly(vinyl acetate) precursor with predictable molecular weight and narrow PDI. The benzyl leaving group allows characterisation by proton NMR spectroscopy as well as SEC.

Hydrazinolysis gives pure PVA after dialysis with a terminal thiol and leaves the benzyl group intact.

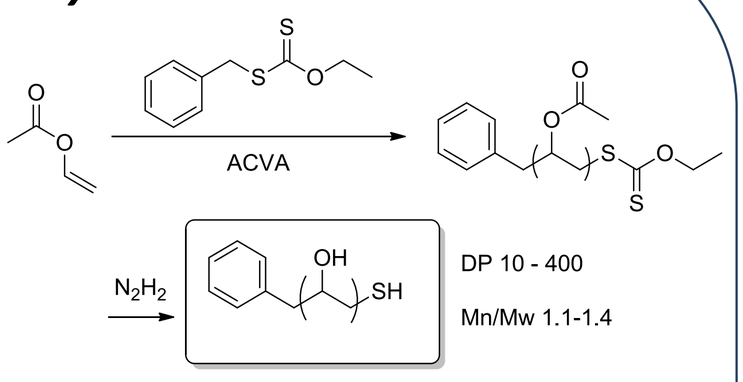
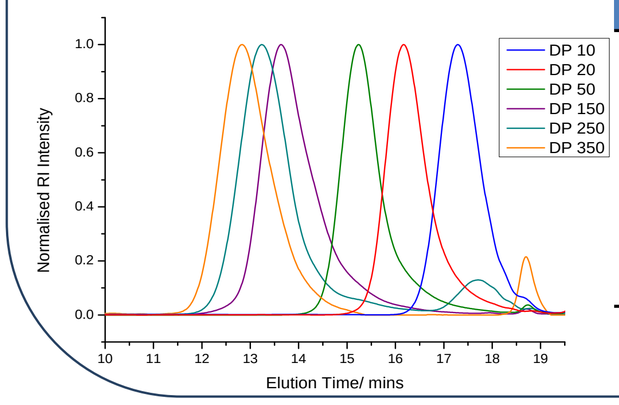


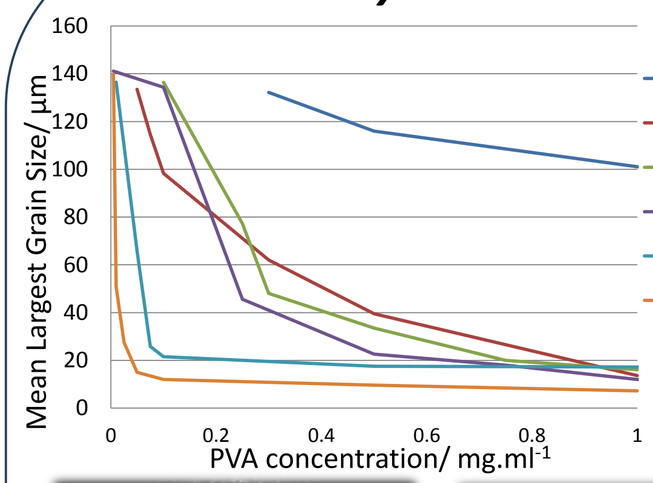
Figure 2*. RAFT/MADIX mediated polymerisation of vinyl acetate, and hydrazinolysis to PVA. Product is purified by dialysis.*



PVA	OH groups By Mn	Mn/ g/mol	Mw/ g/mol	PDI
1	10	570	630	1.12
2	20	870	1000	1.18
3	50	4400	5470	1.24
4	150	12700	18500	1.45
5	250	20700	28900	1.39
6	350	29600	38000	1.28

Table 1*. Typical PVA polymers synthesised for IRI testing.*

Ice Recrystallization Inhibition using PVA



Antifreeze activity is tested using the splat method.

1 mg.ml and lower dilutions were tested. A distinct change in recrystallization activity can be seen over a small change in concentration, with molecular weight and PDI being factors.

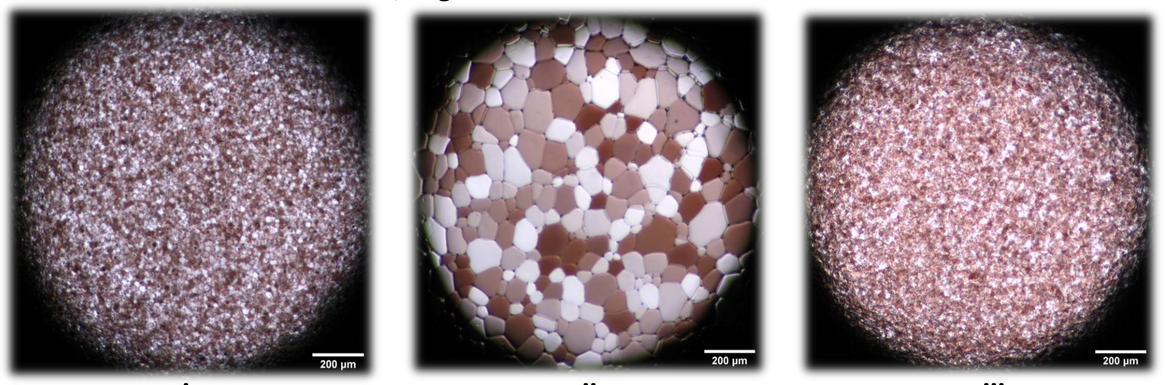
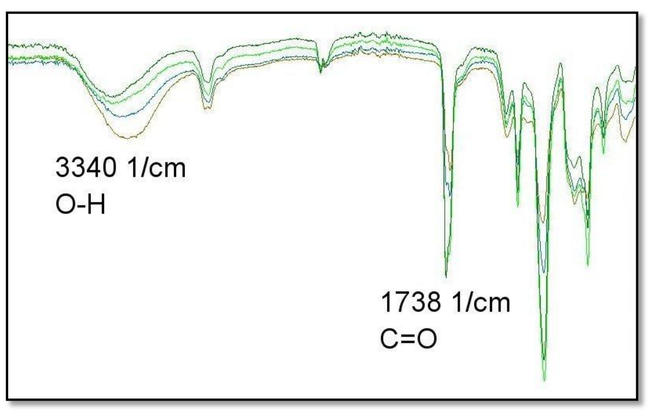


Figure 3*. (i) Initial crystal nucleation and growth in a rapidly frozen PBS solution. (ii) Crystal growth in PBS solution after 30 minutes annealing at -8°C. (iii) Total inhibition of crystal growth after 30 minutes annealing at -8°C caused by 6 kDa PVA at a 1 mg.ml concentration.*

Reducing Activity by Controlled Acetylation

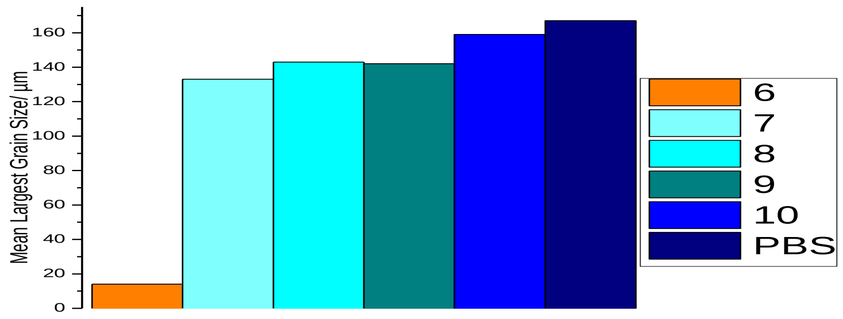
PVA 6 (DP 350) was partially re-acetylated to different degrees by dissolving the polymer in different acetic acid/water mixtures.[3] Acetylation was determined by ^{1}H NMR and IR. The resultant PVA shows a large decrease in IRI activity compared to pure PVA, in terms of alcohol groups.



PVA	H_2O vol %	acetic acid vol %	% acetylation mol. %	OH groups
7	50	50	26.8	270
8	32	68	44.4	210
9	24	76	68.7	180
10	17	83	70.3	140

Table 2*. Modification of PVA 6, by suspending in mixtures of acetic acid and water, with catalytic HCl*

DP 250 has a far greater activity than a partially acetylated PVA with the same number of hydroxyl groups. The average number of repeating OH groups between each acetate group is five, comparable to glucose which has been shown to have similarly weak IRI activity. [4]



Graph 1*. IRI activity of acetylated PVA. IRI assays carried out with 2 mg.ml solutions of polymer additives in PBS*

Conclusion

By using the RAFT/MADIX methodology to synthesise highly controlled, fully hydrolysed PVA we can isolate and examine how the different physical and compositional properties of the polymer affect its IRI.

PVA activity shows a strong dependence on it molecular weight. Low molecular weight ranges show a linear increase, similar to other antifreeze agents.

Higher molecular weight polymers increasingly completely inhibit growth to very low concentrations (0.05 mg.ml with DP 350), and show no gradual loss of activity.

Further Work

Lower levels of acetylation will give greater insight into the structure/property relationships that govern the IRI activity of PVA.

Background References

[1] C. A. Knight, D. Wen and R. A. Laursen, Cryobiology, 1995, 32, 23–34.
[2] M. I. Gibson, M. Cameron, Biomacromolecules, 2009, 10(2), 328-333
[3] F. Fujumoto, K. Hirabayashi, BICR 1951, 24, 92
[4] C. Cappicciotti, R. Ben, Chem. Sci, 1012, 3, 1408-1416

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