Protein Stability during Hot Melt Extrusion: The Effect of Extrusion Temperature, Hydrophilicity of Polymers and Sugar Glass Pre-stabilization

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The Effect of Extrusion Temperature, Hydrophilicity of Polymers and Sugar Glass Pre-stabilization

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Introduction

Biodegradable polymers have been widely investigated for controlled release formulations for protein delivery. However, the processing stability of proteins remains a major challenge. Hot melt extrusion (HME) is a suitable production process for polymeric implants, as it is a simple and continuous process, and lacks the use of organic solvents. However, in particular the high temperatures during HME could damage protein structure.

Purpose

The aim of this research is to assess the impact of the hot melt extrusion process on the activity of a model protein by varying extrusion temperature, hydrophilicity of polymer and pre-stabilization of proteins with a sugar glass, inulin. Additionally, the predictive value of thermal stability testing of the protein is assessed.

Materials and methods

Pre-stabilization
Incorporation of model protein alkaline phosphatase (AP) in inulin (1:10 w/w) by spray drying.

Thermal stability of solid AP
- Dry powder samples of AP-inulin and bare AP.
- Exposure to 55°C, 95°C and 130°C.
- Activity was determined with an enzymatic activity assay.

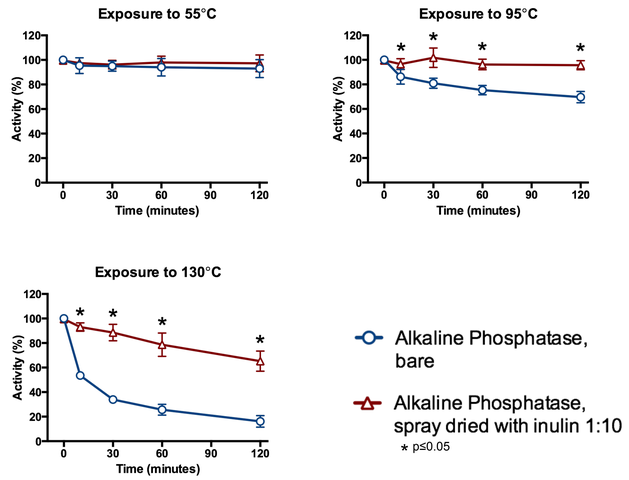
Hot Melt Extrusion
- Physical blend of polymer and AP (1% w/w).
- Extruded in 6 different polymers (Table 1).
- Screw speed was 3-15 rpm, developing a torque of <6 N/m.

Extraction of protein and data analysis
- AP was isolated from extrudates by extraction.
- Activity after extrusion was determined by combining protein content with enzymatic activity data.

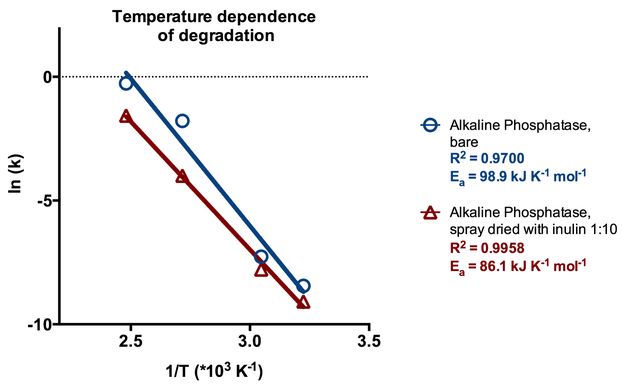
Table 1: Composition and properties of the polymers used for extrusion

Polymer	Composition	Hydrophilicity	Extrusion temperature
PCL	Poly-ε-caprolactone, M_n 45,000	-	55°C
PCL-based	Multiblock co-polymer of PCL and PCL-PEG-PCL blocks	+	55°C
PLGA	Poly (lactic-co-glycolic acid), 50:50 ratio, IV 0.2 dl/g	-	85°C
PCL-PEG	Freeze dried mixture of PCL and PEG	+	85°C
PLGA	Poly (lactic-co-glycolic acid), 50:50 ratio, IV 1.0 dl/g	-	130°C
PLLA-based	Multiblock co-polymer of PLLA and PCL-PEG-PCL blocks	+	130°C

Results – Thermal stability of solid AP



Inulin stabilizes solid AP at 95°C and 130°C

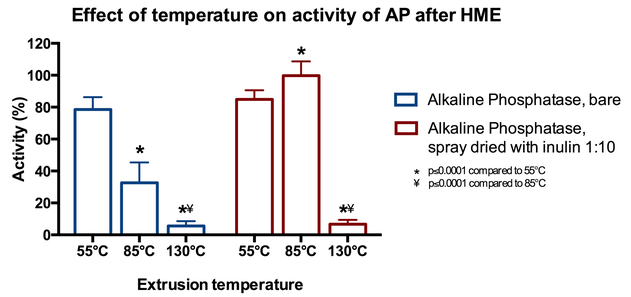


Degradation of solid AP is according to Arrhenius behavior

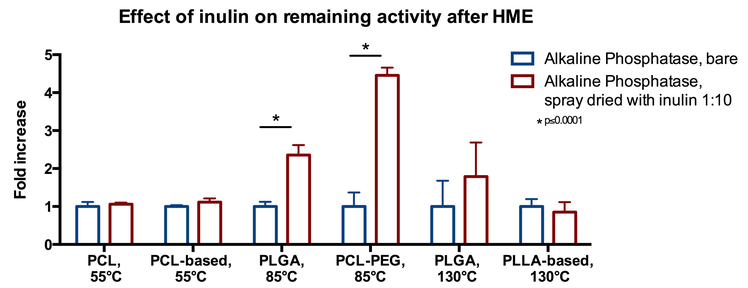
Conclusions

The thermal stability data of proteins can not be used as a predictor for hot melt extrusion formulation development, as the protein activity loss after heat stress does not correlate with the activity loss after extrusion. Although the processes show similar trends, the shear involved in HME could have enhanced the activity loss. Moreover, the protective effect of inulin was lost at high temperatures, which is most probably due to both exposure to shear and passing the the glass transition temperature during the HME process. Nevertheless, the use of inulin as a protective agent can be beneficial at intermediate temperatures.
Polymers with hydrophilic properties (i.e. with PEG incorporated) do not show an improvement of protein stability after HME. The amount of PEG present could be insufficient to prevent hydrophobic interactions or the overall occurrence of hydrophobic interactions is low.

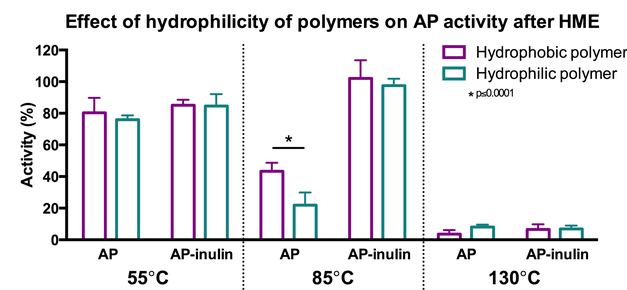
Results – Effects of HME on AP stability



- **The activity of AP after HME decreases with increasing extrusion temperature**
- **Inulin was not able to stabilize AP at high temperature (130°C)**



- **A 2 to 4-fold increase in activity after HME can be achieved by pre-stabilization with inulin at intermediate temperature (85°C)**
- **Pre-stabilization with inulin shows no stabilizing effect with extrusion at low and high temperatures (55°C and 130°C)**



There is no beneficial effect of hydrophilic polymer properties on AP stability

Funding by SNN – The Northern Netherlands Provinces Alliance

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In collaboration with InnoCore Pharmaceuticals