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Gel permeation chromatographic determination of starches using alkaline eluents

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ABSTRACT

By using a dilute solution of sodium hydroxide as eluent in a gel permeation chromatography analysis of natural and modified starches, many of the difficulties encountered with less basic eluent (so-called ghost peaks or memory effects) or with the instability of columns encountered with dimethylsulphoxide, are avoided. The system is shown to give reproducible results and no problems with column stability are encounterd after several months' use. Either ordinary refractive index detection or after-column complexation with iodine and spectrophotometric detection are used. The latter system is especially suited for the analysis of changes that occur on amylose during various treatments (entsymatic or acid hydrolysis or extrusion).

INTRODUCTION

Starches are used in foodstuffs and in various technological applications. For technological purposes, starches are often modified by various chemical, enzymatic or physical treatments, which change their molecular weight distribution and the ratio between the two major components, namely amylose and amylopectin.

The two principal problems in the determination of starches are their high molecular weights and their solubility. On account of the high molecular weight, most liquid chromatographic analyses have been carried out using soft gels and aqueous alkaline eluents, which has meant analysis times of several hours [1–6]. Most work in which modern columns have been employed has been done by using dimethyl sulphoxide (DMSO) as the eluent. The packing materials used have been either silica-based [7,8] or porous glass [9], both showing low capacity and limited separation capability. In the use of styrene–divinylbenzene resins, problems have been encountered with the instability of the column packing material with DMSO [10]. The dissolution of starches into DMSO is also slow, normally requiring several days, and the sensitivity of a refractive index (RI) detector with DMSO is less than that with alkaline aqueous eluents. The use of most water-compatible rigid packings has been handicapped by their low pH stability, which in one experiment led to ghost peaks or a memory effect, obviously due to the low solubility of the samples [11].

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We have been studying the chromatography of starches on μHydrogel columns (cross-linked hydroxylated polymethacrylate gel with residual carboxyl groups). The pH stability of the columns has made it possible to use eluents that are alkaline enough (pH 12.5) for good solubility of the samples and thus no ghost peaks or memory effects are encountered.

The ability of starches to form complexes with iodine has been used for a long time to determine the ratio between amylose and amylopectin [12–14], the complexes showing different absorbance maxima (640 and 525 nm, respectively). We used this for on-line detection by pumping iodine solution into the eluent after separation and utilizing a UV–VIS detector. By utilizing this detection system in addition to ordinary RI detection, we have been able to monitor more precisely what happens to different components of starches during their treatment.

EXPERIMENTAL

The high-performance liquid chromatograph consisted of a Model M-6000A pump, a Model M-710B automatic injector, μHydrogel 2000 and 250 columns (300 × 7.8 mm I.D.) connected in series, a column thermostat and an M-411 refractive index detector.

For detection with iodine complexation, a second Model M-6000A pump was used to pump 1.2 m*M* iodine solution (0.14 g of iodine and 0.34 g of potassium iodide per litre of aqueous 0.5% orthophosphoric acid) at a flow-rate of 0.3 ml/min through a mixing tee, which was situated between the column exit and the M-440 UV–VIS detector monitoring at 658 and 546 nm. All the components that were in contact with iodine solution were carefully passivated by flushing with nitric acid diluted 1:5.

The mobile phase was 50 m*M* sodium hydroxide solution, at a flow-rate of 0.5 ml/min. The mobile phase was carefully deaerated and purged with helium. Samples were dissolved at room temperature in 1 *M* sodium hydroxide solution with a magnetic stirrer for 4 h and then diluted 1:10 with water. All the solutions were carefully deaerated and the samples were stored under an argon blanket. The instrument was controlled and data were processed with a Model M-820 Maxima data station. All the instrumentation was supplied by Millipore–Waters (Milford, MA, U.S.A.).

RESULTS AND DISCUSSION

The molecular weights of most of the samples exceeded the highest commercially available polysaccharide molecular weight standards (dextran T2000 from Pharmacia and pullulan P-800 from Shodex), so only comparisons could be made with the available instrumentation.

With RI detection, the peak area changed linearly in the concentration range 4000–1000 mg/l for both amylose and amylopectin ($r^2 = 0.97$ and 0.99, respectively) and also for injection volumes between 50 and 200 μl. The peak profiles did not change when the column temperature was changed from 60 to 80°C. When a column temperature of 45°C was used, the peaks were less sharp. The remainder of the experiments were therefore carried out at 60°C.

In the detection system where iodine addition was employed, the peak areas

changed linearly for amylopectin in the concentration range 2000–200 mg/l for detection at both 546 and 658 nm (r^2 = 0.97 and 0.99). Moreover, amylose showed linear calibration graphs at 546 and 658 nm (r^2 = 0.97 and 0.99, respectively) in the same concentration range. In all instances the relative standard deviation was less than 8% in triplicate injections, and in most instances the values were less than 3%.

An increase in iodine concentration from 1.2 to 2.4 m*M* did not result in an increase in peak areas but in a slightly inferior baseline at 546 nm because of the increased absorbance of iodine solution at 546 nm. Lowering of the iodine concentration from 1.2 to 0.3 m*M* markedly lowered the detector response for amylopectin samples and also changed the ratio between absorbance at 546 and 658 nm. The detector response for amylose samples, however, remained unchanged. An increase in iodine flow-rate from 0.3 to 0.6 ml/min lowered the peak areas in proportion to the increased dilution, but had no other effect. To check the sufficiency of the reaction time, an additional capillary was added between the mixing tee and the UV–VIS detector, which increased the reaction time from *ca.* 1 to *ca.* 10 s. This did not, however, increase the peak areas, indicating that the original reaction time was sufficient.

With this elution and these detection systems, some commercial amylopectin standards showed two peaks, one of which was in the molecular weight range of several millions and the other *ca.* 80 000, or one peak of molecular weight *ca.* 80 000, which probably means that during their isolation they were seriously degraded. These breakdown products also show an absorbance ratio of 2.2 between detection at 546 and 658 nm. For the peak that eluted with an elution volume indicating a molecular weight of several millions, the absorbance ratio was 1.3 (whereas amylose shows a ratio of 0.5) (Fig. 1). A molecular weight of several millions and a similar absorbance ratio was also obtained for the main peak of a waxy maize sample (containing mostly

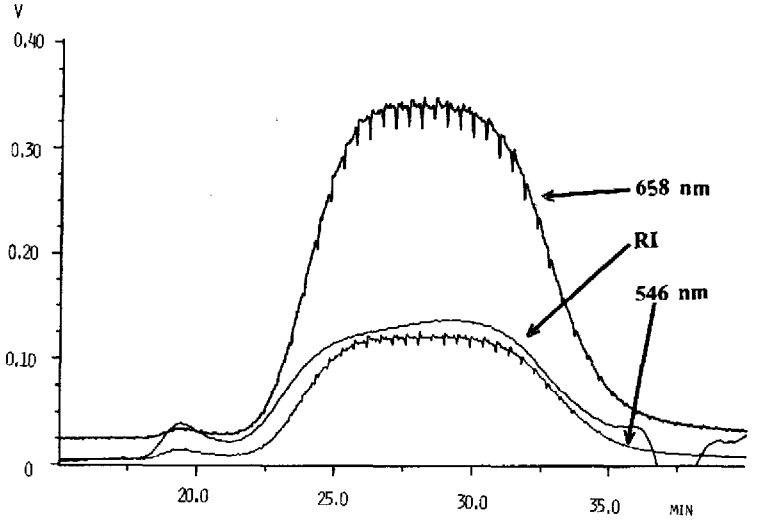

Fig. 1. Chromatogram of amylose from a commercial source. Detection with a refractive index detector (RI) and by spectrophotometry at 546 and 658 nm after complexation with iodine. For other details, see text. A dextran standard of molecular weight 500 000 eluted at 32.0 min. The exclusion volume was 8.7 ml (amylopectin) and the total permeation volume was 19.3 ml (glucose).

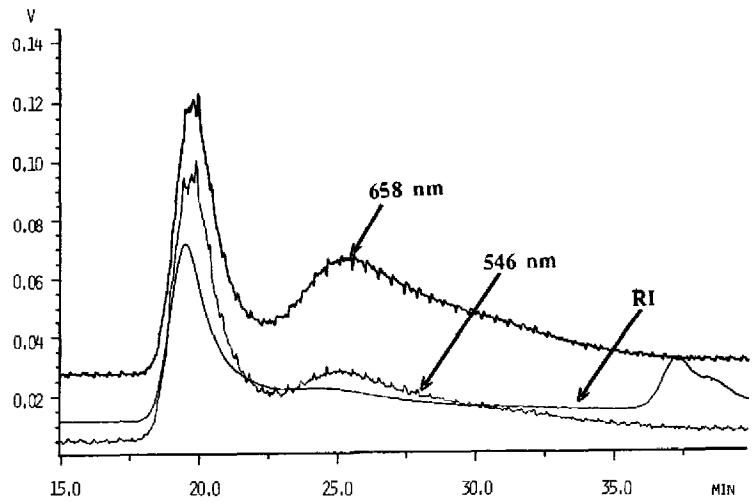

Fig. 2. Chromatogram of potato amylopectin isolated by the method of Schoch [15,16]. Detection with a refractive index detector (RI) and by spectrophotometry at 546 and 658 nm after complexation with iodine. For other details, see text. A dextran standard of molecular weight 500 000 eluted at 32.0 min.

amylopectin) and an amylopectin sample isolated according to the procedure of Schoch [15,16] (Fig. 2). The relative response was 8.8 times higher for amylose at 658 nm than for amylopectin. At 546 nm amylose was detected 2.9 times more sensitively than amylopectin.

We have used this analysis system for several weeks during the last year and the alkaline eluent had no deleterious effect on the column, nor did the iodine solution cause corrosion of the stainless-steel. Moreover, the system has provided valuable information regarding the changes in molecular weight and the different constituents of starches in the native state and after different chemical, enzymatic and physical treatments.

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