Carbohydrate Polymers **6** (1986) 443–462

Molecular Weight Characterization of Starch and Modified Starches as Tricarbanilate Derivatives

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(Received: 16 April 1986)

SUMMARY

Industrial corn starches modified with an oxidant, acid or enzyme have been analyzed by a high-performance size-exclusion chromatography (HPSEC) technique. The procedure involves derivatization of the starch to the corresponding tricarbanilate with separation afforded by commercially available polystyrene–divinylbenzene (PS–DVB) gel columns. Separation time was under 30 min and UV detection at 235 nm allows the analysis of microgram quantities. The elution behavior of amylose and amylopectin tricarbanilates appears to depend on allowed conformational states. Amylopectin tricarbanilate (APTC) is hindered by branching and elutes much earlier than the exclusion limit of the column system according to linear polystyrene standards. Observation of an apparent hydrolysis resistant region of starch and the effects of the various hydrolytic treatments are discussed. Relative molecular weight data are presented utilizing the carbanilate of monodisperse pullulan polysaccharides as primary standards.

INTRODUCTION

The corn wet milling industry is the major supplier of industrial modified starches in the United States (Farris, 1984), and a wide variety of starches are commercially available to satisfy an enormous array of

Carbohydrate Polymers 0144-8617/86/$03.50 —

end use demands. Modification schemes for the depolymerization of the starch macromolecular system include treatments with acid, oxidants and enzymes, thereby providing materials of specific rheological and physical properties.

The classical methods for characterizing modified starch properties relative to molecular size are viscosity, iodine binding and dextrose equivalents (DE). However, these techniques do not fully describe the properties of starches generated by different modification sequences. Hydrolytic depolymerization by α-amylase will produce a different branching distribution relative to a randomly hydrolyzed acid treated starch. It was the goal of this work to characterize the molecular size distribution of several commercially important modified starch products utilizing high-performance liquid chromatography (HPLC).

Size-exclusion chromatography (SEC), otherwise known as gel permeation chromatography (GPC), has become a widely used technique for the analysis of the starch macromolecular system (Young, 1984). Debranching with pullulanase and hydrolysis with amylase (Akai *et al.*, 1971; Mercier, 1973; Colonna & Mercier, 1984) followed by chromatographic analysis on lightly cross-linked polymeric gels such as Sephadex and Bio-Gel has led to a better understanding of the starch matrix. Responses to acid (Robin *et al.*, 1974), oxidants (Bruun & Henriksnas, 1977; Henriksnas & Bruun, 1978) and physical treatment (Craig & Stark, 1984) have also been studied using the methods above. Rigid packings such as porous glass (Dintzis & Tobin, 1974) and deactivated silica (Van Dijk *et al.*, 1976) have been used for the analysis of starch components.

Dimethylsulfoxide (DMSO) is commonly used to disrupt the granular nature of starch, and the subsequent solubility in water has allowed size exclusion to be performed with an aqueous mobile phase. Pulsed-field gradient-NMR (Stejskal & Tanner, 1965) experiments, however, have indicated that amylopectin in water is an aggregate with a volume 400 times the size in DMSO (Callaghan & Lelievre, 1985). Thus DMSO is a better solvent than water for starches. The use of DMSO solvent compatible columns for the analysis of underivatized starches has recently been published (Kobayashi *et al.*, 1985).

Semi-rigid polystyrene–divinylbenzene (PS–DVB) gels have not received the attention that the aforementioned packings have owing to the insolubility of high molecular weight starch in most organic solvents. PS-DVB columns provide high speed analysis of polymers

with organic solubility (Yau *et al.*, 1979). When derivatization of starch under mild conditions to a more soluble form was utilized (in order to be able to employ the HPSEC columns) fast, reproducible analysis was achieved.

Carbanilation

Nucleophilic addition of hydroxyl groups of carbohydrates and polysaccharides to aromatic isocyanates was demonstrated over 35 years ago (Wolff & Rist, 1948). The early work of Wolff and co-workers indicated that the reaction is quantitative with most polysaccharides (Wolff *et al.*, 1952, 1953, 1954). The carbamate functionality provides polymer solubility in solvents such as acetone and tetrahydrofuran (THF) while also tagging the macromolecule with a UV absorbing chromophore. Thus carbanilate derivatization is ideal for the HPLC technique of coupling UV detection and a THF mobile phase. This type of system has already been discussed for the molecular weight analysis of cellulose (Schroeder & Haigh, 1979), and the separation of carbanilated carbohydrates and sugar alcohols by HPLC with UV detection of nanogram quantities has been performed by Bjorkqvist (1981). A general mechanistic pathway has been proposed (Roberts, 1965), and the reaction of phenyl isocyanate (I) with an anhydroglucose unit (II) to form the tricarbanilate (III) is shown in Fig. 1.

Although the original starch carbanilation work used a dry pyridine reaction solvent (Wolff & Rist, 1948), efforts in this laboratory to produce a quantitative reaction by this procedure were unsuccessful. Therefore, DMSO was employed as the reaction solvent to give a rapid and homogeneous reaction. Reaction yields were found to range from 92·8 to 98·7% (Table 1). This is preferable to the heterogeneous pyridine method which requires higher temperatures and longer reaction times.

Molecular weight data

The separation process in SEC is theoretically based solely on the entropic behavior between solute and gel (Yau *et al.*, 1979). Molecular weight distribution (MWD) data are obtained when the system is calibrated against monodisperse and well-characterized standards. Thus

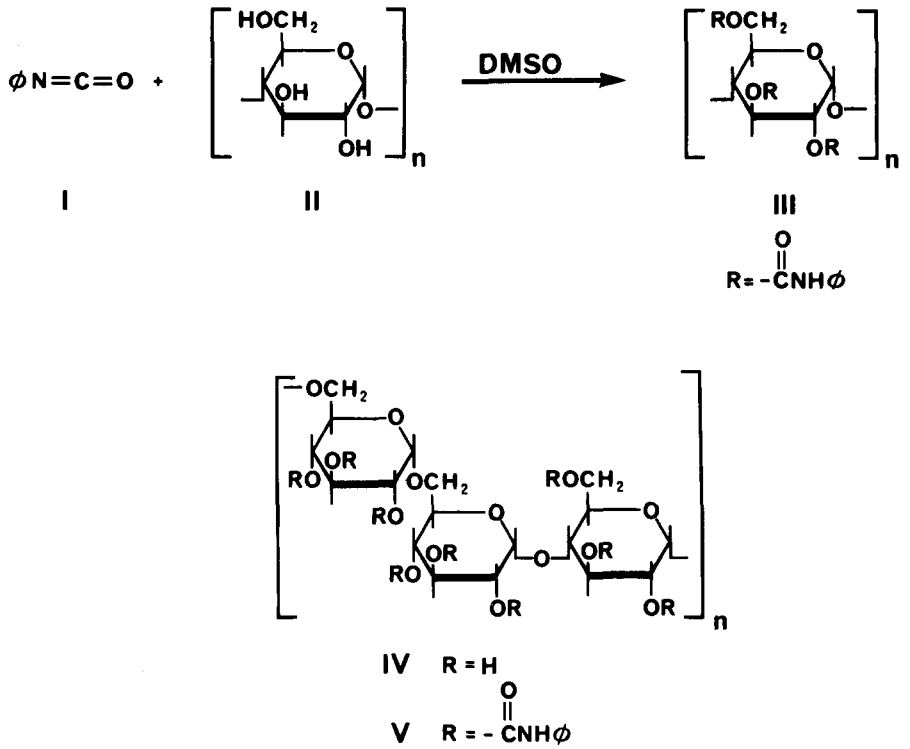

Fig. 1. The carbanilation reaction scheme (I–III), the structure of pullulan (IV) and pullulan tricarbanilate (V).

TABLE 1
Characteristics of the Starch Tricarbanilation Reaction

Starch	*% Theoretical yield*[a]	*Degree of substitution*[b]
U-1	98·7	2·95
A-1	93·4	2·85
A-2	95·8	2·82
A-3	94·3	2·85
Amylose	92·8	2·84
Amylopectin	98·5	2·94

[a] Gravimetric determination: theoretical yield = 519/162 (weight starch reacted).
[b] Modified micro-Kjeldahl.

the molecular weight determination is a secondary method limited by the availability of proper calibration data (Gilding *et al.*, 1981).

Owing to the lack of primary amylopectin and amylose standards, dextran is commonly employed as a relative molecular weight standard (Van Dijk *et al.*, 1976). However, dextran carbanilation yielded incomplete substitution and a polymer insoluble in THF. Therefore, pullulan (IV), shown in Fig. 1, a polymaltotriose produced by *Aureobasidium pullulans*, was employed as a related standard. A set of monodisperse standards is commercially available. The corresponding tricarbanilate (V) was formed and found to be soluble in THF, thus allowing the production of molecular weight data. Underivatized pullulan has been utilized for the standardization of amylose size-exclusion studies by Hizukuri & Tagaki (1984).

EXPERIMENTAL

Corn starch preparation

Raw unmodified corn starch

The raw unmodified starch used in this study was commercially produced by a conventional wet milling process from No. 2 yellow dent corn. The product is referred to as U-1 in this paper. The material was subjected to the carbanilation procedure in a raw granular form. U-1 is considered as the starting material for the modification sequences that follow.

Thermochemically converted starch

Aqueous slurries of raw unmodified corn starch at pH = 6·0 were cooked (gelatinized and dispersed) in a pilot continuous jet cooker operating at 0·7 liters min^{-1} and 152°C with a 5 min retention time. Four starch slurries were prepared to contain levels of starch and ammonium persulfate (AP) on a dry starch basis as shown in Table 2. The starch pastes were lyophilized prior to carbanilation.

Raw acid modified corn starch

These starches were commercially produced via a proprietary treatment of raw unmodified corn starch such as U-1. The acid modifications were effected in the granular state by hydrolyzing aqueous

TABLE 2
Concentrations used in Thermochemical Conversion Sequence

Sample	*% Ammonium[a] persulfate*	*% Starch[a]*
TC-1	0·0	11·4
TC-2	0·1	11·0
TC-3	0·2	18·8
TC-4	0·3	28·4

[a] Dry starch basis.

slurries with mineral acid. Different reaction times and acid concentrations led to the products referred to as A-1, A-2 and A-3. The conditions favoring hydrolysis of the starch macromolecular system were increased respectively. These starches were subjected to the carbanilation procedure as raw, granular starches.

Enzymatically converted starch

Aqueous slurries at 18% solids were treated in a pilot converter. Alpha-amylase (Canalpha, Biocon Inc., Lexington, Kentucky, USA) was added at levels of 0·204, 0·408 and 1·22 SKB g^{-1} (Sandstedt *et al.*, 1939) on a dry starch basis (E-1, E-2 and E-3, respectively). (The value SKG g^{-1} represents the amount of starch (g), under the influence of an excess amount of β-amylase, that is dextrinized in 1 h at 30°C by 1 g of the α-amylase preparation (American Association of Cereal Chemists, 1983).) The conversion cycle entailed heating to 77°C in 15 min and holding the temperature for 20 min. Enzyme deactivation was accomplished by a 10 min heat-up to 97°C, adding Cu_2SO_4 (0·07% on starch), and holding at 97°C for 30 min. The resulting starch pastes were lyophilized prior to carbanilation.

Inherent viscosity

Determination of inherent viscosity was according to the Member Companies of the Corn Industries Research Foundation (1982).

Carbanilation of modified starches and pullulan

The starch samples were dried for at least 48 h *in vacuo* over phosphorous pentoxide. Dried starch (0·5–1·0 g) was added to a heated

flask containing 150 ml DMSO (Aldrich Chemicals; Milwaukee, Wisconsin, USA; 99·9% pure stored over 4A molecular sieves and under dry nitrogen) maintained at 70°C and equipped with a magnetic stirrer, drying tube and thermometer. The solution was stirred slowly until the sample dissolved. A 10 mol excess of phenyl isocyanate (stored over 4A molecular sieves) per polysaccharide hydroxyl group was added and the reaction was allowed to continue for 24 h. The mixture was then cooled to at least 40°C and the excess phenyl isocyanate was destroyed by the slow addition of methanol. (Note: care must be taken when using phenyl isocyanate as it is a toxic lachrymator which reacts rapidly with water.)

The starch carbanilate and associated by-products were precipitated in 8 times the volume of water and isolated on a fine grade sintered glass filter funnel. After washing the material with distilled water, the solid was dried *in vacuo* at temperatures not exceeding 60°C.

The pure carbanilate, excluding the low molecular weight species which were assumed to be lost, was obtained by dissolution in acetone followed by reprecipitation in ethanol (final ethanol:acetone ratio was 8:1). The pure carbanilate was then evaluated spectroscopically (FTIR, NMR) and for total nitrogen content (modified micro-Kjeldahl). The degree of substitution (*DS*) is readily calculated from the % nitrogen value via the following equation (Wolff & Rist, 1948):

$$DS = 162(\%N)/(1400 - 119(\%N))$$

Pullulan standards (Polymer Labs, Amherst, Massachusetts, USA) were prepared in a similar fashion except on a much smaller scale. Dried samples (10 mg) of two different molecular weight standards were added to DMSO (2·0 ml) at 50°C. Phenyl isocyanate (0·5 ml) was added after dissolution, and the reaction was left unstirred for 12 h. The pullulan tricarbanilates were analyzed immediately after quenching the reaction.

Chromatographic analysis

Shodex A 804/S and A 805/S (Showa Denko America, New York, USA) SEC columns were used in series for all separations. A Perkin-Elmer HPLC system consisting of a Series II pump module and a LC-85 UV detector set at 235 nm was used for separation and detection. This equipment was interfaced with a Sigma 15 data station which

acquired and analyzed the data with commercially available molecular weight characterization software (Perkin Elmer, Norwalk, Connecticut, USA).

Standard chromatographic conditions were employed throughout the study. The mobile phase was THF (Burdick and Jackson, Muskegon, Michigan, USA), at a flow rate of 0·8 ml min^{-1} and ambient temperatures. The water precipitate (10–20 mg, containing 12–14% derivatized polysaccharide) was dissolved in THF (5 ml) and filtered through a 1·0 μm membrane filter. In order to avoid precipitation losses with the pullulan tricarbanilates, a portion of the reaction mixture (0·1 g) was dissolved in 5 ml of THF, filtered through a 1·0 μm filter and injected directly onto the columns. All samples could not be analyzed with this simpler direct procedure due to DMSO column overloading effects which decrease the life expectancy of the columns. Injection volumes ranged from 10–40 μl. The relationship between absorbance and concentration was assumed to be linear and no concentration effects were observed.

The calibration curve used for the determination of the MWD is shown in Fig. 2. Since the degree of polymerization of the pullulan standards ranged from 36 to 5265, it was necessary to extrapolate the curve at both ends. Recalibration during the extent of the study showed no change in column character.

RESULTS AND DISCUSSION

Reaction characterization

The derivatization data for several starch samples are shown in Table 1. The degree of substitution (*DS*) ranged from 2·82 to 2·95. The yields reported in Table 1 are based on the weight of the ethanol insoluble precipitate from which a small portion of the very low molecular weight material is assumed lost.

Fourier transform infrared spectroscopy (FTIR) of starch and its corresponding tricarbanilate (Fig. 3) show the introduction of the carbamate functionality. There are the expected drop in hydroxyl absorption at 3400–3300 cm^{-1}, a carbonyl stretch at 1740 cm^{-1}, the C—C(=0)—0 stretch at 1235 cm^{-1}, the amide II band at 1600 cm^{-1},

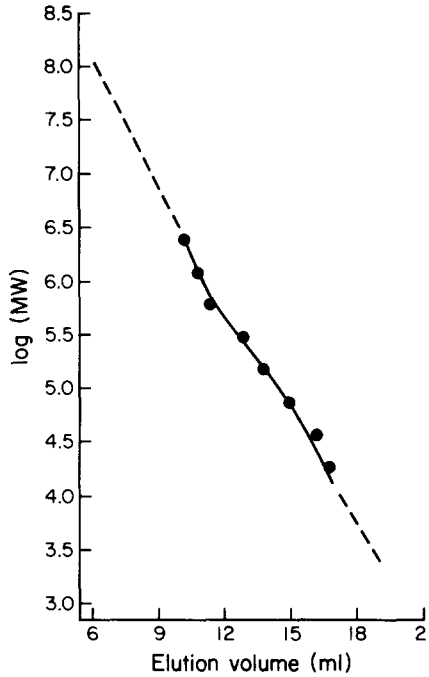

Fig. 2. Calibration curve of the Shodex A 804/S and A 805/S separation system: ●, pullulan tricarbanilate; - - - -, computer extrapolation.

and the monosubstituted aromatic ring bendings at 760 and 700 cm^{-1}.

The elution pattern of amylopectin tricarbanilate (APTC)

Commercial amylose and amylopectin (Sigma Chemical, St Louis, Missouri, USA) were analyzed as model compounds, and the chromatograms are presented in Fig. 4. An off-scale peak at high elution volumes (20 ml, not shown) is due to the reaction by-products which cannot be eliminated without loss of the low molecular weight species. There is a marked difference in the elution patterns of amylose tricarbanilate (ATC) and amylopectin tricarbanilate (APTC), with the APTC eluting much sooner than its linear counterpart.

This behavior is complicated by the fact that the exclusion limit for the system employed is $MW = 5\,000\,000$ for a linear polystyrene

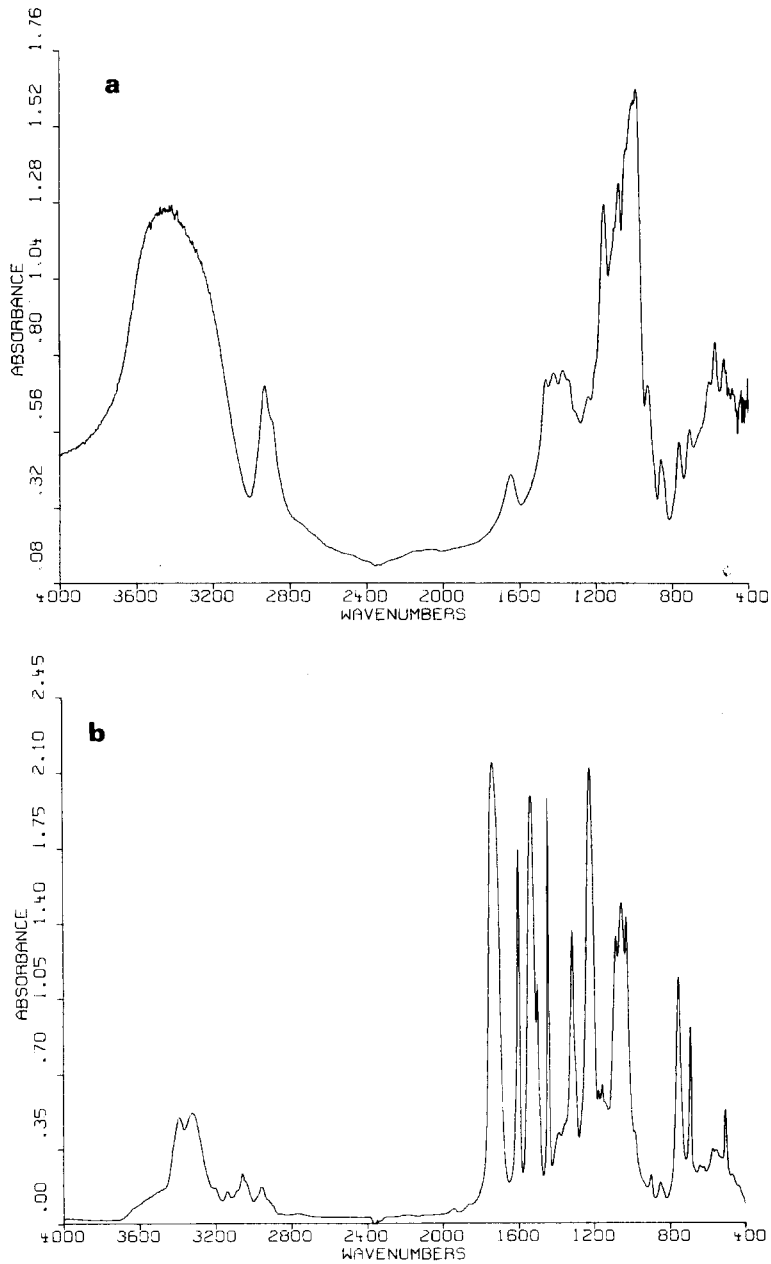

Fig. 3. FTIR spectra of raw unmodified corn starch: (a) native state; (b) tricarbanilate.

standard. The elution volume associated with this standard is 9·50 ml whereas the Sigma APTC and U-1 have a major peak eluting at 7·2 ml (Fig. 4). Several theoretical explanations of these results are presented below.

A possible reason for this behavior would be the formation of starch microgels which elute as highly agglomerated units. Cellulose diacetate is known to yield 'prehumps' (Kamide *et al.*, 1974, 1979) or an early eluting peak when SEC is employed to obtain a macromolecular distribution. This has been attributed to a gelation effect.

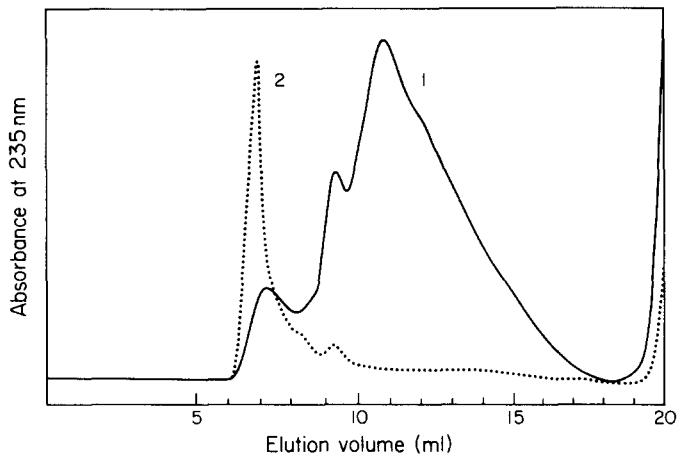

Fig. 4. HPSEC chromatograms of amylose (1), and amylopectin (2) tricarbanilates. Degree of polymerization data are presented in Table 3.

Price and co-workers (Price *et al.*, 1977; Booth *et al.*, 1980; Styring *et al.*, 1985) have recently studied the separation of polystyrene microgels in THF on columns quite similar to the ones employed in this work. They showed that the high molecular weight networks were absorbed onto the stationary phase therefore eluting later than their linear counterparts, or not at all. However, if the starch tricarbanilates were in solution as microgels it would probably not be possible to see the microstructural changes shown on the chromatograms in Figs 4 to 8 at various levels of hydrolytic treatment, unless the microgels were sensitive to the overall starch structure of each specific modification sequence. The microgel would also have to be smaller than 1 μm in order to pass through the pre-injection filtration step.

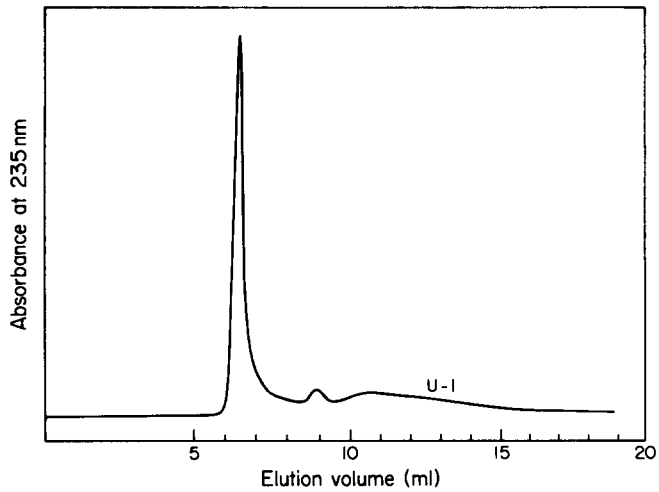

Fig. 5. HPSEC chromatogram of raw unmodified corn starch, U-1. Degree of polymerization data are presented in Table 3.

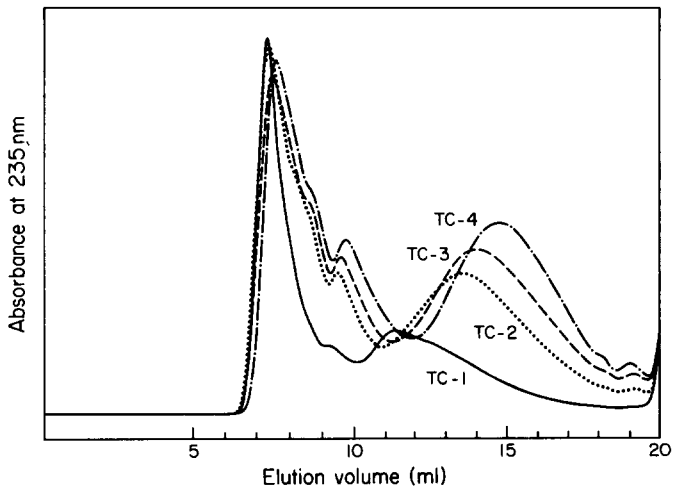

Fig. 6. HPSEC chromatograms of thermochemically converted corn starch tricarbanilates. Aqueous slurries at pH6 were gelatinized and dispersed in a pilot plant continuous jet cooker under the following conditions: 152°C, 0·7 liters min^{-1} and a 5 min retention time. Several levels of ammonium persulfate (AP) were used to effect depolymerization, providing samples TC-1 to TC-4 (Table 2). Degree of polymerization data are presented in Table 3.

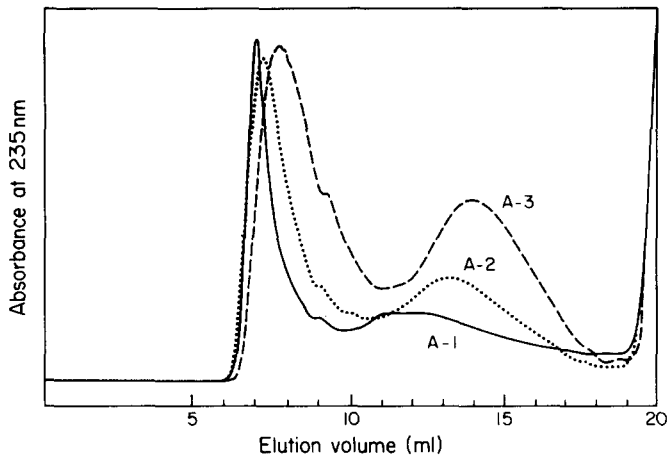

Fig. 7. HPSEC chromatograms of acid treated corn starch tricarbanilates. A proprietary acid modification sequence was employed, with conditions favoring depolymerization increasing from A-1 to A-3, respectively. Degree of polymerization data are presented in Table 3.

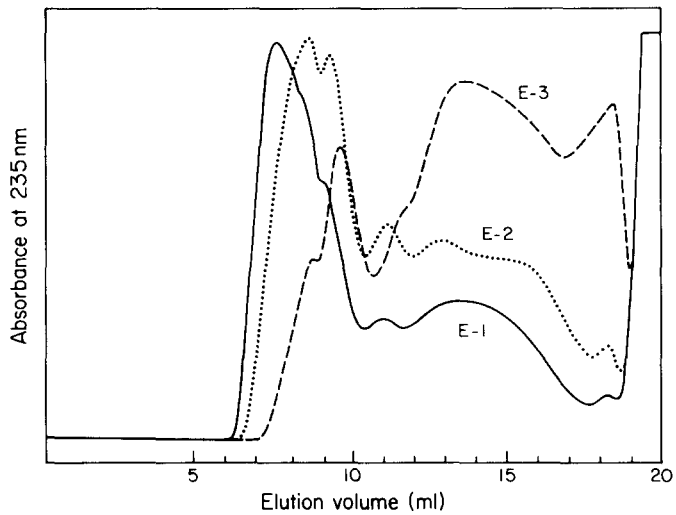

Fig. 8. HPSEC chromatograms of enzymatically treated corn starch tricarbanilates. Slurries of raw unmodified corn starch (18% solids) were treated with α-amylase at 0·204 (E-1), 0·408 (E-2) and 1·22 (E-3) SKB g^{-1} and 77°C. Degree of polymerization data are shown in Table 3.

Another possible explanation for the observed results is that high molecular weight linear polystyrene standards are under the influence of both size-exclusion and physical separation mechanisms. This scenario would then imply that the polystyrene does not reflect the 'true' exclusion limit of the columns whereas the branched tricarbanilates do so to a greater extent. Linear high molecular weight polymers would therefore have a larger partition coefficient for absorption relative to highly branched systems. It is known from this work that pullulan and amylose tricarbanilates act similarly to the polystyrene during the separation process. Therefore, it seems safe to assume that if polymer absorption does indeed occur it is not a major contributor to the observed elution behavior.

The hydrodynamic effects of (1-6)-branching may also explain the elution behavior of starch tricarbanilate. A Gaussian distribution of pore sizes will theoretically be sampled by each solute molecule which, depending on its size, will interact with a certain percentage of the pores whose dimensions are larger than the molecule. Two solute molecules of the same size will sample the stationary mobile phase equally and will thus, assuming the effects of diffusion are negligible, elute at the same time. Linear systems will exist as random coils with the extent of coiling or agglomeration dependent on the solvating power of the solvent. The polymer will elute at its corresponding average hydrodynamic volume. However, a branched polymer such as APTC will exist in a restricted spherical shape with fewer degrees of freedom. The hydrodynamic volume of APTC will remain relatively constant. Large linear polymers therefore sample pore sizes less efficiently than a high MW branched system. We conjecture that the ATC molecules are not effectively differentiated by the larger pores because they readily change conformation. APTC, with restraint induced by branching, remains in a rigid form. The larger pores, between which ATC systems cannot effectively differentiate, are utilized by APTC for purposes of separation. Thus the exclusion limit for this branched polymer is higher than its linear counterpart.

The preceding discussion is helpful for interpretation of the chromatograms of ATC and APTC in Fig. 4. ATC, considered to be essentially free of amylopectin, was found to contain 8·5% branched polymer. The results indicate that the high molecular weight amylopectin and amylose tricarbanilate derivatives can be separated

TABLE 3
Calculated Degrees of Polymerization for Several Modified Starch Tricarbanilates[a]

Starch	$\overline{DP}_w$	$\overline{DP}_n$	$\overline{DP}_w/\overline{DP}_n$	DP_{50}[b]	*Inherent viscosity* ($dl\,g^{-1}$)
Amylose	10460	622	17	1890	—
Amylopectin	89020	1130	79	95400	—
Raw unmodified					
U-1	98170	1620	61	117770	2·37
Thermochemical[c]					
TC-1	38535	888	43	23025	1·84
TC-2	33330	431	77	11410	0·94
TC-3	26590	288	92	6690	0·69
TC-4	21290	248	86	4820	0·46
Acid modified[d]					
A-1	57800	1230	49	35010	1·70
A-2	41715	538	78	17020	1·01
A-3	20085	369	65	5520	0·69
Enzymatic[e]					
E-1	34450	722	48	17440	0·74
E-2	18940	491	39	7320	0·49
E-3	3695	54	69	320	0·23

[a] $\overline{DP}=\overline{MW}/519$.
[b] $DP_{50} = DP$ at 50% total peak area elution slice.
[c] See Fig. 6 for explanation of treatments.
[d] See Fig. 7 for explanation of treatments.
[e] See Fig. 8 for explanation of treatments.

chromatographically in relatively high purity with larger preparative columns (Yamada & Taki, 1976; Takeda *et al.*, 1984).

The molecular weight distribution of modified starches

The chromatograms of the modified starches analyzed are shown in Figs 5 to 8 with the molecular weight data presented in Table 3. The DP_{50} values of Table 3 represent the degree of polymerization (DP) associated with the elution slice at 50% total peak area as calculated by the computer software. This value is considered a better representation of the true DP as it is less sensitive to the high and low molecu-

lar weight tails of this polydisperse system relative to $\overline{DP}_w$ and $\overline{DP}_n$, respectively (Young, 1984).

Modified starches contain an apparently inaccessible region which becomes more prevalent under harsher hydrolytic treatments. This 'microstructure' has been observed in other studies (Rollings & Thompson, 1984). This peak or shoulder at 9·5 ml has several possible explanations: (a) a truly inaccessible or resistant starch fraction, (b) an intermediate fraction, or (c) the exclusion limit of amylose. Since it is generally assumed that amylose is degraded rapidly (Robin *et al.*, 1974), the material is probably not a high molecular weight amylose. Although the intermediate fraction theory cannot be disproven, the logical assumption is that the material arises from the highly crystalline amylopectin regions of the macromolecular system which are unavailable for degradation during the modification sequences. The results of this investigation support the idea that the peak at 9·5 ml is indeed an inaccessible region of the original starch granule and possibly the 'core' of the heterogeneously branched cluster model (French, 1972; Robin *et al.*, 1974). Further studies are necessary to verify this conclusion.

It should be noted that because of the anomolous behavior of APTC it is not known if the first eluting peak is at the exclusion limit, i.e. the highest molecular weight peak could be an artifact generated by column restrictions. The development of a system with a higher exclusion limit (based on polystyrene) is currently underway and will be reported.

Analysis of the hydrolytic treatments

The raw unmodified starch of Fig. 5 was found to contain exceedingly high molecular weight material along with an amylose fraction of a broad molecular weight distribution. A very large drop in both viscosity and molecular weight were effected without chemical treatment by only the shear of the jet and the nominal 5 min retention at the conditions of pH 6 and 152°C for the 0% ammonium persulfate (AP) treatment of Fig. 6 (TC-1). Successively larger doses of AP to the starch slurry brought about corresponding decreases in viscosity and molecular weight. It is also evident from the chromatograms that there was a steady decrease in the high molecular weight amylopectin fraction

with a concomitant increase in the lower molecular weight branched degradation products.

The mechanism of degradation has recently been suggested by Chen and co-workers (1985) to occur by free radical formation through C–H abstraction. The generation of hydrogen ions provides an acidic medium which effects random glycosidic bond cleavage.

Thermochemical conversion of starch is the predominant technique used for the preparation of starch sizing adhesives in the paper industry. The thermochemically converted starches in Fig. 6 show modifications comparable to that expected for on-site paper mill preparation. Whereas a higher *DP* is thought to provide better binding ability, this higher total chain length could lead to a higher rate of agglomeration upon storage. Burchard (1963) has proposed a 'dissolving gap', or a range of insolubility, and this has been alluded to recently by other investigators (Kodama *et al.*, 1978; Rollings & Thompson, 1984). Viscosity stability is achieved in commercial practice by controlling the temperature and pH of the starch pastes.

The data in Table 3 show the products of the granular state acid modification of corn starch (Fig. 7) decreased in molecular size as the conditions favoring hydrolysis were increased. Studies support the idea that hydrolysis is preferential to the amorphous region (Mussulman & Wagoner, 1968). Salemis & Rinuado (1984*b*) have suggested that the rate of amylopectin acid hydrolysis is a two-step process with the production of 'two well defined types of molecules'. The $\overline{DP}_w$ for these two groups were approximately 2000 and 400. This corresponds to the shoulder at 9–10 ml and the branched degradation products peak at 13–14 ml, respectively.

The action of α-amylase is considered to be a multiple attack mechanism (Robyt & French, 1967) where some very short oligosaccharides are cleaved from a polymer chain followed by enzyme transfer to another chain and continued oligosaccharide release. Glycosidic bond cleavage occurs anywhere except near branch points (Rollings, 1985), and penetration into the crystalline regions will be slow. Figure 8 dramatically reveals the production of the lower molecular weight material at higher enzyme concentrations along with the appearance of the resistant starch fraction. At the most severe enzymatic treatment the lower molecular weight branched material becomes the predominant species with a $\overline{DP}_{50}$ of 320. It can also be

seen in Table 3 that at equivalent inherent viscosities the enzymatic starches have a much larger total chain length. Since α-amylase breaks (1-4)-glycosidic bonds, the relative proportion of (1-6)-linkages increases. Thus the branched nature of the system allows a higher total chain length to exist at low viscosities.

The degree of polymerization data presented in Table 3 reveal a broad distribution of both $\overline{DP}$ and polydispersity. This is to be expected for a heterogeneous system such as the starch tricarbanilates where molecular sizes range from the largest APTC molecule to a small oligosaccharide hydrolysis product (Erlander & French, 1956). The $\overline{DP}_{50}$ value counteracts the weighting factors involved in the calculation of weight- and number-average $\overline{DP}$. Although extrapolation of the calibration curve introduces an unknown degree of error, the data generated in this work were very similar to those reported by Salemis & Rinuado (1984*a*) utilizing a GPC–LALLS (gel permeation chromatography–low angle laser light scattering) technique. This method, after instrument calibration, requires only the determination of the refractive index increment of the polymer being studied (Ouano & Kaye, 1974). Therefore, weight average MW information is obtained without primary standard calibration. Salemis & Rinaudo (1984*a*) reported the $\overline{DP}_w$ for a waxy maize amylopectin sample to be 93000. This is quite similar to the $\overline{DP}_w$ of 89020 for a commercial corn amylopectin sample presented in Table 3.

CONCLUSION

A new technique has been developed which employs derivatization of the starch macromolecular system to its corresponding tricarbanilate. Separation is performed by HPSEC. Relative *DP* values have been generated for this polydisperse system which are quite similar to data recently published utilizing the GPC–LALLS technique, without investment in this expensive instrumentation. Amylopectin tricarbanilate is characterized by an anomolously early elution volume due to its inherent hydrodynamic properties. Viscosity gives incomplete information on the total chain length distribution of industrially modified starches. Enzymatic treatment yields large polymers at low viscosity. The technique has been proven useful in the analysis of commercial starch preparations for industrial utilization, and may aid in monitor-

ing on-site modification processes or in the development of new modification schemes.

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