Antifreeze (Glyco)Protein Mimetic Behaviour of Synthetic Macromolecules: Structure-Property Relationships

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Antifreeze proteins found in both plants and animals have many possible applications industrially and in healthcare. However the specific mechanisms and features of these proteins are poorly understood, and recent studies have shown their incompatibility with several cell and organs types. Here we present a detailed study on the ability of polymers to act as a bio-mimetic surrogates for antifreeze(glyco)proteins (AF(G)Ps), with a focus on the specific property of ice-recrystallisation inhibition (IRI).

Poly(Vinyl Alcohol) (PVA) as an Antifreeze Agent

- Surprisingly little work has been carried out into the antifreeze potential of synthetic macromolecules.
- The first experiments demonstrating that poly(vinyl alcohol) (PVA) displayed Ice Recrystallization Inhibition (IRI), a major component of antifreeze activity, 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] It is nontoxic and readily available.
- PVA also does not display dynamic ice shaping, a property of AF(G)PS that cause the growth of needle-like ice crystals that could damage cell membranes.

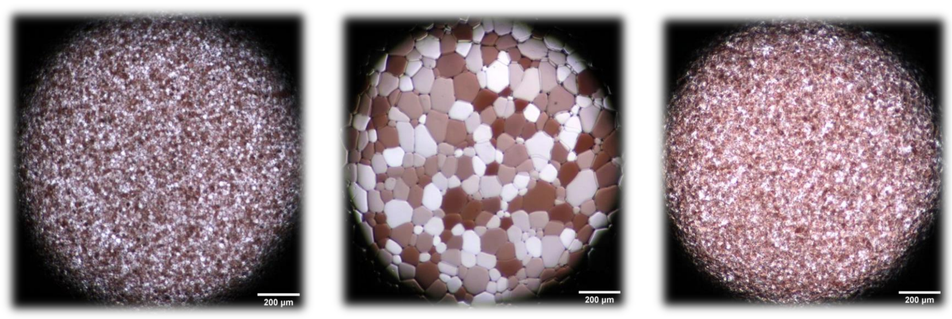

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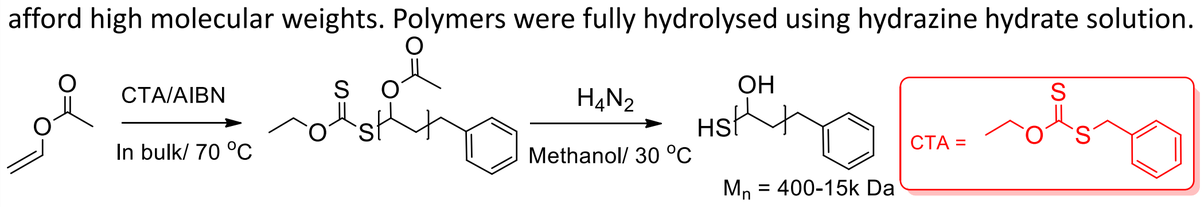
Figure 1. *Ice recrystallization inhibition activity of PVA homopolymers as measured by the splat assay. (i) Initial crystal nucleation and growth in a rapidly frozen PBS solution. (ii) Crystal growth in PBS solution after 30 minutes annealing at -8°C. (iii) Total inhibition of crystal growth after 30 minutes annealing at -8°C caused by 6 kDa PVA at a 1 mg.ml concentration. Scale bar = 200 µm*

RAFT Polymerisation Affords Highly Controlled PVA For Molecular Weight Dependant IRI Analysis

Scant reports suggest a correlation between chain length and activity. These reports used poorly defined commercial PVA samples. In order to gain a better insight RAFT polymerisation was used to control the molecular weight dispersity. All samples displayed narrow polydispersities and high conversions for bulk polymerisation, and in the case of PVA 350 could be chain extended to afford high molecular weights. Polymers were fully hydrolysed using hydrazine hydrate solution.



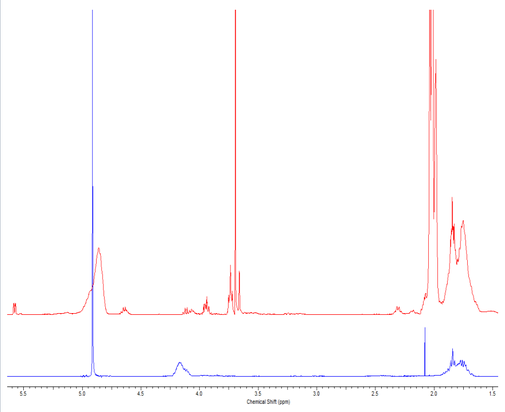


Figure 2. *^{1}H NMR of PVAc (red) and PVA (blue). Conversion determined from the integral of the acetate methyl protons at 2.0-2.1 ppm, and the α backbone proton of the alcohol at 4.1-4.25 ppm. Integrals taken from the PVA (blue) trace shows that hydrolysis > 99%.*

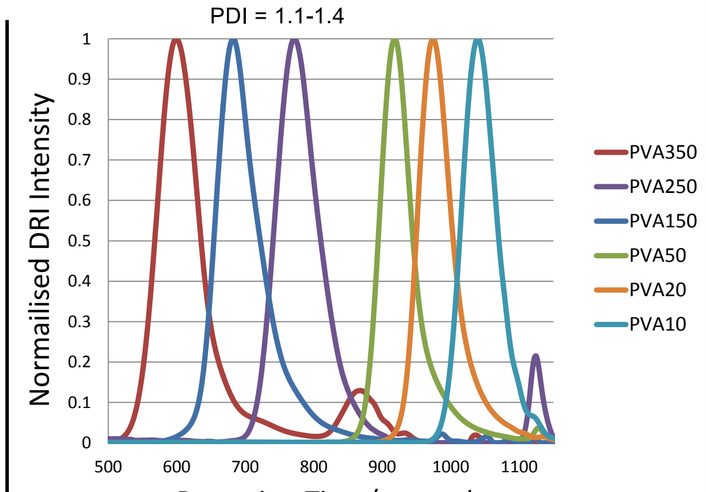


Figure 3. *SEC traces for a selection of Poly(vinyl acetate) polymers prepared as above. These polymers where then fully hydrolysed using hydrazine hydrate and purified by dialysis and then freeze drying, affording pure PVA.*

The IRI Activity Of PVA Shows A Far Higher Molecular Weight Dependence Than Previously Thought

The graph below shows the concentration and molecular weight dependant activity of the PVA samples prepared by RAFT polymerisation. There is a stark difference between the activity of PVA with 10 units and 20; which follows the same behaviour as larger PVA. High molecular weight PVA show strong inhibition at very low concentrations.

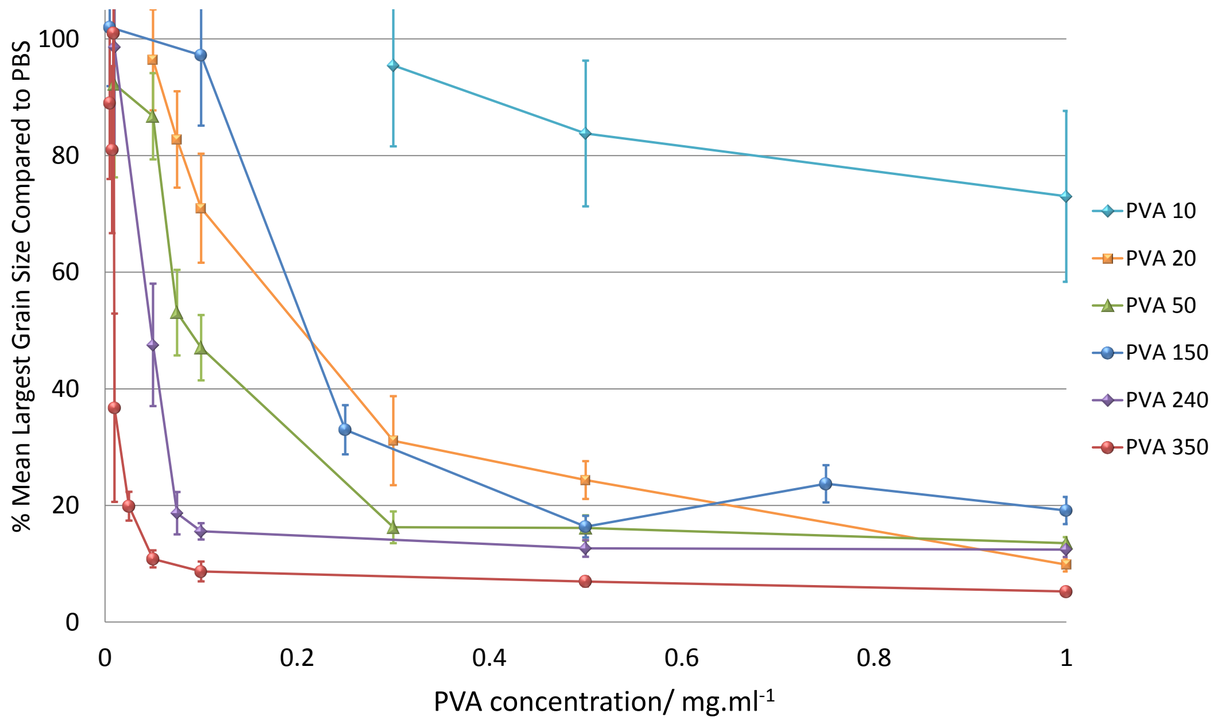


Figure 4. *Ice recrystallization inhibition activity of PVA homopolymers as measured by the splat assay. Graphical analysis IRI activity as a function of polymer concentration. MLGS = mean largest grain size relative to a PBS control, expressed as %. Errors bars represent the standard deviation from at least three ice wafer image measurements.*

Hydrophobic Modifications Reduce The IRI Activity…

Vinyl acetate can be copolymerised with isopropenyl acetate, or the hydroxyl groups on PVA can be modified via post-polymerisation modification. Substitution of the hydroxyl group led to a dramatic decrease in activity above 10 mol% inclusion, as did inclusion of methyl groups along the polymer backbone, which greatly affected solubility of the copolymer past 10% inclusion.

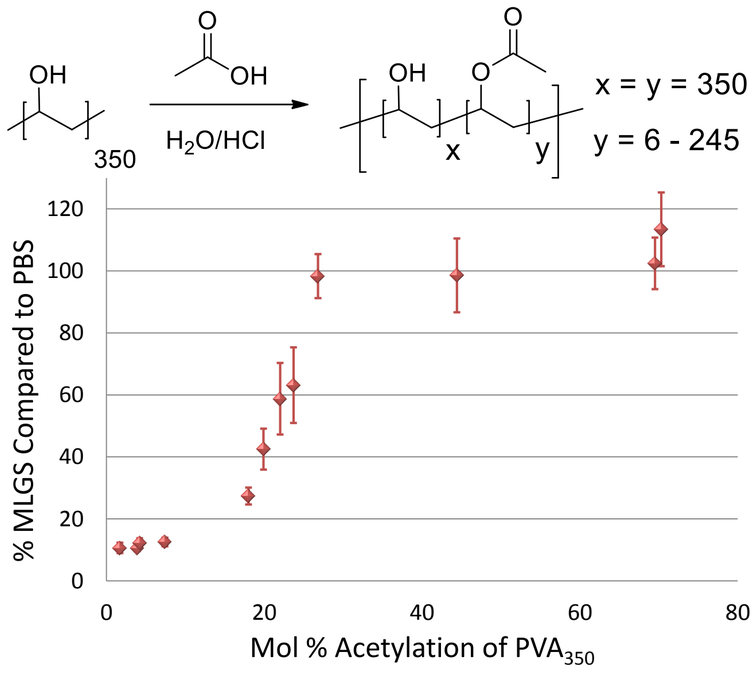
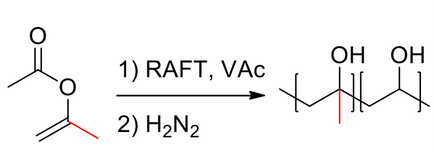


Figure 5. *Ice recrystallization inhibition activity of PVA/PVAc as measured by the splat assay. Errors bars represent the standard deviation from three ice wafer image measurements.*



Isopropenyl acetate is highly inactive towards propagation in RAFT mediated polymerisation, and the resultant hydrolysed polymer is insoluble in water, but a 90:10 PVA:P^{i}PA copolymer is soluble at 2 mg.mL^{-1} or lower concentration.

It was theorised that the methyl group would aid in mimicking the amphiphilic effect that some AF(G)Ps display an is attributed to their high IRI activity.

This was not the case as activity decreased compared to homo PVA.

…As Do Hydrophillic Modifications

Vinyl acetate is often copolymerised with vinyl pyrrolidone to make block copolymers , and The preparation of random copolymers is facile so long as the conversions are kept low due to the different reactivity ratios. Poly(vinyl pyrrolidone) (PVP) is highly water soluble and is used in a number of applications similar to PVA. PVP has been previously shown to have no IRI activity, but increasing the solubility of the polymer would give greater insight into the factors affecting the IRI activity of synthetic macromolecules.

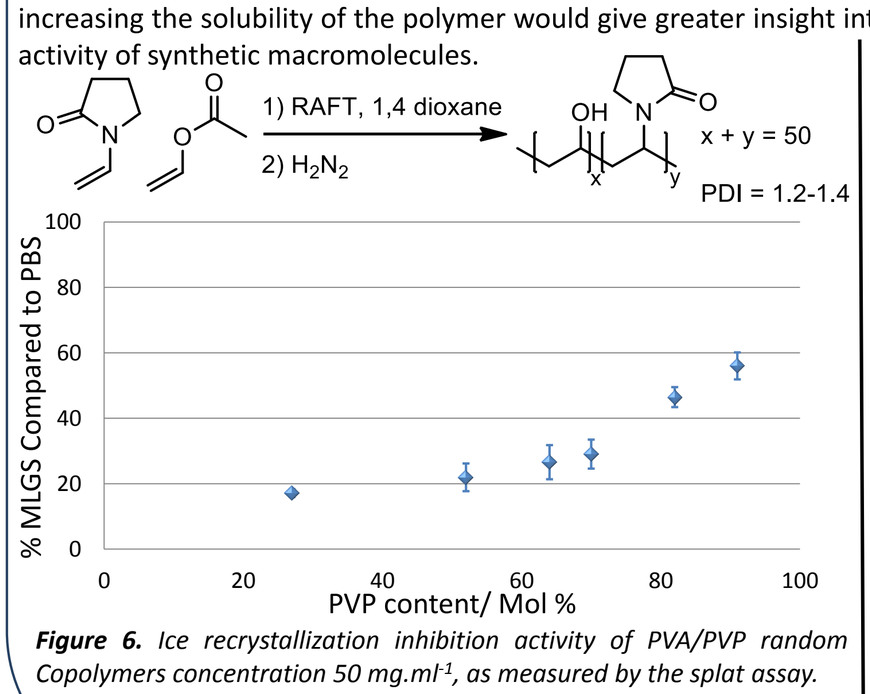


Figure 6. *Ice recrystallization inhibition activity of PVA/PVP random Copolymers concentration 50 mg.ml^{-1}, as measured by the splat assay. Mol % PVA is the molar ratio of repeat units of the total polymer. All polymers consist of roughly 50 repeat units and have a PDI = 1.2-1.4.*

Conclusions

PVA exhibits IRI activity was between ten and twenty repeat units implying a minimum repeat sequence is essential for strong interactions with the ice surface.

Addition of either vinyl acetate or vinyl pyrrolidone comonomers both lead to a reduction in activity above approximately 20 mol% incorporation showing that only very small quantities of additional functional groups can be tolerated in the main-chain structure if activity is to be maintained

Background References

[1] C. A. Knight, D. Wen and R. A. Laursen, Cryobiology, 1995, 32, 23–34.
[2] Inada, T.; Lu, S.-S. *Cryst. Growth Des.* **2003,** *3*, 747-752.
[3] Gibson, M. I.; Barker, C. A.; Spain, S. G.; Albertin, L.; Cameron, N. R. *Biomacromolecules* **2009,** *10*, 328-333.
[4] Gibson, M. I. *Polym. Chem.* **2010,** *1*, 1141-1152.

Acknowledgements

MIG Group
- *Robert Deller*
- *Dan Phillips*
- *Caroline Moore*
- *Tom Congdon*
- *Alaina Emmanuella*
- *Sarah-Jane Richards*
- *Lucienne Otten*
- *With thanks to Birmingham Science City Advanced Materials 2*

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