Carbohydrate Polymers 5 (1985) 201–213

A Study of Some Factors Influencing Amylose Gelation

H.S. Ellis and S.G. Ring

AFRC, Food Research Institute, Colney Lane, Norwich NR4 7UA, UK

(Received: 14 June 1984)

SUMMARY

Amylose fractions were prepared by aqueous leaching from pea, maize and potato starch granules. The fractions were characterised by iodine binding, β-amylolysis and viscometry. Amylose starts to form a gel rather than a precipitate on cooling aqueous solutions to room temperature at concentrations above the coil overlap concentration C. Amylose gels are almost purely elastic, with negligible viscous flow at room temperature. The rigidity modulus is strongly dependent on concentration, c, in that above 1·5% w/w the modulus increases as a function of c^7. The modulus of a matured gel falls only slightly with increasing temperature; at temperatures below 100°C the gel could not be melted. The non-equilibrium nature of the system is shown by the dependence of rigidity on thermal history. The shear modulus is also dependent on amylose type; higher molecular weight amylose fractions produced less rigid gels at a given concentration.*

INTRODUCTION

Starch is a major component of many foods and can have an important influence on texture and hence acceptability. Starch-containing foods are usually processed by heating in the presence of water. Above a characteristic temperature known as the gelatinisation temperature, starch granules irreversibly swell to many times their original size. At the same time one of the starch polysaccharides, amylose, is preferentially solubilised. If concentrated suspensions of gelatinised starch are cooled amylose molecules associate to form an interpenetrating gel matrix in which are embedded swollen gelatinised granules. In foods the matrix may contain other polysaccharides and proteins in association

Carbohydrate Polymers 0144-8617/85/$03.30 –

with the amylose. These materials are composites (Ring & Stainsby, 1982); their rigidity at small deformations is dependent on the volume occupied by the particles (in this case the gelatinised granules), their shape (Richardson *et al.*, 1982), their deformability and the rigidity of the matrix gel. As yet the factors which influence the rigidity of an amylose gel matrix are not fully understood.

Amylose is an essentially linear $\alpha(1\rightarrow4)$ linked D-glucan which can be conveniently defined (Banks & Greenwood, 1975) as that starch polysaccharide which under standard conditions binds 19·5% w/w iodine. Some of the molecules which fit this definition are incompletely degraded to maltose by the exo-acting enzyme β-amylase. As the restriction to hydrolysis is removed by pretreatment with an $\alpha(1\rightarrow6)$ glucan hydrolase it follows that amylose can contain the $\alpha(1\rightarrow6)$ linkage as an interchain linkage or branch point (Peat *et al.*, 1949). Recent work (Hizukuri *et al.*, 1981) on potato amylose favours the latter view. Additionally the amylose solubilised during gelatinisation contains molecular species varying in degree of polymerisation.

In aqueous solution amylose behaves as a flexible coil. At room temperature these solutions are inherently unstable. Whereas a precipitate gradually develops with time from dilute solutions, concentrated solutions produce a white friable elastic gel on cooling. The aim of this study was to investigate, using well characterised amylose fractions, some of the factors which influence the rigidity of amylose gels at small deformations.

EXPERIMENTAL

The starches were isolated by the aqueous extraction procedure of Adkins & Greenwood (1966) from smooth-seeded leafless peas (variety Filby), potatoes (variety Desirée), and maize. The amylose contents of the purified starches, determined from their iodine binding capacity, were 26% w/w, 17% w/w and 25% w/w respectively. Amylose fractions were prepared by sequential aqueous leaching of the intact granules. Swollen gelatinised granules were removed by mild centrifugation (2000 ***g***) and filtration through a glass sinter (porosity 3). Amylose was precipitated as its *n*-butanol complex by the addition of *n*-butanol (8 g/100 g) to the filtrate. After 24 h, the *n*-butanol complex was collected by centrifugation (2000 ***g***). Amylose solutions were regener-

ated from the complex by heating to 95°C followed by removal of the *n*-butanol in a heated nitrogen stream. Amylose gels were prepared by rapidly quenching concentrated amylose solutions to 20°C followed by maturation at this temperature for 24 h. Amylose concentrations in the gel were determined from measurements of dry weight (vacuum oven at 60°C).

Analytical

The iodine binding behaviour of the starches and amylose fractions was determined using a semi-micro differential potentiometric technique as described by Banks *et al.* (1972). β-Amylolysis was performed by incubating 20 units of a crystalline sweet potato β-amylase with 10 ml of a 0·1% w/w amylose solution in 0·02 M acetate buffer at pH 5 for 4 h at 30°C. The relatively high level of β-amylase was employed in order to ensure a rapid β-amylolysis and thus minimise the chance of retrogradation occurring during the experiment. The maltose which was released was determined from the reducing sugar value of the digest by the method of Nelson and Somogyi (Whistler & Wolfrom, 1962). The β-amylase preparation was checked for the presence of contaminating enzymes, α-amylase and α-glucosidase (Marshall, 1974). The preparation did not increase the reducing value of a maltose solution; α-glucosidase was therefore inferred to be absent. Additionally, as the β-amylase preparation did not release the chromophore from amylose azure as low molecular weight fragments, α-amylase was also inferred to be absent.

Amylose concentrations were determined either from measurements of dry weight or by the phenol–sulphuric acid colorimetric method (DuBois *et al.*, 1956).

Rheological measurements

Saunders and Ward tube

The modified (Stainsby *et al.*, 1984) Saunders and Ward (Saunders & Ward, 1954) tube is a simple apparatus of modular design. It consists of a wide bore tube (approximate radius 0·7 cm) whose inner surface is coated with glass chips, which is connected to a capillary of radius r. The gel was set and matured in the wide bore tube. Air pressure was applied to the gel via a manometer and the movement, h, of an index

liquid (CCl_4) in the capillary noted. The shear modulus G was calculated from

$$G = \frac{PR^4}{8lr^2h}$$

where P is the applied pressure and l the length of the gel. The effective radius, R, of the wide bore tube was calculated from measurements on gelatin gels of known rigidity.

Pulse shearometer

The instrument was obtained commercially from Rank Brothers, Cambridge. It is based upon a design of Van Olphen (Van Olphen, 1956) whcih was subsequently modified at the University of Bristol (Buscall *et al.*, 1982). The material to be tested is positioned between two perspex discs which are mechanically coupled to piezo-electric crystals. A square pulse is applied to one crystal, causing a torsional force to be transmitted to the drive disc which then starts to oscillate at its resonant frequency. This generates a shear wave travelling through the sample to the other disc and crystal. The output from the receiving crystal, a damped sine wave, and the timing of the pulse are displayed on a dual beam oscilloscope. The shear storage modulus, G', is given by

$$G' = \rho v^2(1-r^2)/(1+r^2)^2$$

where ρ is the density of the material, v the velocity of the shear wave and $r = \alpha\lambda/2\pi$ where λ is the wavelength of the shear wave and α its attenuation in the sample. For amylose gels the damping of the shear wave was small and therefore the correction factor, $(1-r^2)/(1+r^2)^2$, was neglected.

Viscosity measurements

Specific viscosity was measured at 30°C using suspended level (Ubbelohde) capillary viscometers. Extrapolation to zero concentration was performed by the combined methods of Huggins and Kraemer (Morris & Ross-Murphy, 1981), from data in the range 0·5–5 mg ml^{-1}. The measured viscosity of solutions of asymmetric or easily deformable polymers may show a marked dependence on shear rate. The specific viscosity of aqueous amylose solutions in the concentration range tested (0·5–5 mg ml^{-1}) was found to be essentially independent of shear

rate in the range 400–2400 s^{-1}. Therefore no corrections were made for shear effects.

As concentrated solutions of amylose retrograde rapidly at lower temperatures, when solutions above 1% were used in an experiment all measurements were carried out at 65°C. The value of intrinsic viscosity obtained at this temperature was similar to that found for the same sample at lower temperatures.

RESULTS AND DISCUSSION

Amylose solutions were regenerated from the *n*-butanol complex by heating to 90°C followed by removal of the *n*-butanol in a hot nitrogen stream. This procedure for preparing aqueous amylose solutions was used in preference to other methods such as dissolution in alkali followed by neutralisation, which is potentially degradative, or autoclaving at 120°C which results in incomplete solution.

At an extrapolated zero concentration of free iodine all fractions bound 19·5 ± 0·5 mg of iodine per 100 mg of polysaccharide and therefore can be considered to be of high purity (Table 1). Additionally, the extent of hydrolysis of the purified fractions by a crystalline sweet potato β-amylase was determined by measuring the release of maltose (Table 1). The pea and maize amylose fractions leached at tempera-

TABLE 1
Chemical Characteristics of Amylose Fractions

Fraction	*Leach temperature (°C)*	*Iodine binding capacity (% w/w)*	*β-Amylolysis limit (%)*	$[\eta]$ *(ml g^{-1})*	*Calculated* $\langle M_w \rangle$ *(g mol^{-1})*
Pea	60-70	19·8	100	80	5×10^5
Pea	70-80	19·5	100	98	$7{\cdot}5 \times 10^5$
Pea	80-90	19·5	80	110	$9{\cdot}5 \times 10^5$
Potato	60-75	19·5	75	96	$7{\cdot}2 \times 10^5$
Potato	75-90	19·0	75	126	$1{\cdot}24 \times 10^6$
Maize	75	19·5	100	80	$7{\cdot}8 \times 10^5$

tures below 80°C were quantitatively hydrolysed to maltose. The pea amylose leached from 80 to 90°C and the potato amylose fractions were incompletely degraded. By analogy with other published work (Peat *et al.*, 1949; Hizukuri *et al.*, 1981) they were assumed to contain molecular species of amylose which were branched.

The intrinsic viscosity, $[\eta]$, of a dilute aqueous amylose solution is an index of the average size of the macromolecule in solution and, if the amylose is linear, can give a convenient estimate of the weight-average molecular weight $\bar{M}_w$ using the relationship (Banks & Greenwood, 1975),

$$[\eta] = K\bar{M}_w^a$$

where K and a are constants with values of 0·113 and 0·5 (if $[\eta]$ is in ml g^{-1} (Banks & Greenwood, 1975)). The fractions of amylose for which this relationship was derived had a limited polydispersity, typically $\bar{M}_w/\bar{M}_n \simeq 1{\cdot}5$. The intrinsic viscosity of the amylose fractions ranged from 80 to 126 ml g^{-1} and calculated weight-average molecular weights ranged from 500 000 g mol^{-1} to over $1{\cdot}2 \times 10^6$ g mol^{-1} (Table 1). The fractions leached at higher temperatures were of larger molecular size showing also a decrease in susceptibility to hydrolysis by β-amylase.

Rheological measurements

Initial experiments were performed on the fraction of amylose leached from pea starch at 70°C. Determination of the shear modulus of amylose gels is difficult because they synerese, i.e. they form a film of water at the gel surface preventing adhesion between the gel and measuring device. As a consequence they have usually been tested using empirical methods. Recently two simple methods have been developed which largely overcome this problem. The first is the Saunders and Ward tube, an apparatus which has been widely used to measure the shear modulus of weak gelatin gels; this has been modified to permit measurement on gels such as amylose. In this method a static shear stress is applied to the gel, usually for 30 s, and the deformation noted, the stress is removed and the gel allowed to recover for 5 min. The procedure is repeated at different applied stresses. Figure 1 shows the stress–strain behaviour of a 3% w/w pea amylose gel prepared by rapidly quenching the amylose solution to 20°C followed by maturation at this tempera-

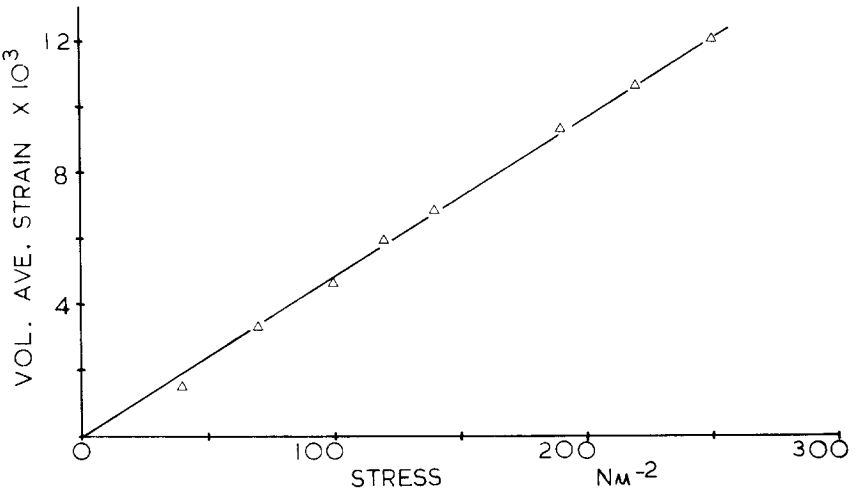

Fig. 1. Stress-strain plot for a 3% w/w pea amylose gel.

ture for 24 h. Upon application of a stress a virtually immediate response was observed which remained constant for 30 s. Upon removal of the stress the deformation was recovered within 30 s. In the modified Saunders and Ward tube the strain varies from a maximum at the wall of the tube to zero in the middle. Below a maximum strain of 0·18, corresponding to a volume-average strain of 0·12, there was a linear relationship between stress and strain; the gel behaved at these small deformations as a Hookean solid. It was therefore possible to assign a single value to the shear modulus. No significant change in shear modulus with time was detectable during the time period 24 to 48 h.

A characteristic feature of the precipitation of amylose from neutral aqueous solution was its thermal irreversibility. Even at elevated temperatures and pressures (120°C in an autoclave) the precipitated amylose was only partially soluble in water. Most biopolymer gels display some hysteresis in their melting and setting temperatures; with amylose this behaviour is exaggerated. Figure 2 shows the dependence of shear modulus on temperature for a 3·2% w/w amylose gel which was matured for 24 h at 20°C; 30 min was allowed at each temperature for the gel to reach thermal equilibrium. Even at 100°C the gel does not melt. In the temperature range 20°–80°C there was a slight decrease in

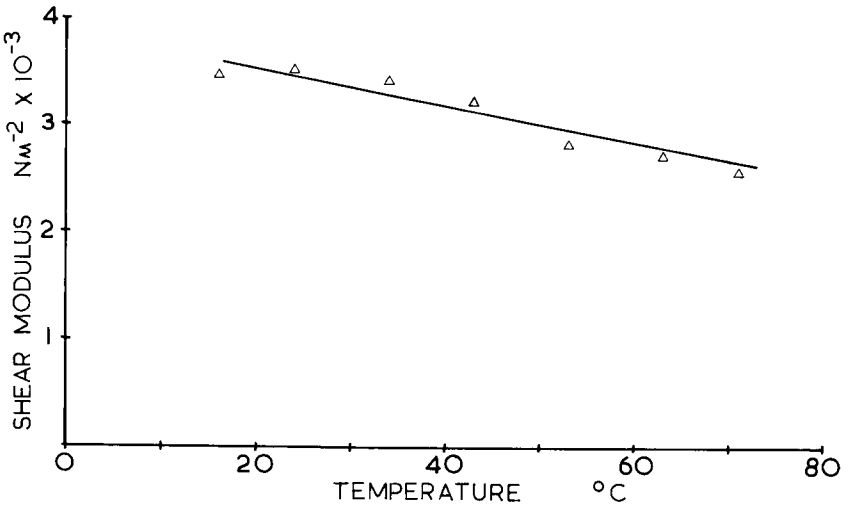

Fig. 2. Graph of shear modulus as a function of temperature for a 3·2% w/w amylose gel.

modulus with rising temperature. The stress–strain behaviour of the gel was linear throughout this temperature range.

The modified Saunders and Ward tube can be usefully employed to examine the stress–strain behaviour of amylose gels in the concentration range 2–3·5% w/w. Below this concentration range the gels are too weak while, above, syneresis causes problems even with the modified tube.

The remaining measurements of the shear modulus were determined using the pulse shearometer by measuring the velocity of a small amplitude shear wave, frequency 200 Hz, transmitted through the gel. As the measurement is complete within a fraction of a second any structure lasting longer than this time can contribute to the observed elasticity. Parallel determinations of the shear modulus of 3% w/w amylose gels using both rheological methods gave results which were within 10% of each other. It was inferred that the shear modulus of amylose gels is largely independent of frequency in this range. Figure 3 shows the dependence of shear modulus on amylose concentration, as a double logarithmic plot. The amylose starts to form a gel rather than a precipitate at concentrations of 1·5% w/w; above this concentration the modulus showed a seventh power dependence on concentration.

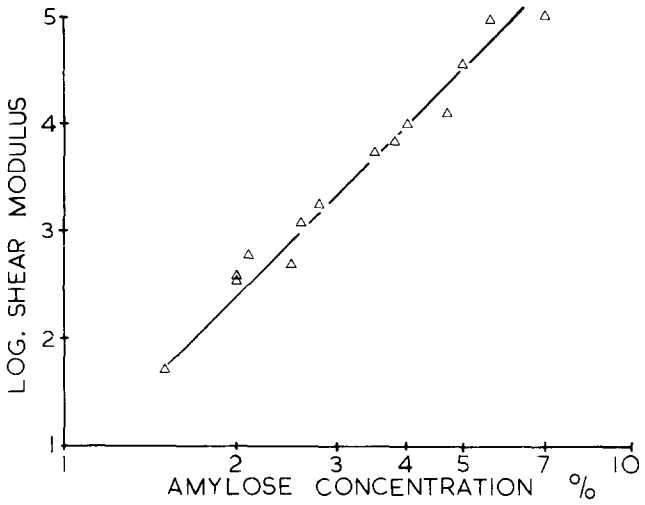

Fig. 3. Double logarithmic plot of shear modulus of pea amylose gels as a function of amylose concentration.

The high (seventh power) dependence of modulus on concentration for amylose gels is unusual; other biopolymer gels, e.g. gelatin (Ferry, 1948), show a square dependence. Most analyses of the relationship between modulus and concentration start from the theory of rubber elasticity. Unfortunately there are several objections to applying it to systems such as the amylose gel. The theory requires that the mechanism of energy storage of the gel is purely entropic and predicts a linear increase in modulus with absolute temperature. For the amylose gel, despite the fact that it cannot be melted at temperatures below 100°C, the modulus falls with increasing temperature, suggesting that there are energetic contributions to the observed elasticity. Additionally it has been suggested (Ring, 1983) that the amylose gel network consists of bundles of amylose chains, not individual network strands as is the case for a rubber-like material. Until these objections are adequately accounted for in any theory relating modulus to network structure, it is probably not profitable to analyse the concentration dependence of modulus for the amylose gel.

An indication that the amylose gel is a non-equilibrium system came from measurements of the shear modulus of amylose gels with different thermal histories. The shear modulus of a 3·4% w/w gel which was

rapidly quenched to 25°C and matured at this temperature for 24 h was 3500 N m^{-2}. That of a corresponding, slowly cooled gel (4 h to approach 25°C) was 5300 N m^{-2}, so there is a marked difference.

As the gel is not at equilibrium it is worthwhile to characterise it at the moment of preparation. Figure 4 shows the dependence of specific viscosity on concentration, as a double logarithmic plot for amylose in aqueous solution at 65°C. In dilute solution the average volume available for each molecule is sufficient for the bulk of the chains to act independently of the others. The viscosity of this type of solution arises from the hydrodynamic volume of the molecules and is proportional to amylose concentration. At a concentration of 1·5%, however, there

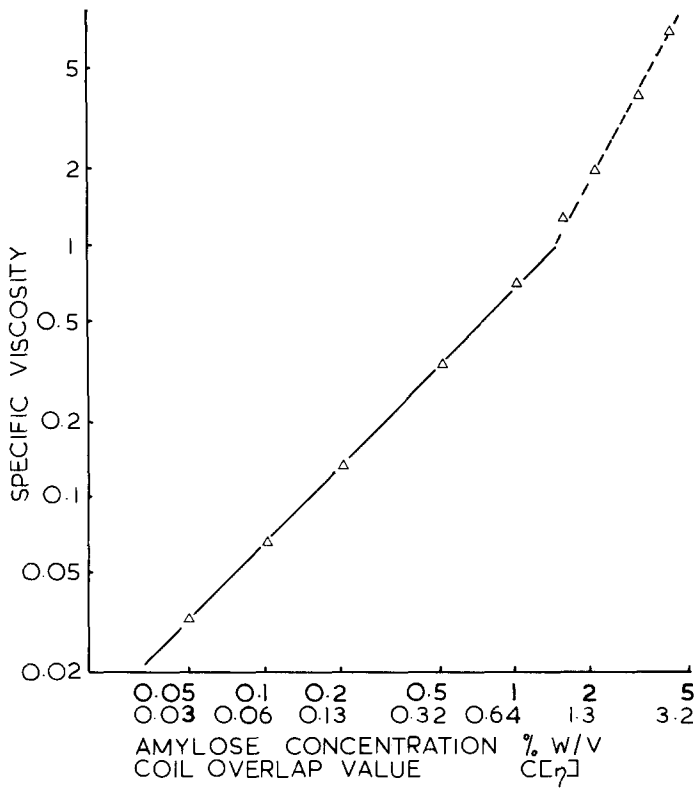

Fig. 4. Double logarithmic plot of specific viscosity of amylose as a function of pea amylose concentration (65°C).

was a marked change in viscous behaviour. Above this level the viscosity assumed an approximately second power dependence on concentration. The change occurs in a region where the volume not occupied by the molecules tumbling in the solution is reduced to the extent that parts of adjacent molecules must temporarily overlap. At these higher concentrations the overlap restricts the free movement of the molecules, so causing a greater increase in viscosity. The gelation of amylose therefore occurred during the precipitation of amylose from an entangled polymer network.

If the radius of gyration is calculated from the data from Fig. 4, the number of molecules that will occupy a given volume may be calculated. From this and the average molar mass a concentration at which overlap (i.e. interpenetration of the molecular volumes) will occur can be estimated (Morris & Ross-Murphy, 1981). This calculation gives a value for the onset of overlap of 2·4%, whereas Fig. 4 indicates an experimental value of approximately 1·5%. In view of the polydispersity of the sample used, as against the monodispersity assumed by the calculation, and the possibility that shear thinning may be more pronounced at concentrations above C^*, the agreement is acceptable.

If the coil overlap parameter ($C[\eta]$) is used as the abscissa of Fig. 4 it can be seen that the onset of overlap occurs at $C[\eta] = 1$. It is more usual for this value to be nearer to 4 for polysaccharides (Morris *et al.*, 1981), although the value obtained from Fig. 4 is closer to the theoretical result of $C[\eta] = 1{\cdot}5$ proposed by Morris & Ross-Murphy (1981).

The previous measurements were performed on the fraction of amylose leached from pea starch granules at 70°C. Starches from different sources differ in their gelling behaviour so it is useful to examine if these differences are due to the type of amylose which was solubilised during gelatinisation. Above the minimum gelling concentration all the amyloses tested showed a seventh power dependence of shear modulus on concentration. At a fixed gel concentration there were clear differences between the shear modulus values (Table 2) which range from 1×10^3 to $4 \times 10^3\,\mathrm{N\,m^{-2}}$ for 3·5% w/w gels. The amylose preparations differ in two main aspects, degree of average polymerisation and linearity. It was not possible entirely to separate these factors (see above). For the linear fractions of amylose with increasing molecular size there was a reduction in the shear modulus of the gel. This factor would substantially account for all the observed

TABLE 2
Shear Modulus of 3·5% w/w Amylose Gels

Fraction	*Leach temperature (°C)*	*Shear modulus ($N m^{-2}$)*
Pea	60-70	4×10^3
Pea	70-80	$2{\cdot}2 \times 10^3$
Pea	80-90	$1{\cdot}3 \times 10^3$
Potato	60-75	$1{\cdot}5 \times 10^3$
Potato	75-90	$1{\cdot}0 \times 10^3$
Maize	75	$3{\cdot}4 \times 10^3$

differences in shear modulus. It was not possible from the present results to identify if there was a small but significant effect due to chain branching. Whilst the higher molecular weight fractions may have a stronger driving force favouring precipitation, the present results indicate that the rate of diffusion of the macromolecule was an important determinant of gel structure under the conditions studied.

CONCLUSIONS

This study has examined some of the major factors which influence the shear modulus of pure amylose gels. Gelation only occurs from entangled solutions (above C^*). At small deformations amylose gels were almost purely elastic. The shear modulus of the gel showed a very high (seventh power) dependence on concentration. The botanical source of the starch and the temperature of extraction of the amylose have been shown to affect the degree of branching and molecular mass of amylose solubilised during gelatinisation. These factors also have an influence on the shear modulus of the amylose gel.

ACKNOWLEDGEMENT

One of us (S.G.R.) would like to thank Dr G. Stainsby, University of Leeds, for advice and encouragement.

REFERENCES

Adkins, G. K. & Greenwood, C. T. (1966). *Stärke* **18**, 213.

Banks, W. & Greenwood, C. T. (1975). *Starch and its components*, Edinburgh University Press.

Banks, W., Greenwood, C. T. & Sloss, J. (1969). *Carbohydr. Res.* **11**, 399.

Banks, W., Greenwood, C. T. & Muir, D. D. (1972). *Stärke* **23**, 118.

Buscall, R., Goodwin, J. W., Hawkins, M. W. & Ottewill, R. H. (1982). *J. Chem. Soc. Faraday Trans.* **78**, 2873.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). *Anal. Chem.* **28**, 350.

Ferry, J. D. (1948). *Adv. Protein Chem.* **4**, 1.

Hizukuri, S., Takeda, Y., Yasuda, M. & Suzuki, A. (1981). *Carbohydr. Res.* **94**, 205.

Marshall, T. T. (1974). *Adv. Carbohydr. Chem. Biochem.* **30**, 257.

Morris, E. R. & Ross-Murphy, S. B. (1981). *Techniques in carbohydrate metabolism* B310 1, Amsterdam, Elsevier/North Holland.

Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A. & Price, J. (1981). *Carbohydr. Polym.* **1**, 5.

Peat, S., Whelan, W. T. & Pirt, S. T. (1949). *Nature* **164**, 499.

Richardson, R. K., Robinson, R., Ross-Murphy, S. B. & Todd, S. (1982). *Polym. Bull.* **4**, 541.

Ring, S. G. (1983). Ph.D. Thesis, University of Leeds.

Ring, S. G. & Stainsby, G. (1982). *Fd Nutr. Sci.* **6**, 323.

Saunders, P. R. & Ward, A. G. (1954). *Proc. Ind. Int. Cong. on Rheology* 284.

Stainsby, G., Ring, S. G. & Chilvers, G. R. (1984). *J. Text. Stud.* (in press).

Van Olphen, H. (1956). *Clays and Clay Minerals* **4**, 204.

Whistler, R. L. & Wolfrom, M. L. (Eds) (1962). *Methods in carbohydrate chemistry*, New York and London, Academic Press.