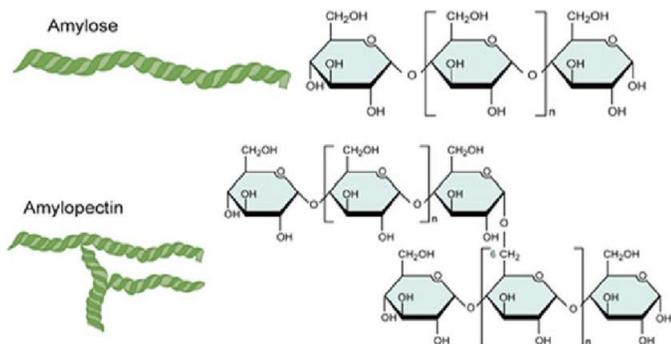


# AN1311: SEC-MALS analysis of linear and branched synthetic amyloses

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## Introduction

Amylose is an occasionally-branched biopolymer and, together with amylopectin, the hyper-branched component, a constituent of starch. Determination of branching in amylopectin, an important property affecting viscosity, stiffness and thermo-mechanical performance, requires a fully-linear amylose analog. This analysis may be performed with the help of synthetic amyloses. This application note describes how synthetic amyloses from enzymatic (phosphorolytic) reaction were checked for their linearity using SEC-MALS.



## Materials and Methods

Synthetic amyloses were dispersed in 1 M KOH at room temperature to 100mg/ml, then diluted with water, filtered to 0.22 $\mu$ m, agitated for 24h under argon, and ultracentrifuged at 100k x g. Separations were performed on Shodex OHPak HQ-806, -805, -804 and -803 size-exclusion chromatography (SEC) columns. Eluant was 0.3 M NaNO<sub>3</sub>, pH 10, run at flow rate 0.5 mL/min.

A DAWN® multi-angle light scattering (MALS) detector and Optilab® differential refractometer were placed downstream of the columns to analyze molar mass and size of each eluting fraction. Data were acquired and

analyzed by ASTRA® chromatography software in order to calculate differential weight distributions, conformation plots of plot mean-square radius vs. molar mass, and branching per molecule.

The molar mass averages  $M$  ( $M_w$ ), and root-mean-square radius  $R$  ( $R_w$ ) were calculated at each slice using the Zimm formalism with first-order fit with respect to  $M$  and  $R$ , and second virial coefficient  $A_2 = 0$ . The refractive index increment value was  $dn/dc = 0.146$  mL/g, and the slice size 6.25 $\mu$ L. At least three data sets per sample were averaged together.

## Results and Discussion

Branching calculations [Branching ratio  $g_M$ , branches per molecule  $B$  and long-term branching  $\lambda$ ] were performed using the Zimm-Stockmayer equations (trifunctional random branching).

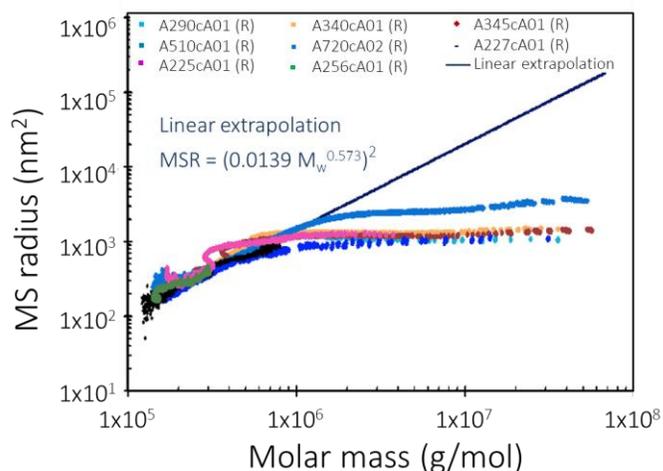


Figure 1. Eight synthetic amyloses and linear extrapolation of the equation obtained from LMM amyloses to 108 g/mol.

The molar mass distributions of low-molar-mass (LMM) synthetic amyloses are narrow in the range of  $1\text{-}4 \times 10^5$  g/mol, and the branching calculations show that these polymers are strictly linear.

The differential weight distribution plots of high-molar-mass (HMM) amyloses show a broader distribution extending into the  $10^6$  g/mol range. A distinct halt to the rise of  $R$  with increasing  $M$  is noticeable beginning around 1 million g/mol (Fig.1). Rigorous analysis shows that branching starts ( $B=e^0$ ) at approximately  $8 \times 10^5$  g/mol (Fig.2).

## Conclusions

A fully linear amylose was synthesized for use in branching analysis of naturally hyperbranched amylopectin. The synthetic amylose samples analyzed here were linear in the low-molar-mass range up to  $\sim 800,000$  g/mol, but exhibited branching at higher molar masses. The origin of branching during the enzymatic reaction remains to be investigated.

Even though the linear samples ended at 800,000 g/mol, the conformation plot of linear amylose can be safely

extrapolated up to  $10^8$  g/mol, enabling analysis of quite large amylopectins. Hence the SEC-MALS method using a DAWN and Optilab has proved to be useful to check branching in HMM biopolymers on the basis of linear LMM biopolymers and, by extension, in hyperbranched biopolymers as well.

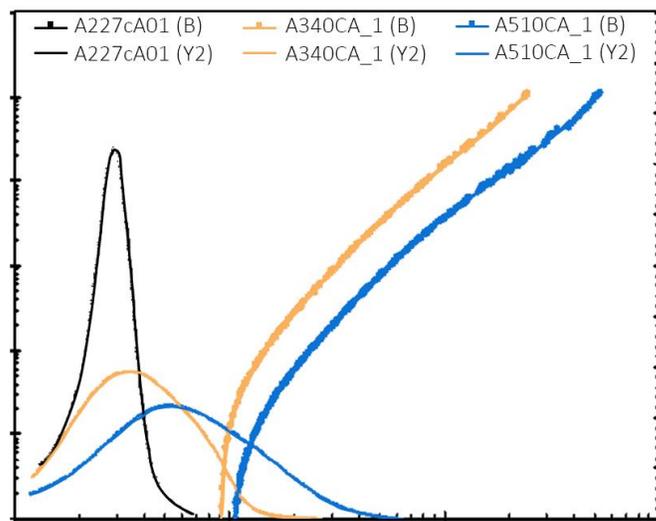
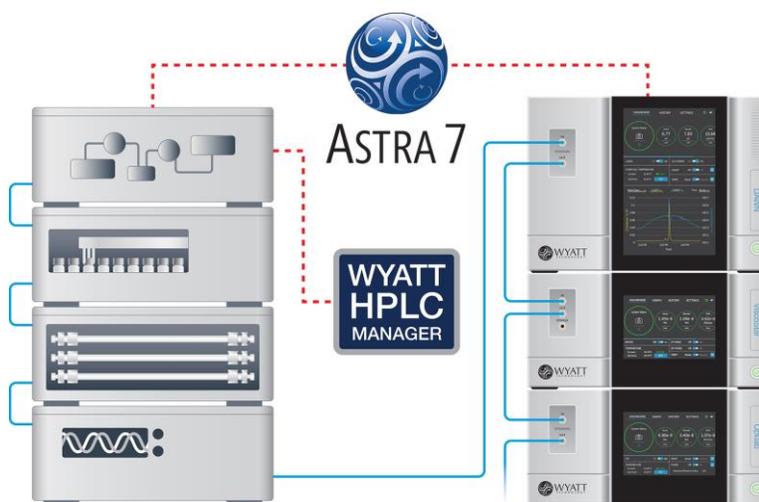


Figure 2. Differential weight distribution (Y2) and branches per molecule (B) in three synthetic amyloses.



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