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Aqueous size exclusion chromatography of hydroxyethyl-amylopectin

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A modified starch component, hydroxylated amylopectin (HAP), was analysed by a low angle laser light scattering apparatus used with high-performance steric exclusion chromatography. The correct weight-average molecular weight and molecular weight distribution (MWD) have been obtained and compared with calibration methods used in conventional chromatography. The modified amylopectin was first chemically scissored and afterwards fractionated using a low-pressure apparatus with crosslinked agarose gel and with a fraction collector on line. For comparing with conventional methods the calibration curves were obtained from polyethylene oxide and polysaccharides provided by Polymer Laboratories. The HAP fractions were separated using a three-columns system and degased buffered filtered water as the mobile phase. This paper discusses the different valuable methods of calculations leading to the molecular weight and MWD characterisation. Failure in the universal calibration is observed for grafted carbohydrates.

INTRODUCTION

The fast and accurate characterisation of hydroxyethyl starch (HAP) has already been the object of numerous studies (Sommermeyer *et al.*, 1987; Kobayashi *et al.*, 1985; Praznik *et al.*, 1985). At first, low-pressure size exclusion chromatography (SEC) has frequently been used but the analysis required several hours per sample. The developments of high-pressure-resistant phases, either porous glass or synthetic gels have allowed a decrease in the measurement times.

Determination of molecular weight by SEC requires a calibration curve for which a set of standards are usually used.

To resolve the main problem of the calibration, several methods are possible.

- A universal calibration relating the elution volume, V_e, to the hydrodynamic volume, $[\eta]\, M_w$ of fractionated standards of chemical nature different from that of the sample under study (Benoit *et al.*, 1967).
- A direct calibration obtained with fractions of the same polymer. The fractionation of HAP can be performed by precipitation from aqueous solution with acetone and isopropyl alcohol or by preparative SEC (Sarazin *et al.*, 1992).
- The direct coupling with a mass molecular weight detector such as a low angle laser light scattering (LALLS) (Kaye, 1973; Ouano & Kaye, 1974; Ouano, 1976). This technique has been very useful for the characterisation of various hydrosoluble polymers such as dextrans, poly(ethylene oxide) (PEO) or polysaccharides (PSA) (Jordan, 1980; Kim *et al.*, 1982).

However, the fractionation is a long and difficult process in the absence of commercially available fractions of HAP. On another hand, LALLS apparatus is very expensive and very difficult to use continuously in aqueous medium for current analysis.

The first method remains the most simple and the aim of this paper is to establish its limitations, by systematically comparing results obtained from the

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