THE NATURE OF AMYLOSE-LIPID INTERACTIONS AND THEIR EFFECTS ON THE RHEOLOGICAL PROPERTIES OF STARCH

by

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ABBREVIATIONS

AGU	anhydrous glucose unit
AMG	amyloglucosidase
CD	circular dichroism
CMC	critical micelle concentration
DMSO	dimethylsulphoxide
DP	degree of polymerisation
DSC	differential scanning calorimetry
FSD	full scale deflection
GLC	gas liquid chromatography
NMR	nuclear magnetic resonance

CONTENTS

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SUMMARY

The interaction of amylose with lipids and the formation of helical inclusion complexes were studied by viscometry, surface tension measurements, enzymic techniques, gas-liquid chromatography, X-ray crystallography and differential scanning calorimetry (DSC). The effect of fatty acids on the rheology of starch gels, and associated creep phenomena, were investigated by means of a viscoelastic analyser.

The viscosity number of amylose solutions at pH 12 decreased with increasing concentration of added fatty acids (12:0 to 22:0) and reached a constant value presumably because of saturation of the amylose helix. At saturation, the molar ratio of fatty acid/amylose was dependent on the chain length of the fatty acid; the longer the chain length the lower the saturation molar ratio. The same stoichiometric relationship was valid for the insoluble complexes prepared at pH 4·6 with fatty acids and their monoglycerides. The precipitated complexes were completely degraded to glucose by amyloglucosidase. It was demonstrated that the mass of lipid required for saturation of a given mass of amylose could be predicted from the chain length of the lipid.

It was found by DSC that there was a gradual increase in the dissociation temperature of the complexes which was related to the molecular weight of the fatty acids, but the dissociation enthalpy was essentially the same irrespective of the fatty acid chain length, suggesting a close structural similarity in the conformation. Complexes heated below their dissociation temperature showed structural

transitions, probably from 7 to 6 glucosyl residues per helix, which were dependent on the chain length and melting point of the fatty acids.

Viscosity measurements (at pH 12) with starch solutions containing added fatty acids, as well as creep and dynamic experiments with amylose and starch gels with added fatty acids, indicated that both the viscosity of solutions and the mechanical properties of the gels were dependent on the chain length and the concentration of the fatty acids, the concentration of the glucans and the ratio of amylopectin/amylose. Rheological tests provided evidence that amylopectin may interact with fatty acids, especially with those with a long chain. It was shown that structural failure of gels may be responsible for the inconsistent data reported in the literature.

1 INTRODUCTION

1.1 The starch granule

Starch is a major component of plants. It is found inside small cells, the plastids, in the form of particles that are insoluble in cold water, known as starch granules. They vary in size from 2 - 150 μm and maybe round, oval or irregular in shape, depending on the botanical origin. The granule is largely composed of two polyglucan molecules, amylose and amylopectin, organized into a radially anisotropic semicrystalline unit. The radial anisotropy is responsible for the birefringence exhibited when the granules are observed in polarised light. The semicrystalline nature is evident when the granule is studied by X-ray diffraction and reflects the presence of both ordered and amorphous regions. Starch occurs naturally in three crystalline modifications as revealed by X-ray crystallography and is designated as A(cereal), B(tuber) and C(smooth pea and various beans). Starch precipitated from solution, or complexed with iodine or various organic molecules, adopts the so called V-structure.

Starch granules swell in water. This swelling is reversible, until at a certain so called gelatinization temperature, material is leached from the granule and structural order is irreversibly lost as evidenced by the loss of birefringence. Gelatinisation is one of the most important properties of starch with regard to its use in the food industry. The property of starch to form viscous solutions and gels finds extensive applications in a wide range of food products.

1.1.1 The fine structure of amylose

Amylose is a mixture of completely linear chains of α -(1⟶4)-linked D-glucose units together with molecules possessing a very limited amount of branching. The branch points appear to be α-(1⟶6)-linkages and they occur to the extent of only one per several thousand glucose units. Undegraded amylose is a very large molecule with an average degree of polymerisation from 100 to 10000. Amylose can also exist in the A,B,C and V-structures (Whistler and Daniel, 1984). Amylose, which has been precipitated from aqueous solution yields an X-ray powder diffraction pattern consistent with that observed for the B-type crystalline form (Rundle, Daasch and French, 1944). It is generally believed that amylose, in the solid state, exists as a left handed helical polymer with six α-D-glucopyranosyl units per turn. Fig. 1 shows α -D-glucopyranosyl units in the minimum energy $^4C_1$ conformation.

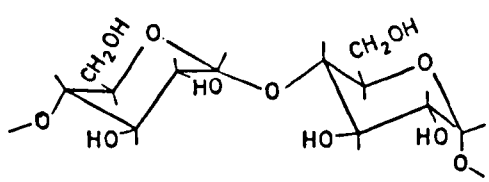

Fig. 1 The $^4C_1$ conformation of α-D-glucopyranosyl units (α- 1→4) linked (Morris, 1979).

1.1.2 The fine structure of amylopectin

Amylopectin is a highly branched macromolecule which consists of short chains of (1⟶4)-linked -α-D-glucose with (1⟶6)-α-linked

branches which correspond to an average length of unit-chain of 20 - 25 glucose units. Several models have been proposed for amylopectin to account for its physicochemical properties. The earliest of these included the laminated structure of Haworth, Hirst and Isherwood (1937) and the so called "herring bone" model of Staudinger and Huseman (1937). Later, Meyer and Bernfield (1940) proposed a randomly branched structure of amylopectin and recently a tassel-on-a-string model has been suggested by French (1972,1973) and Nikuni (1975). The models are characterised by having three distinct types of (1⟶4)-α-D-glucan chains, except for the herring-bone structure which has only two. By definition the A-chains are those that are linked to the rest of the molecule only through their reducing ends and the C-chain is the one that bears the reducing end group. Fig. 2 shows the various representations proposed for amylopectin molecule. A better picture of the amylopectin molecule is given by the "cluster" models proposed by a number of workers during the last fifteen years or so as shown in Fig. 3.

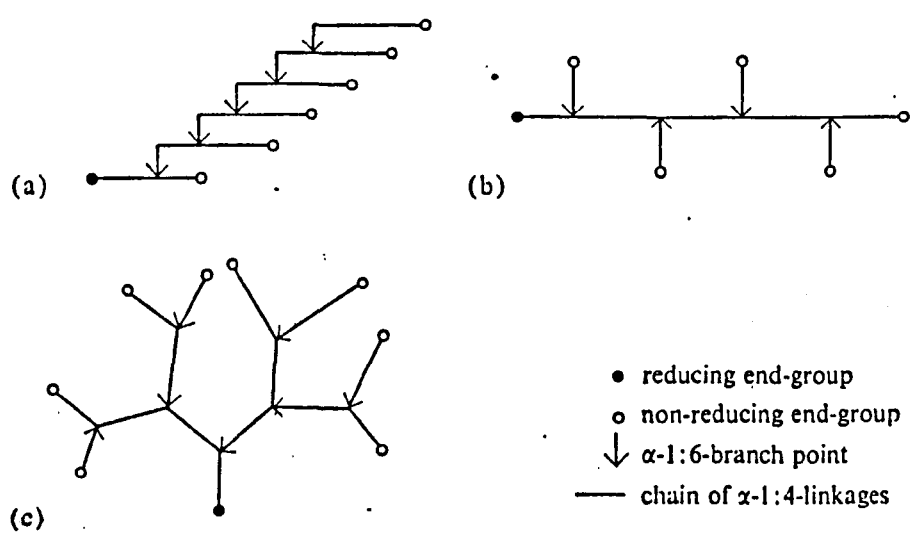

Fig. 2 Proposed models for amylopectin: (a) laminated structure; (b) herring-bone structure; (c) randomly-branched structure. (Banks and Greenwood, 1975)

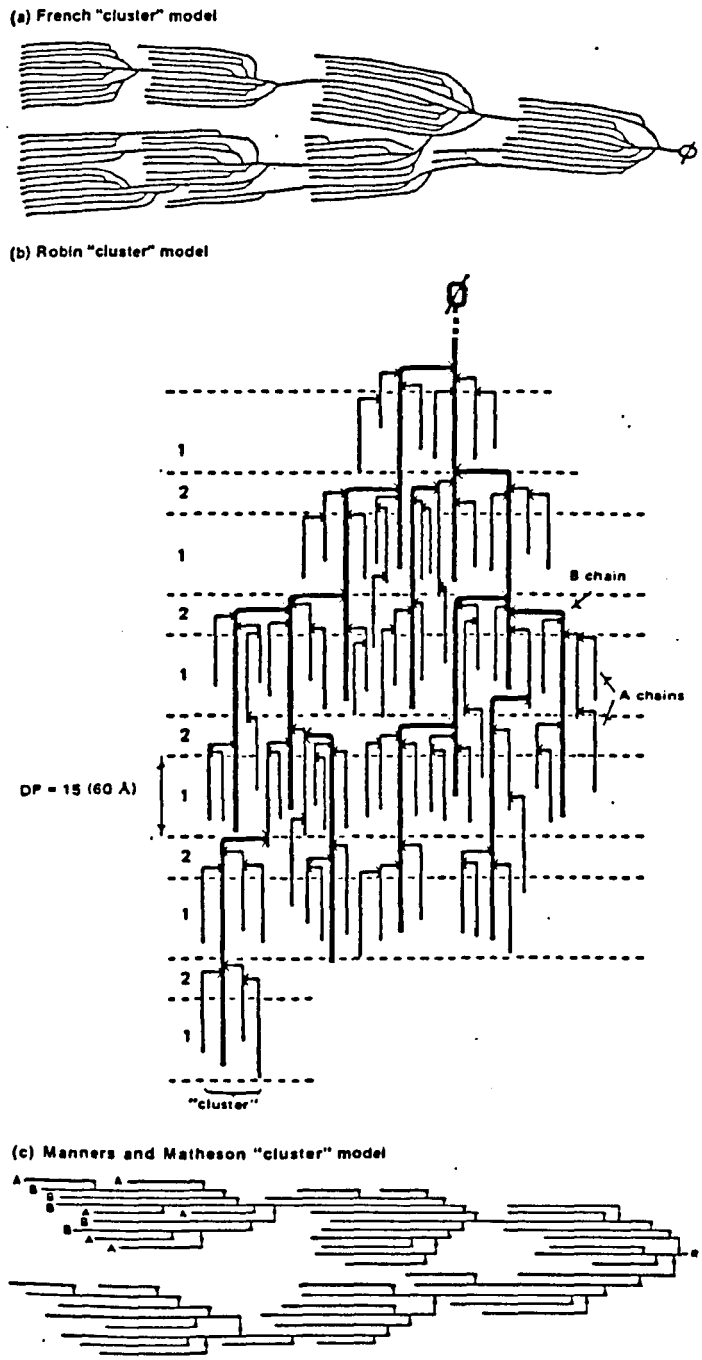

Fig. 3 Structural models for amylopectin, as proposed by French (a), Robin and coworkers (b) and Manners and Matheson (c). (Manners, 1985)

Our knowledge of the exact structure and physical size of amylopectin is still limited, although measurements indicate that it is one of the largest molecules in nature with a degree of polymerisation of many millions.

1.2 The conformation of amylose in solution

The conformation of amylose in solution has been in controversy for many years. Various researchers have proposed a range of models in aqueous solution which vary from helical (stiff, rod-like or loosely wound) through interrupted helix to random coil. Holló and Szejtli (1958) proposed the interrupted helix in which the amylose molecule is regarded as being composed of long helical sections each containing approximately 120 glucose residues with short regions of random coil. They based their conclusions on viscometric measurements of amylose and on its interaction with iodine. Rao and Foster (1963) proposed a deformed helix based on results from hydrodynamic studies. In a review article, Szejtli and Augustat (1966) concluded that amylose in aqueous solution adopts a random coil conformation which consists of linear segments of helical structure. The helical segments are built of 2-20 helical turns all containing 6 - 8 glucosyl residues (AGU). The same authors believe that the formation of hydrogen bonds between the neighbouring glucosyl units helps to stabilise the helix. Banks and Greenwood (1968a) postulated that amylose in aqueous solution behaves as a random coil. The authors based their views on data from light-scattering, viscometric and ultracentrifugal measurements. They interpreted their results using classical theories for polymer

conformation and hydrodynamic behaviour. The same authors (1968b) showed that amylose also assumes a random coil conformation in solvents such as formamide, DMSO and aqueous KOH, and summarised their conclusions in a review article (1971a) as follows:

Conformation of amylose in various solvent systems

Random coil: in water and neutral aqueous potassium chloride.

Expanded coil: in formamide, dimethylsulphoxide and aqueous alkali.

Helical: in neutral or alkaline solution in the presence of a complexing agent and in aqueous solution at pH 12 in the presence of 0·3M potassium chloride.

Kodama, Noda and Kamata (1978) reported that the conformation of amylose in aqueous solution depends on its molecular weight. When the molecular weight is outside the so-called "dissolving gap" it behaves as a random coil, when the molecular weight is within the "dissolving gap", it easily aggregates forming a rigid coil which corresponds to the B-type pattern of retrograded amylose. It has been suggested by several authors that the conformation of retrograded amylose is that of a double helix. These suggestions were based on results obtained by light scattering, sedimentation equilibrium techniques and fibre-diffraction studies. The term "dissolving gap" was introduced by Burchard (1963) who enzymatically synthesised water soluble amylose and found that amylose in a certain molecular weight region i.e. the "dissolving gap" ($6500 < M_w < 160000$) is insoluble in water and that amylose is soluble only if its molecular weight is outside this region. Hydrodynamic studies carried out by Senior and Hamori (1973) suggested that the amylose chain conformation is characterized by loose, extended helical regions which are interrupted

by short disordered regions. Hydrogen bonds between O(2) and O(3) of neighbouring residues are assumed to account for stabilisation. PfannemÜller and Ziegast (1981) employed circular dichroism to investigate the conformation of aqueous amylose. Although their results do not suggest the existence of a helix in solution, nevertheless they were unable to completely rule out this possibility. During retrogradation they did observe the formation of a double helix which they believe probably takes place simultaneously with precipitation. Jordan, Brant and Cesaro (1978) used the Monte Carlo method to generate theoretically representative sample chain conformations to be consistent with known experimental characteristics of amylose chains. They compared the graphic representation with the reported configurations. Their study produced a model which is a random coil possessing perceptible regions of left handed pseudo-helical backbone trajectory.

1.3 The concept of molecular interactions

The concept of intermolecular interactions was introduced for the first time by van der Waals in 1873 in an attempt to explain the deviation of real gases with regard to the ideal gas. In order to apply the ideal gas law equation to the behaviour of real gases he made allowance for the attractive and repulsive forces occurring between the molecules. Since then the dipole moment theory of Debye (1912) and the induced dipole theory of London (1930) have been the main driving forces of research in intermolecular interactions.

The types of bonds holding matter together may be classified into

two main groups: The strong or primary bonds which have an energy content of the order of 100 - 200 kcal/mol while the weak or secondary bonds have an energy content of the order 0·1 - 10 kcal/mol. Primary bonds include ionic or heteropolar bonds, covalent or homopolar bonds and metallic bonds. The main secondary bonds include Coulomb forces, hydrogen bonding forces, van der Waals forces, change transfer forces, exchange repulsion and hydrophobic interactions (Houwink, de Decker and van den Tempel, 1971; Tsuchida and Abe, 1982).

1.3.1 Coulomb forces (Electrostatic interactions)

The Coulomb force acts between two charged molecules: According to the point charge model the energy is described by Coulomb's law: $E_{es} = Z_A.Z_B.e^2/R$ where Z_A and Z_B are valencies of charges of A and B and R is the distance between A and B. This interaction is characterised by comparatively long-range and relatively strong forces, being about few tens of kcal/mol and differing from other interaction forces. In polyelectrolyte systems the theory is modified either to the point charge model, assuming a distribution of point charges on the polymer chain, or to the dipole-ion theory considering an ion pair as a dipole.

1.3.2 Hydrogen bonds

Hydrogen bonds are formed through interaction between electron-deficient hydrogen atoms and atoms of high electron density. There are two main types of hydrogen bonds: i) Those which connect atoms with

an electronegativity higher than that of hydrogen e.g. in $H_2O....H-OH$, and ii) those which connect atoms of lower electronegativity such as B - H - B bonds e.g. in boranes. The potential energy of the hydrogen bond is described by the Stockmayer equation, based on the electrostatic potential, the Lippincott-Schroeder equation, based on chemical bonds and the Scheraga equation, based on van der Waals and Coulomb interactions. The hydrogen bond energy is relatively low 3 - 6 kcal/mol.

1.3.3 Van der Waals forces

These are relatively short range forces between molecules with permanent dipoles or molecules with induced dipoles, i.e. they almost occur between all molecules. These interactions include dipole-dipole interactions, induced dipole interactions and dispersion energy. Orientation energy is due to dipole-dipole interactions. If molecules with a permanent dipole assume a random arrangement the average potential energy should be zero. Generally, however, the probability of assuming an arrangement of low energy is high and this results in attraction forces. Induced forces act between a polar molecule with a permanent dipole and a neutral molecule with an induced dipole due to the electric field of the permanent dipole. Dispersion energy is observed with certain molecules i.e. even in completely symmetrical molecules e.g. He and Ar, the electron distribution rapidly becomes asymmetric so that an instantaneous dipole moment appears. The formation of the transient dipole induces dipoles in other molecules leading to interactions among them. Van der Waals forces are at most

about 1 kcal/mol and relatively short range.

1.3.4 Charge-transfer interactions

These are attractive forces caused by charge-transfer between an electron donor and an electron acceptor.

1.3.5 Exchange repulsion

When two molecules come so close to each other that both electron clouds overlap, electron exchange takes place which gives rise to repulsive forces.

1.3.6 Hydrophobic interactions

These occur when hydrocarbons are dissolved in an aqueous medium. They essentially differ from the interaction forces mentioned before because they are not caused by direct cohesive forces between molecules but by the specific structure of water molecules. A water molecule forms four hydrogen bonds with neighbouring water molecules to give a cluster. When low molecular weight hydrocarbons are transferred from a non polar solvent to water, the changes of the thermodynamic parameters are $\Delta S<0$, $\Delta H<0$ and $\Delta F>0$. Thus, the low stability of hydrocarbons in water is due to the large decrease of entropy. In order to minimise the contact surface area of hydrophobic groups with water, which results in a decrease of the entropy, hydrophobic groups aggregate with each other. Factors determining hydrophobic interactions are, in

addition to the change of the structure of water mentioned before, van der Waals forces between hydrophobic groups and the restraint of the internal degree of freedom (mainly rotation) of hydrophobic groups caused by clustering of water molecules. Hydrophobic bonds are very important in stabilising micelles. In the latter, the polar part of the detergent or lipid molecule is exposed to the aqueous solvent and the non polar parts are aggregated together. Such molecules with both polar and non-polar parts are sometimes called amphiphilic (Richards, 1980). Similarly, attractive forces called "solvophobic interactions" exist when the interaction forces between solvent molecules are stronger than those between solvent and solute molecules.

1.3.7 The effect of solvent on molecular interactions

In an intermolecular interaction system it is difficult to investigate separately the effective secondary binding forces and it should be noted that the total force of interaction might not be the sum of the individual forces. Macromolecular chains normally interact in solution, except in the ideal state, the so-called theta state. This refers to the effect of the solvent on the solute i.e. the dissolved macromolecules. This is of great importance in the study of intermolecular interactions, because the solute-solvent interaction must be taken into account as well. Solvents can be classified in general as good, poor or ideal. Ideal solvents are also referred to as theta solvents (Tanford, 1961; Blanshard, 1982). In a good solvent attraction between polymer segments and solvent molecules is greater than that between polymer segments themselves. Conformations allowing

the maximum amount of solvent-solute interaction will be favoured, whereas for the solute-solute interactions, whose effect is to increase the free energy of the system, the conformations leading to such interactions are suppressed. In this instance, temperature has no effect on the structure and the solvent is called "athermal". In poor solvents, solute-solute interactions will be preferred and the requirement of minimum free energy will favour compact conformations and phase separation may occur. When for a given solvent there is an exact cancellation between attractive and repulsive forces then intermolecular interactions between the polymer molecules vanish at a certain temperature. This solvent is called theta solvent and the temperature is the theta temperature. The theta solvent is thermodynamically ideal and the polymer dissolved in it adopts its unperturbed dimensions. The methods employed for the study of intermolecular interactions vary depending on the nature of the interactions themselves. In general, the most common analytical methods involve conductivity, potentiometry, electrophoresis, viscometry, sedimentation, diffusion, centrifugation, light scattering, turbidimetry, I.R. spectroscopy, X-ray diffraction, optical and electron microscopy, chiroptics, NMR and Raman spectroscopy, thermal analysis and absorption spectrophotometry. The study of intermolecular interactions is of great importance for the understanding of biological functions. Many biopolymers participate in biological systems and, for example, the activity and high selectivity of enzymes are achieved by intermacromolecular interactions. In recent years extensive studies on the higher order structures of biopolymers have been performed. However a general rule for molecular interaction has not yet been established in biological systems because of the complexity

and specificity of biopolymers. In contrast to the biopolymers, synthetic polymers have rather simple structures. Thus, they are advantageously used for the detection and understanding of the fundamental phenomena of the complicated reactions occurring *in vivo*. For this reason, studies of the mechanism of complex formation between synthetic macromolecules might give important suggestions for the modes of function of polymer chains, which in turn could be used to explain the mechanism of molecular interactions in biopolymer systems (Flory, 1961).

1.4 Interactions of amylose with inorganic and organic molecules

It has been known for over a century and a half that amylose interacts with iodine to give a blue complex. Hanes (1937) was the first to suggest that the complex consists of a helical polysaccharide molecule with iodine occupying the helical cavity. Rundle and Baldwin (1943) presented the first experimental evidence for the existence of the helical structure of complexed amylose. They observed that solutions of starch-iodine show flow birefringence i.e. the complex molecules become positioned parallel to the direction of flow and thus absorb the light vector in the parallel direction more strongly than in the perpendicular direction. This could be attributed to the helical nature of the complex in which iodine occupies the cavity of the amylose molecules. Rundle and French (1943a,b) studied the optical properties of the precipitated amylose-iodine complex and reported that the crystals are highly birefringent. X-ray diffraction analysis revealed that, at least in the solid-state, the complex has a helical

structure; they were able to calculate the dimensions of the helix. They concluded that the helices formed an hexagonal packing. Schoch (1942), in his experiments on starch fractionation, employed butan-1-ol to precipitate a fraction of starch from a hot dispersion. He reported that the butanol precipitate showed birefringence under polarised light and that it formed spherulites. Bear (1942) and Rundle and Edwards (1943) studied with X-ray diffraction analysis the structure of dried butanol precipitate. They reported that the complex had a helical structure with the butanol occupying the helical cavity. The helices formed a packing whose unit cell was of the orthorhombic type. The complex could absorb many organic molecules and when it absorbed iodine vapour it contracted in volume. Schoch and Williams (1944) noticed that the iodine uptake of starch was markedly suppressed by the presence of fatty acids. They presumed that fatty acids could be absorbed on the linear chain component of starch i.e. amylose. Bear (1944) studied the interactions of starch with a number of organic molecules. The molecules he used included normal and branched alcohols, ketones, aromatic alcohols, n-valeric, n-butyric and α-bromopropionic acids. On account of his crystallographic findings he concluded that not only the straight chain molecules could form helical complexes with starch, but also the branched chain molecules could be accommodated inside the helices. In this case the diameter of the helix should be larger than that estimated for the iodine and normal alcohol complexes but he was unable to determine this change in the dimensions of the helix. Following Schoch's example, Whistler and Hilbert (1945) employed nitroparaffins to separate amylose from amylopectin. They endorsed the view that the formation of amylose complexes with organic

molecules involved hydrogen bonding as the binding force. They based their view on the fact that amylose easily complexed with compounds which possess nitro-, ester, ketone, mercapto-, carboxylic groups and cyclic nitrogen (=N-) e.g. pyridine. They postulated that although the amylose-nitroparaffin complexes have identical crystal structures, nevertheless they differ from the structure of the butanol complex. Mikus, Hixon and Rundle (1946) proved conclusively that fatty acids interact with amylose to form helical complexes and investigated their structure by X-ray analysis. They concluded that the structure is identical to that of iodine and butanol complexes, the helices being packed in an orthorhombic unit. They also suggested that dipole interactions may be responsible for the stability of the complexes. Studies of the action of monoglycerides (Strandine et al., 1951) and polyoxyethylene monostearate (Lord, 1950) have shown that these surface active agents inhibit the swelling of the starch granules and cause starch gels containing them to be soft and easily broken. A fairly systematic study of a series of amylose complexes with surfactants was made by Osman, Leith and Flês (1961). They used mono-, di- and tri-glycerides in their studies. Their investigation included the effect of surfactants on the iodine affinity of amylose and the X-ray patterns of the complexes in the solid state. The di- and tri-glycerides did not complex or complexed very little with amylose, whereas lecithin complexed almost as efficiently as the majority of monoglycerides. The X-ray diffraction patterns of the complexes were very similar to those of amylose-fatty acid complexes reported by Mikus et al. (1946).

So far, it was believed that it is necessary for a balance to

exist between the hydrophobic and hydrophilic properties of the complexing agent, as was assumed by Schoch (1942), and that only polar compounds could form complexes with amylose. Based on the ability of non-polar compounds to precipitate amylose, French, Pulley and Whelan (1963) found that a large number of hydrocarbons, among them CCl_4, $CHCl_3$ and benzene complexed with amylose whereas others like toluene, carbon disulphide and paraffin did not form complexes. X-ray analysis of the dried complexes revealed that their structure is of the known helical type, the only difference being for at least the 1,1,2,2-tetrabromoethane complex whose helical configuration seems to contain approximately 7-glucosyl units per turn, rather than the 6-unit helix characteristic of the normal aliphatic alcohols. Bumb and Zaslow (1967) examined by X-ray diffraction analysis a number of halogen-substituted hydrocarbons complexed with amylose. They reported that the conformation of these compexes is essentially the same as that of amylose-alcohol complexes. However, the dimensions of the unit cell of the wet precipitated complexes were different from those of the same complexes after drying. They suggested that the wet complex unit cell has eight glucosyl units per helix turn, whereas the dried one has seven glucosyl units per turn. French and Zobel (1967) investigated the amylose-DMSO complex in the form of orientated fibres by means of X-ray diffraction analysis. They reported that the complex occurs in a six glucosyl unit helix with alternate "up" and "down" chains packed in a square array i.e. pseudotetragonal. Jackobs, Bumb and Zaslow (1968) extended the study of the structure of amylose-KBr complex which was initiated by Senti and Witnauer (1952). They employed three dimensional crystallographic analysis and based their conclusions on

stereochemical considerations. They reported that the complex forms a left handed helix with 6-glucosyl units (AGU) per turn, whose unit cell is orthorhombic. Bittiger and Husemann (1968) investigated the influence of various solvents on the molecular and super molecular structures of amylose complexes by means of electron microscopy. They employed phosphorylase-synthesised amylose with a degree of polymerisation (DP) from 2000 to 12000. With methanol, amylose did not form a complex. With ethanol, amylose formed hexagonal crystals with a thickness of 4 - 5 nm. With butan-1-ol, orthogonal crystals were formed with a thickness of 7 - 8 nm. The crystal formed with cyclohexanol had a thickness of 8 - 10 nm. However, on complexing with iodine, depending on the concentration of the amylose, only rods and fibrils were formed, but no crystals. They presumed the following process should take place during crystallisation: i) the transition of the coiled molecules to helical chains ii) the nucleation, i.e. the formation of primary nuclei from the helices and iii) the growth of nuclei to larger crystals. In contrast to the alcohol complexes, the nuclei of the iodine must be very small. They attempted to extend their ideas regarding solid state to solutions. They suggested that folded helices exist in solution with the folding length depending on the type of the complexing agent. Kuge and Takeo (1968) attempted to form complexes of amylose with organic compounds by means of gas solid chromatography. They packed a gas chromatographic column with a mixture of amylose and an inert solid (Teflon powder). The complexing agents in the vapour state were transported through the column, by means of helium which was used as the carrier gas at $80^{o}C$. Their results did not entirely agree with those of French *et al.* (1963). They concluded that non-polar compounds

scarcely interact with solid amylose and that the complexing ability increases with increasing degree of polarity. From their results they presumed that complexation would be mainly due to dipolar-cooperative interaction.

Banks and Greenwood (1971b) studied the amylose interactions with iodine and butan-1-ol in neutral aqueous solutions. They showed that the addition of either iodine or butanol to amylose solutions causes a decrease in the viscosity number and hence a reduction in the hydrodynamic volume of the macromolecule. The minimum viscosity number occurs when the expected stoichiometric amount of reagent necessary to form the complex has been added. Simpson, Dintzis and Taylor (1972) investigated by X-ray analysis, the conformation of amylose-DMSO complex, in the solid state. They concluded that the complex has an orthogonal unit cell with a sevenfold helical structure. On the other hand, Winter and Sarko (1974) reported that the amylose-DMSO complex crystallises in a pseudotetragonal unit cell with sixfold helical structure. Osman-Ismail and Solms (1973) studied the formation of starch and amylose complexes with flavour substances, among them, pinene, limonene and menthol, using chemical methods. They claimed that potato starch showed the best complexing ability followed by rice starch, maize starch and potato amylose. On account of their quantitative studies, they suggested that not only amylose but amylopectin as well forms complexes to a certain extent. Nakatani et al. (1977) studied the interaction of amylose with a hydrophobic fluorescent probe (2-p-toluidinyl naphthalene-6-sulphonate, TNS). In the presence of amylose the fluorescence of TNS was enhanced. They investigated

the dependence of relative TNS fluorescence intensity per glucose unit on the chain length of the macromolecule. The stoichiometry of the complex was determined by fluorescence titration. The fluorescence intensity of TNS in the presence of amylose was decreased by the action of glucoamylase and increased with increasing ionic strength. Amylopectin enhanced TNS fluorescence rather more strongly than amylose at the same concentration. They postulated that the enhancement in fluorescence indicated the presence of a hydrophobic environment.

The crystal structure of amylose-KOH complex was investigated by Sarko and Biloski (1980). X-ray diffraction analysis showed that the complex crystallises in an orthorhombic unit-cell and the conformation of the amylose chain is a distorted left handed helix with 6-AGU per turn. The structure is extensively hydrogen bonded although largely through water molecules which account for its ready water solubility. Pfannemüller and Ziegast (1981) studied the amylose-iodine complex in solution by Raman spectroscopy and circular dichroism (CD). The Raman spectra did not give any information about the nature of the complex. The results from CD could be explained by assuming that a transition occurs from a loosely wound helical chain to a well organised helix when iodine is present in a dilute solution of short chain amylose. When the chains are longer an additional effect from chain folding may take place. Hayashi, Kinoshita and Kotani (1983) investigated the phase change of amylose-butanol complex in solution of 10% DMSO as a function of temperature. The phase change was determined by turbidometric, fluorescent depolarisation and viscometric measurements. The results suggested that, when the solution cools from a high temperature,

amylose molecules change in conformation from a random coil to an interrupted helix and they separate into two phases. Coacervate particles resulting from the phase separation coalesce with each other to yield precipitates.

Jane and Robyt (1984) studied the structure of amylose complexes with alcohols by enzymic degradation. The authors used a series of α-amylases to hydrolyse the complexes and then fractionated the resultant fragments by gel filtration. According to their findings, the complexes consist of helices of six AGU per turn with a folding length of 10 nm for the butanol complex, seven AGU per turn with a folding length of 10 nm for the tert-butanol and eight AGU per turn with a folding length of 12 nm for the 1-naphthol. Jane, Robyt and Huang (1985) studied the conformation of amylose complexes in solution by ^{13}C-NMR spectroscopy. As complexing agents they used DMSO, potassium triiodide and a number of alcohols. The addition of the complexing agents to amylose solutions caused a downfield change in the chemical shifts of carbons 1 and 4 of the D-glucopyranose residues as detected by ^{13}C-NMR spectra. This shift has been interpreted as a change in the torsion angle of the α-1 $\rightarrow$ 4 glucosidic linkage due to the formation of a helical structure. Differences in the ratio of the downfield shifts of carbons 1 and 4 could distinguish an extended helix from a compact one.

The world literature on amylose interactions with various compounds is vast and the present account of the subject is by no means exhaustive. Nevertheless, every effort has been made to ensure that the most

important and original papers have been reviewed.

1.5 Amylose-lipid complexes in starch granules

It has been known for some time that non-waxy cereal starches contain lipids unlike starches from tubers and beans (Morrison, 1978). According to Morrison, the starch lipids should be distinguished in relation to their location. The lipids inside the starch granule should be called starch internal lipids. These lipids may be involved in starch biosynthesis and they are not readily extracted with solvents. The starch internal lipids are mostly composed by lysophospholipids and free fatty acids. Starch surface lipids are mostly monoacyl lipids originating from the non-starch lipid fraction which has been adsorbed onto the surface of the granules (Morrison and Milligan, 1982). These monoacyl lipids are all capable of forming inclusion complexes with amylose (Morrison, 1985). Nevertheless, evidence for the existence of amylose-lipid complexes, in native starch granules, is inconclusive. Evidence on the state of lipids in starch granules originates from DSC and X-ray diffraction measurements. In the following sections, the formation of amylose-lipid complexes will be discussed in detail.

1.5.1 The complexes of fatty acids with amylose

Relatively little work has been done on amylose-fatty acid interactions in comparison with the work done on the iodine complex. This is probably due to the difficulty in obtaining the fatty acids in a form suitable for interaction with amylose. In section 1.4 it was

mentioned that Mikus et al. (1946) were the first to prove the helical structure of amylose-fatty acid complexes in the solid state. They prepared the complexes by autoclaving starch pastes to disintegrate granular structure, then the fatty acids were added dissolved in a suitable solvent such as dioxane or methanol followed by slow cooling to room temperature under continuous stirring. The complex was precipitated as a microcrystalline floc. The precipitates were examined by X-rays either wet or after drying at 100°C over P_2O_5 under vacuum. It was found that the diameter of the helix of the wet sample was 1·37 nm and that of the dried sample 1·30 nm. Potentiometric titration of amylose-fatty acid complexes with iodine was employed in an attempt to determine the amount of iodine bound by the complex. The authors were able to show that there was a stoichiometric relationship between the fatty acid chain length and the number of glucosyl units participating in helix formation. In another experiment, it was found that iodine vapours were taken up quantitatively by the amylose-palmitic acid complex at 78°C. Subsequent washing of the complex with CCl_4 removed 78% of the original amount of the acid present in the complex, thus leading to the conclusion that iodine and fatty acid occupy the same position in the complex. Amylopectin and retrograded amylose could not complex with fatty acids, but amylose already complexed with fatty acid after defatting by solvent extraction could regain part of the acid extracted by reintroducing it into the amylose by means of a suitable solvent e.g. CCl_4. Methanol was much more effective than CCl_4 in extracting fatty acids from the complexes, and this could be attributed to the small size of the molecule which can enter the cavity of the helix and compete with the acid in the helix.

Takeo, Tokumura and Kuge (1973) investigated the structure of amylose-fatty acid complexes by X-ray diffraction analysis both in the wet and the dry state in order to clarify the effect of the molecular size of fatty acids on the dimensions of the amylose helix. They prepared the complexes by dissolving amylose in NaOH. The solution was neutralised with HCl, heated to boiling and excess of fatty acid was added. The mixture was placed in a Dewar flask containing boiling water and left to cool slowly to room temperature. The precipitate was removed by centrifugation. Samples were prepared either as wet or dry precipitates and monobasic, dibasic and unsaturated fatty acids were used. X-ray diffraction analysis revealed that both 6 and 7-glucosyl units per turn helical conformations were present and that the conformation depended on the chain length of the acid molecule. The orthorhombic unit cell was proposed for the 7-fold helix of the wet complexes. The dibasic acids formed complexes with either the hexagonal or the orthorhombic type of unit cell. The n-propionic complex showed various patterns depending on the amount of n-propionic acid added to amylose solution. It could form simultaneously two types of crystals with 6 and 7-glucosyl units or crystals of either type. Szejtli and Banky-Elöd (1975), investigated the stability of unsaturated fatty acids complexed with amylose, against oxidation. They prepared the complexes in two ways: i) amylose was dissolved in KOH, the solution was heated at 60^{o}C, neutralised with HCl and the fatty acid added in the form of ethanolic solution. The mixture was cooled to room temperature and the precipitate recovered and dried at 100^{o}C over P_2O_5 under vacuum. ii) to the alkaline solution, the alcoholic solution of fatty acid was added at room temperature and then neutralised with HCl.

The precipitate was treated as mentioned before. By washing the complex with dioxane or petroleum ether only the fatty acid adsorbed was removed. The fatty acid could be removed, with ethanol, from the complex. The complexed unsaturated fatty acids were practically completely protected against oxidation even in an atmosphere of pure oxygen. Szejtli and Banky-Elöd (1978) determined the dissociation constants of amylose-fatty acid complexes. The method they employed was based on photometric titration of the blue complex formed by the interaction of iodine and amylose. The amount of iodine bound to the complex depended on the concentration of guest molecules in the mixture. Readiness of the guest molecule to form amylose compexes was characterised by the dissociation constant. The fatty acids used were stearic, oleic and elaidic. Their dissociation constants ranged from 10^{-6} to 10^{-8}. The most stable complex was that of stearic acid. Oleic acid showed lower complexing ability than elaidic acid perhaps due to its cis-double bond. Kugimiya, Donovan and Wong (1980) studied the amylose-palmitic acid and starch lysolecithin complexes using differential scanning calorimetry (DSC). Amylose was dissolved in NaOH and mixed with palmitic acid dissolved in ethanol. The mixture was neutralised with HCl and heated at $70^{o}C$ for 4 hours. After cooling the recovered precipitate was dried in air at room temperature. On heating, in a differential scanning calorimeter, the complex showed a bimodal endothermic transition with peaks at $97^{o}C$ and $104^{o}C$. On cooling and reheating, it showed one peak at $97^{o}C$. On heating for a third time the endothermic peak observed the second time of heating, appeared again. X-ray diffraction analysis confirmed that it was an amylose-palmitic acid complex. When potato starch was heated with water and

lysolecithin in a DSC calorimeter, then apart from the characteristic gelatinisation endotherm, a second endotherm was shown near 100°C which was attributed to the formation of a starch-lysolecithin complex. When the same experiment was repeated with palmitic acid instead of lysolecithin, the second endotherm was not observed. The authors suggested that the palmitic acid being insoluble in water, in contrast to lysolecithin, did not participate in complex formation. Bhide, Karve and Kale (1981) studied the competing action of the sodium salts of dodecyl sulphate (SDS), stearic, palmitic and myristic acids on the amylose-iodine complex in solution. They observed the reduction in absorbance of the solution, of the iodine complex, accompanied by the blue shift in the absorption spectrum. The results suggested that, when iodine is added to a solution of amylose complex, the iodine displaces part of the guest molecule in the helix. Perturbation studies of the complexes using sodium thiosulphate, to extract the iodine from the helix, revealed that the transition from helix to coil is rather sluggish and that hydrophobic interactions should play an important role in the stability of the complexes. In other words, amylose retains its helical structure for quite a long time after the removal of the guest molecule, i.e. it displays a "memory" effect.

Bulpin, Welsh and Morris (1982) studied the amylose-sodium palmitate complex in solution, using NMR spectroscopy and optical rotation measurements. The samples were prepared by dissolving amylose in DMSO, diluting with water and adding the soap at 45°C. The NMR spectra showed a dramatic reduction in the amylose signal intensity at 45°C on addition of the soap. On heating the solution at 90°C, the

signal almost restored, suggesting that the complex is thermoreversible. Chiroptical measurements showed that the addition of the soap to an amylose solution caused a change in the optical activity at 45°C. By heating the solution to 90°C the formation and melting of the complex could be monitored by optical rotation. DSC measurements confirmed the thermoreversibility of the complex. The same authors suggested that the complex may be a helix with a right handed V-structure. Stute and Konieczny-Janda (1983) investigated the thermal properties of amylose-fatty acid complexes by DSC. The complexes were prepared by dissolving the amylose in NaOH, neutralising with HCl and adding the fatty acid to the solution at 72°C. The mixtures were allowed to cool to room temperature and the precipitates recovered by centrifugation and freeze-dried. The complexes, on heating in a DSC calorimeter, showed a shift in the dissociation temperature of the complex, depending on the fatty acid chain length, being the lowest for capric and the highest for stearic. The same shift was observed for the unsaturated fatty acids depending on the number of the double bonds, the lowest being that of linolenic ($C_{18:3}$) with oleic ($C_{18:1}$) being the highest, which in turn was lower than that of stearic ($C_{18:0}$). In another series of experiments they investigated the thermal stability of the amylose-lauric acid complex. After heating the complex beyond its dissociation temperature (135°C), it was kept for long periods of time (16h) at 90°C. Then it was cooled to room temperature and during heating in a DSC calorimeter the endothermic peak was shifted from its original temperature to a higher one. Furthermore, another endothermic peak appeared at 150°C which was attributed to crystalline uncomplexed amylose.

Bulpin, Cutler and Lips (1986) investigated the physical properties of amylose-sodium myristate complex in solution. Optical rotation measurements showed a large change in amylose conformation on the addition of fatty acid sodium salts. This change was also seen on the addition of fatty acid soaps containing 12 to 18 carbon atoms, but it was not very pronounced for the shorter fatty acid chains. The complex was thermally reversible which was also confirmed by ^{13}C and ^{23}Na-NMR spectroscopy. Surface tension measurements demonstrated that the presence of amylose prevents expression of the surface active properties of the sodium myristate, consistent with its inclusion within the central cavity of the amylose helix. Comparison of the surface tension results with those obtained from the optical rotation measurements suggested that changes in surface tension of the complex solution occur only after the amylose helix was saturated with complexed sodium myristate.

1.5.2 Complexes of amylose with monoglycerides and surfactants

Amylose complexes with surfactants have been studied more extensively than complexes with fatty acids because of their importance with regard to the texture of bread and the retardation of staling (Lord, 1950). Osman *et al.* (1961) studied the iodine affinity of amylose in the presence of surfactants. They prepared the complexes by dissolving amylose (heavily contaminated with amylopectin) in KOH, then neutralising it with HCl and keeping the solution at 65°C. A quantity of solid surfactant was added and the mixture kept for 3h at 65°C with stirring. After slow cooling to room temperature the precipitate was

removed by centrifugation, washed with CCl_4 amd dried under vacuum at 65°C. Potentiometric measurements of the iodine affinity showed that monoglycerides, lecithin and monoesters of fatty acids complex with amylose but not the di- and tri-glycerides. Complex formation was confirmed by X-ray analysis. Krog and Nybo-Jensen (1970) studied the effect of the physical state of glycerol-monostearate on its ability to complex with amylose. Complexes were prepared by mixing amylose solutions with dispersions of monostearate in water or with monostearate in powder form, followed by spectrophotometric measurement of iodine affinity. They used the term "complexing index" defined as

$$\frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

as a measure of the degree of complex formation. According to their results it was shown that the preparations which involved hydration of the monoglyceride had the higher complexing ability and comparatively showed the best antifirming effect in bread. Lagendijk and Pennings (1970) investigated the complex formation of amylose and amylopectin with a series of monoglycerides ranging from monolaurate to monoarachidate as well as monooleate and monolinoleate. The complexes were prepared by mixing amylose or amylopectin and monoglyceride in water at 80°C and keeping the mixture for 4h under stirring. The results suggested that for amylose, the monopalmitate has the greatest complexing ability and this ability decreases with increasing degree of unsaturation. Amylopectin binds monoglycerides to a lesser degree than amylose and the longer the chain of monoglyceride the more it is bound by amylopectin. Krog (1971), investigated the complexing ability of amylose with monoglycerides (C_8 to C_{22}) and a number of commercial

emulsifiers. He employed two methods. In the first method, to a mixture of dissolved amylose and emulsifier in aqueous dispersion, potassium iodide was added and the final mixture was potentiometrically titrated with standard iodine solution. In the second method, the emulsifier in dispersion was added to amylose in solution and the precipitate was removed by centrifugation. The supernatant was titrated amperometrically with standard iodine solution. The results suggested that monomyristin has the highest amylose complexing index and monobehenin ($C_{22:0}$) the lowest, with monocaprylin being nil. The results from the second method, although consistent, were a little higher than those from the first method. Van Lokhuysen and Blakenstijn (1974) studied the interaction of starches with commercial emulsifiers. They prepared the complexes by adding starch to a suspension of emulsifier in water and by heating samples of the mixture at 30°C and at 90°C. The amount of the emulsifier bound to starch was determined indirectly by extracting the free emulsifier with diethyl ether and determining it by GLC. The results suggested that various starches bind different quantities of emulsifier and the higher the temperature of the interaction the higher the amount of the emulsifier bound to a specific starch. Kim and Robinson (1979) studied the effect of surfactants on amylose by a variety of methods. They prepared their samples by dissolving the amylose in KOH, neutralising with HCl and adding the complexing agent at room temperature under stirring. Viscosity measurements showed that the intrinsic viscosity of amylose decreased slightly. It was assumed that the amylose in aqueous salt solution has helical conformation and the surfactants enter into the already existing helical cavity of amylose molecules. β-amylolysis was inhibited due

to the presence of the complexes and potentiometric titration with iodine showed that the amylose helices were partly filled with surfactant molecules. Carlson et al. (1979) investigated the amylose-monostearate complex by means of Raman spectroscopy and X-ray analysis. The complex was prepared by mixing amylose in aqueous solution with monostearin in an ultrasonic bath for 1h at 60°C. The precipitate was recovered by centrifugation and examined in the wet state. The results suggested that the hydrocarbon chain inside the helix is extended and ordered in a way similar to that in the crystalline dry state, and the geometry of the amylose V-form of the complex corresponds to nearly three turns of the helix per hydrocarbon chain. The authors claimed that a part of the monoglyceride molecule, i.e. the polar group, remains outside the helix due to its bulkiness since it cannot be accommodated in the narrow cavity of the helix. Hoover and Hadziyev (1981,a) investigated the increased stability of the starch granule when it interacts with monoglycerides or fatty acids to form clathrates. They examined a series of fatty acid potassium salts and monoglycerides (with carbon atoms C_8 - C_{18}) using X-ray diffraction, viscosity, turbidimetry and differential scanning calorimetry techniques. The highest stability was achieved with monomyristin, hence such granules had the lowest solubility, swelling power, water binding capacity and viscosity based on leached amylose. The same authors (1981,b) assessed the ability of two commercial emulsifiers to complex with amylose in a simulated potato starch process for preparation of mash potato. They employed scanning electron microscopy (SEM), infrared spectroscopy, iodine titration and X-ray diffraction. The emulsifier based on monopalmitate (90%) proved to be more efficient to complex with amylose

compared with the one based on monostearate. Kugimiya and Donovan (1981) investigated the possibility of devising a method for the determination of amylose content of starches by DSC based on the formation and melting of the amylose-lysolecithin complex. The amylose content of a starch was calculated from the enthalpy of melting of its lysolecithin complex. Amylose contents of starches from various sources were in good agreement with values obtained by iodine binding, although considerably higher values were found for maize and wheat starches.

Ghiasi, Hoseney and Varriano-Marston (1982,a) studied the effect of surfactants and commercial monoglycerides on the gelatinisation of wheat starch. Both inhibited the swelling and solubility of the starch up to 85°C. Iodine affinity values, β-amylolysis limits and gel filtration showed that amylose was leached from untreated starch at temperatures below 95°C. The surfactants and monoglycerides effectively stopped the leaching of amylose. The same authors (1982,b) found that X-ray diffraction patterns of the insoluble residues of wheat starch heated to 60°, 70° or 80°C with monoglycerides or surfactants show an amylose-surfactant complex. The formation of this complex suggests that, at a temperature below 80°C the surfactant can enter the starch granule and complex with the amylose. At 95°C, the complex was no longer observed. Dissociation of the complex at 95°C was shown by an increase in the starch-iodine absorbance after the complex was heated at 95°C in the presence of iodine, followed by cooling. It was hypothesised that iodine and surfactant compete for the same binding site. Riisom, Krog and Eriksen (1984) studied the ability of cis-

and trans-unsaturated monoglycerides to complex with amylose. They found that the physical state of the monoglyceride plays an important role in their complexing capacity. When used in the form of liposomes unsaturated monoglycerides complex equally well as the saturated monoglycerides e.g. monopalmitate. When dissolved in DMSO unsaturated monoglycerides are 20 - 30% more effective as complexing agents than saturated monoglycerides. Eliasson and Krog (1985) investigated, by DSC, the thermal properties of amylose complexeswith monoglycerides differing in fatty acid chain length and unsaturation. They found, that thermal stability increases with increasing chain length and decreases with increasing unsaturation. Biliaderis et al. (1985) studied the thermal behaviour of amylose complexes with monopalmitin, lysolecithin and lauric acid in various water concentrations, using DSC. The lysolecithin complex showed an endothermic peak at all water contents. The monopalmitin and lauric complexes showed two peaks at low and intermediate water contents. They explained the observed phenomena on the assumption that a new structural order develops upon heating, after partial melting of the initial crystalline structure of the complex. They concluded that the overall behaviour of the complexes is typical of that of semicrystalline synthetic polymers.

1.6 Rheology of polysaccharides - Fundamental aspects

Rheology has been defined as a science devoted to the study of deformation and flow of liquids and solids (Whorlow, 1980). The fundamental operation in all rheological testing is to apply a force to the sample under investigation and measure its deformation, or

equivalently, to apply a deformation and measure the resistance. Before dealing with the behaviour of real polymer solutions and gels, it is appropriate to define two idealised extremes: Perfect solids are those which respond to a force by deforming and on removal of the force by returning to their original shape. This type of response is termed elastic. Perfect liquids are those which deform and continue to deform as long as the force is present. This type of response is termed viscous. Materials do not always fall readily into one or other of the above mentioned categories. An additional parameter is needed to describe material response more fully and that is time. As a general rule the faster the deformation the closer the response is to being elastic, the slower the deformation, the closer the response is to being viscous. Concentrated solutions and gels of polysaccharides, and food products in which they are incorporated, have both solid like and liquid like character i.e. they are viscoelastic.

1.6.1 Viscosity and molecular structure

The steady shear viscosity is perhaps the only rheological property of concentrated polymer solutions which can be investigated with any confidence. Experimental results for elastic properties and extensional flows are of limited range and their accuracy is open to some doubt. The viscosity of liquids is related to the structure of molecules of which they are composed. The effect of molecular structure on viscosity can be understood if there is knowledge of the effect of molecular structure on well defined motions of molecules in liquids (Graessley, 1974). Three groups of factors can be distinguished as affecting the

structure and macroscopic behaviour of linear polymer systems (Ziabicki, 1976): i) Intramolecular interactions: the result of these interactions is mainly the effect of entropy mechanisms on the elasticity of a chain (Treloar, 1975). Existence of rotational barriers leads to some energetic contributions to elasticity and to the dissipation of energy when the chain is subjected to deformation ("internal viscosity"). In polar systems, long range potential interactions between individual parts of the macromolecule can also affect its conformation and behaviour. Such interactions are specially important with polyelectrolytes. ii) Intermolecular interactions (polymer-solvent interactions): these include thermodynamic effects like excluded volume, coil-expansion coefficients and polymer-solvent friction. The latter is usually described as the force acting on a fragment of a chain moving with velocity ΔV, in a Newtonian fluid. An analogue to polymer-solvent friction, is polymer-polymer friction. Interactions in concentrated polymer systems also include crosslinks and entanglements. Entanglement junctions provide a different type of interaction specific for long chain systems. An entanglement is non-localised in the space of the component macromolecules which can slide past one another. This leads to a complex behaviour combining transmission of elastic forces and possible chain breakage at very high tension with frictional interactions.

iii) Kinetics of reactions between structural elements: this plays an important role in network systems. A kinetic process or "reaction" between two structural units in the system occurs, when collision of such elements brings about an instantaneous change in their conformation e.g. retrogradation of amylose.

1.6.2 Viscoelasticity

As mentioned previously, food polysaccharides in concentrated solutions or gels, are viscoelastic materials. In order to separate out the elastic component from the viscous component, a sample is subjected to an oscillatory strain or stress and the viscoelastic parameters extracted by comparing the strain or stress with the resultant oscillating stress or strain respectively (Ferry, 1980). If a perfectly elastic solid is subjected to an oscillating shear, the stress generated in resistance to the applied deformation is exactly in phase with the imposed strain. For a purely viscous liquid (Newtonian), by contrast, the stress will be exactly 90^{o} out of phase with the imposed deformation because it will have its maximum when the rate of change of strain with time is at its maximum value. For real systems, the degree of solid like and liquid like character may be measured by resolving the resultant stress to its in-phase and out-of-phase components. The ratio of in-phase stress to applied strain is the elastic modulus (G'), while the corresponding parameter for the out-of-phase response is the viscous modulus (G'') (Morris, 1984). The energy used in deforming an elastic solid is recovered as the sample springs back to its original shape, i.e. it is stored, while for a perfect liquid there is no such recovery and the energy is lost. Hence G' and G'' are also known as the "storage" and "loss" moduli respectively. The overall response of the sample may be characterised by the complex modulus G* defined by the equation $G^* = (G'^2 + G''^2)^{\frac{1}{2}}$. Besides the dynamic oscillatory measurements to characterise the viscoelastic behaviour of materials important information can also be obtained by measuring the response of materials

as a function of time. These are the creep and stress relaxation measurements. In a creep experiment, a sample is subjected to a constant stress and the amount of deformation is monitored continuously as a function of time. In the converse stress relaxation experiment, a sample is subjected to an initial fixed deformation which produces a stress in the sample, this stress then tends to decrease as a function of time as viscous flow allows the sample to relax (Ross-Murphy, 1984). By analogy to the shear moduli obtained in oscillatory measurements, a shear creep compliance $J(t)$ can be defined as the ratio of shear strain to shear stress at any given time t when the stress is kept constant as well as a shear relaxation modulus $G(t)$ is defined as the ratio of shear stress to shear strain at any time t when strain is kept constant (Mohsenin, 1970).

1.6.3 Polysaccharide gels

A gel can be regarded as a sample capable of supporting its own weight against gravity i.e. maintaining its shape, over a practical time scale of days or weeks (Morris, 1984). In molecular terms this behaviour normally results from the association of long stretches of the polymer chain into conformationally ordered "junction zones" stabilised by co-operation arrays of non-covalent interactions or by hydrophobic interactions. Dissociation of such junctions then requires an energy well in excess of that available from Brownian motion, thus conferring long term stability to the resulting cross-linked network. If a gel sample is deformed by a fixed amount and held there over a long period of time, the stress required to maintain

this constant strain will gradually decrease (stress relaxation) and on release of the stress the sample will only partially recover its original geometry. Conversely if a finite stress is applied, the resulting strain will gradually increase (creep compliance) with again only partial recovery. This behaviour may be understood in terms of gradual network rearrangement to accommodate the applied deformation, by dissociation of pre-existing inter-chain junctions and their replacement by new interactions between different chain partners. On a shorter time scale the behaviour of non-covalently cross-linked gels approximates to that expected for a permanent network.

1.7. Rheological properties of starch

Most of the research done on the rheology of starch is related to the gelatinisation of starch ganules. As mentioned in section 1.1, the term gelatinisation describes the swelling and hydration of granular starches during heating over a certain range of temperatures normally from 55^{o} to 70^{o}C depending on the origin of the starch. During heating the viscosity of a starch dispersion increases due to the swelling of the granules and the leaching of amylose and reaches its peak around the peak temperature of gelatinisation. Hence, viscometry is a technique which could be used for the determination of starch gelatinisation. One of the first instruments developed and still widely used for the measurement of the viscosity of gelatinised starch, the so-called paste viscosity, is the Brabender Visco/amylograph. This instrument is an empirical rheometer in the sense that it records the torque required to balance the resistance (viscosity)

that develops when a starch slurry is subjected to a programmed heating and cooling cycle. The sensitivity is low, there is no precisely defined shear rate and the paste viscosity is measured in arbitrary Brabender units. Nevertheless, the bulk of world literature on starch rheology is based on measurements taken with this instrument and only by the end of the past decade other true rheometers were employed for a more thorough study of the rheological properties of gelatinised starches.

Nedonchelle and Schutz (1968) used the "power law" to describe the rheological behaviour of starch pastes. They examined the physical importance of parameters K and α of the power law $\tau = K\dot{\gamma}^{\alpha}$, (where, τ = shear stress and $\dot{\gamma}$ = shear rate) and developed a procedure for correlating viscosity data obtained on different coaxial cylindrical rheometers. In the case of the influence of concentration, the parameter α remains constant for starch concentrations from 2 to 10%, whereas the parameter K considerably varies. The same authors also studied the phenomenon of retrogradation and postulated that during retrogradation the power law is still valid, providing the starch concentration is low enough to avoid gelation. D'Appolonia (1972) studied the effect of various ingredients used in bread making on the gelatinisation of starch. He used the Brabender amylograph which showed that the viscosity of a wheat starch sample during gelatinisation increased when dried skimmed milk and sucrose were added and decreased on the addition of shortenings. Olkku, Fletcher and Rha (1978) investigated the gelatinisation of wheat starch in the presence of sucrose using a modified Brabender amylograph. They used the least squares method for

fitting a large number of data points in order to correlate the pasting temperature of the starch and the sucrose concentration. The effect of sweeteners on the ageing characteristics of starch gels was studied by Maxwell and Zobel (1978) using the Instron Universal Testing Machine to measure the rigidity of starch gels. They employed the non-linear least squares method for fitting experimental data and used the Avrami analysis for crystallisation of polymers to explain the mechanism of starch retrogradation in the presence of sucrose or glucose. The rheology of gelatinised starch suspensions was studied by Evans and Haisman (1979). They used the Haake-Rotovisco viscometer for high steady shear rates and the Weissenberg rheogoniometer for low steady shear rates and for oscillatory experiments. They measured the pseudo-plasticity constants, yield values and elastic moduli of potato, maize and tapioca starches and wheat flour at 60^{o}C. The authors proposed that the gelatinised starch systems comprise a suspension of swollen granules in a continuous phase known to contain soluble macromolecular material. These suspensions differ from most other suspensions in that the swollen granules are not only deformable but also compressible. Hence, starch suspensions remain "liquid" at concentrations well in excess of the concentration at which the volume of the swollen granules just fills the total volume of the system. According to these authors, the rheological measurements showed that elasticity and yield stress first appear at a concentration of closely packed granules. The elastic modulus is much greater than the loss modulus and almost independent of the frequency over the range 0•002 - 2•5 Hz. Doublier (1981) studied the flow behaviour of wheat starch pastes as a function of concentration and temperature using the Rheomat 30 viscometer and

the highly sensitive viscometer Low-shear 30. The flow curves indicated a non-Newtonian and shear thinning behaviour for concentrations over 1•5%. Below that concentration, the behaviour was that of a Newtonian liquid at least in the lower shear rates ($<1•0$ 1/s). At a concentration above 1•5%, the flow curves could be described by an Ostwald-De Waele equation over a limited range of shear rates. Moreover, for the highest concentrations, a tendency to a yield stress was exhibited. During gelatinisation, it was also found that the size of the swollen granules and the composition of the matrix in which they were dispersed, play an important role on the flow behaviour displayed by the starch pastes. The influence of starch concentration, source of starch and method of paste preparation on the viscoelastic behaviour of wheat starch pastes was investigated by Wong and Lelievre (1981). They employed a custom-made coaxial cylinder viscometer operating over a frequency range of 0•02 to 5•0 rad/s. The results suggested that, under the conditions of study, differences in the dynamic rigidity and viscosity of pastes may be attributed to differences in the number fraction of large granules and the volume fraction of swollen particles they contain. In continuation of their work, Wong and Lelievre (1982) examined the effect of ageing on the viscoelastic properties of wheat starch pastes. Using the custom made rheometer mentioned before, the behaviour of starch pastes was monitored over a range of storage temperatures (10 - 30^{o}C) and ageing times up to 150h. The results suggested that a crystallisation process was responsible for the observed increase in dynamic rigidity with time. The authors employed the Avrami equation to interpret the results in an attempt to establish the mechanism of crystallisation. They concluded that although the

variation in dynamic rigidity with time obeyed the Avrami equation, the mechanism of crystallisation remains uncertain.

Bagley and Christianson (1982) studied the effect of heating time, temperature and concentration on the swelling of wheat starch granules and its relationship to starch suspension viscosity. The temperatures they used for heating the starch ranged from 60 to 75^{o}C and the time of heating ranged from 15 to 75 min. The viscosity of the suspensions was measured with a Haake-Rotovisco viscometer at 60^{o}C and 23^{o}C. The results showed that the heating time strongly affects the viscosities of starch suspensions. The character of flow curves changed significantly with heating time often showing dilatancy effects. At the temperature range at which the samples were heated, the granules swell markedly and the flow curves are typically non-Newtonian with viscosity decreasing at steadily increasing rate with shear rate. Since during flow granules deform and move past each other, no volume change takes place. If the particles are not readily deformed then dilatancy will be observed and this is particularly pronounced in the shorter heating times when the granules are more rigid. Eliasson and Bohlin (1982) performed shear stress relaxation experiments on wheat starch gels, using a custom-made instrument in order to investigate the effect of water content on the rheological properties of the gels prepared at various heating temperatures. The results showed that during the gelatinisation of starch the stress relaxation modulus G and the half relaxation time $T_{\frac{1}{2}}$ increased. When the starch was heated in excess of water $T_{\frac{1}{2}}$ and G did not change for gels heated above complete gelatinisation. When the starch was heated in the presence of limited

amounts of water, G was lower and $T_{\frac{1}{2}}$ was longer for gels heated above the gelatinisation temperature. Moreover, it was found that the particle size distribution did play a significant role on the rheological properties. Thus G and $T_{\frac{1}{2}}$ increased in samples having mainly large granules. Christianson and Bagley (1983) determined the apparent viscosities of dispersions of swollen maize starch ganules, using the same techniques and methods which they used for their previous work on wheat starch (1982). The results showed that the behaviour of the maize starch dispersions, at the same levels of granule swelling, is identical to the behaviour of wheat starch dispersions. Maher (1983) employed a Brookfield viscometer to study the alkali gelatinisation of starches. By measuring apparent viscosities of dispersions of various alkali to starch ratio he tried to establish the ideal conditions of complete gelatinisation in regard to time. Bagley and Christianson (1983) investigated the existence of yield stresses in heated wheat starch dispersions using the techniques and methods mentioned before. According to their results, no yield points were observed in the flow behaviour at 60°C. However at 23°C, viscosity measurements showed yield points with varying values depending on heating time, heating temperature, concentration and cooling history of the starch dispersions. Navickis and Bagley (1983) investigated yield stresses in concentrated dispersions of gelatinised wheat starch granules using the Rheometrics mechanical spectrometer with parallel geometry. Starch gels were prepared from dispersions with 14% starch, heated at 70°C for 90 min. To overcome the problem of slip between the gel and the parallel plates, the hot dispersion was placed on sanded plates or the cool preformed gel was attached to the rheometer's platens using cyano-

acrylate ester cohesives. The shear stress-strain curves showed an initial linear region from which a small deformation modulus was obtained. As shear-strain increased, a yield point was observed where the stress-strain curve showed an inflection point. At higher strains the curve rose again and forces normal to the direction of shear were generated. Failure finally occurred at strains of 25 - 50%.

The effects of starch concentration and conditions of gel preparation on the retrogradation of concentrated wheat starch gels were investigated by Krüsi and Neukom (1984) using the Instron tester. An increase in starch concentration from 40 to 55% caused a corresponding increase in gel strength of the fresh gels and an enhanced increase in gel strength during storage. The time of heating during gel preparation had little effect on retrogradation. The influence of temperature of heating was dependent on starch concentration. The water binding capacity of the insoluble starch fraction of the gel was decreased during storage demonstrating that retrogradation also occurs in the insoluble starch and significantly contributes to the increase in gel strength. The authors concluded that retrogradation in concentrated starch gels proceeds inside and on the surface of the swollen starch granules as well as in the solubilised starch leached out during the heating process. Christianson et al. (1984) investigated the development of normal forces in a series of steady shear experiments on starch and starch/hydrocolloid mixture gels using the Rheometrics mechanical spectrometer. The starches were completely gelatinised and the concentration of starch in the gels was 20% $^{w}/_{w}$. For the interpretation of the results, the authors made use of the statistical theory of

rubber elasticity (Treloar, 1975) and particularly of the equations devised by Mooney and Rivlin on normal stresses. The authors concluded that not all the results obtained could fit the equations, hence they can not be used unambiguously for measuring the cross linked density of the gels. Bagley, Christianson and Wolf (1985) in continuation of their studies on starch gels, examined their samples under uniaxial compression, using the Instron machine. To overcome the problem of friction effects between the samples and the instrument's platens, they applied a thin layer of lubricant on the platen-sample interfaces. The lubrication allowed uniform lateral expansion of the samples. The authors expressed the opinion that data obtained from uniaxial compression experiments could be described very effectively using the Mooney-Rivlin relation. Miles et al. (1985) investigated the roles of amylose and amylopectin on the gelation and retrogradation of starch using, among other techniques, a modified pulse shearometer to measure the development of shear modulus of gels with time. The starch gels were considered as composites containing gelatinised granules embedded in an amylose matrix. The short term development of gel structure and crystallinity in starch gels was found to be dominated by irreversible gelation and crystallisation within the amylose matrix. Long term increases in the modulus of starch gels were linked to a reversible crystallisation of amylopectin within the granules on aging. It was considered that the crystallisation resulted in an increase in the rigidity of the granules and thus enhanced reinforcement of the amylose matrix.

1.7.1 Rheological properties of amylose

The rheological study of amylose in solution presents problems mainly, because of its tendency to retrograde. At room temperature dilute aqueous solutions of amylose are inherently unstable and after a limited period of time, depending on polymer concentration and molecular weight, amylose forms an insoluble precipitate. When concentrated solutions of amylose are cooled to room temperature, the samples form opaque elastic gels. The initial gelation is followed by crystallisation of amylose and three dimensional polymer network is formed.

Ott and Hester (1965) determined the amount of soluble amylose needed to form gels in relation to the degree of swelling and the relative size of hydrated starch granules. Amylose prepared from maize starch, defatted with methanol for 3h, was added to various amounts of waxy maize starch (amylopectin). They found that the amount of amylose needed for gels of equivalent strength was about three times greater in the absence of amylopectin granules than in the presence of well hydrated and intact granules. The results suggested that soluble amylose was the main component of the gel network and provided the binding material that linked together intact starch granules or fragments. The rheological properties of concentrated amylose solutions in good solvents such as DMSO, DMSO/water mixtures and aqueous KOH solutions of different concentrations were studied by Geller et al. (1975). The viscosity values were influenced by the solvent type and character and polymer concentration. The anomalies in viscosity and the structural

mechanical properties increased in the DMSO/water mixtures as the water content increased and in the aqueous KOH solutions these properties increased as the alkali concentration decreased. Amari, Watanabe and Nakamura (1976) studied the viscoelastic properties of aqueous solutions of the amylose-iodine complex at ultrasonic frequencies. Dynamic measurements were carried out for aqueous solutions of amylose-iodine, amylose-iodine-urea and amylose-iodine-ethanol systems. The results showed that the addition of urea reduced the rigidity of the amylose-iodine complex and the addition of ethanol changed the conformation of the non-complexed amylose. Amari and Nakamura (1979) measured the stress relaxation moduli G(t) of aqueous gels of amylose, amylopectin and their mixtures at various magnitudes of shear strain with a relaxometer of the cone and plate type. The magnitude and shear strain dependence of G(t) for amylose gels were markedly affected by the rate of cooling. The relaxation behaviour of amylopectin gels was dependent on the magnitude of shear strain. By adding amylose to amylopectin the relaxation modulus of gels was remarkably enhanced. Welsh et al. (1982) investigated the intermolecular associations of amylose solutions and gels with dynamic measurements of rigidity modulus. The amylose gels were prepared either by heating amylose dispersions to 150°C and then cooling at room temperature, or by dissolving in 1M NaOH followed by neutralisation with an equal volume of 1M HCl. The development of gel rigidity modulus measured using a torsional rheometer and the gel strength measured on an Instron machine were dependent on the molecular weight of the polymer and the method of preparation of amylose solutions. The gel network developed more slowly and was weaker for the neutralised amylose sample than for the sample prepared by heating an amylose

dispersion at 150°C. Ring and Stainsby (1982) studied the effect of reinforcement of pure amylose gels with swollen starch granules. The authors used as fillers starch granules which had been gelatinised in a range of temperatures from 65° to 95°C. It was found that the gels reinforced with granules swollen at 65°C had the highest shear modulus and the gels containing granules swollen at 95°C had the lowest shear modulus. The results supported the view of Ott and Hester (1965) that gel formation in starch was primarily related to amylose concentration and the degree of swelling. Hayashi et al. (1983) investigated the phase changes of amylose solution in the binary solvent system of DMSO/water, under various conditions, from sol to gel. The results suggested that gel formation occurred at the phase separation point when the amylose concentration was higher than a certain critical value. In the case of amylose in aqueous solution the same result could be obtained if the amylose concentration was high enough to form a gel. Moreover, the phase separation point agreed with the starting point of retrogradation. Miles, Morris and Ring (1985) studied the gelation of amylose by DSC, X-ray diffraction, turbidimetry and viscometry. They also measured the shear modulus with a pulse shearometer. Viscosity measurements suggested that polymer - polymer entanglements occurred at a concentration designated as C*. At concentrations below C* amylose precipitation was observed from solutions. At polymer concentrations over C* opaque gels were formed on cooling hot aqueous solutions. The development of opacity appeared to be related to the irreversible phase separating into polymer rich and polymer deficient regions. The development of a phase separated system and the development of the network structure of the gel were strongly correlated i.e.

for concentrations over C* phase separations resulted in a continuous network of polymer-rich phase. Gelation on the early stages of retrogradation appeared to involve phase separation followed by a slow crystallisation in the polymer rich phase.

1.7.2 Rheological properties of amylose-lipid interactions

The most pronounced effect, caused by the addition of lipids to starch systems is the inhibition of swelling of the granules and the significant increase in the gelatinisation temperature. By inhibiting gelatinisation, lipids usually prevent the leaching of amylose from the granule and thus it is believed they act as antistaling agents. Another effect of lipids is to cause textural changes in starch and flour systems. Relatively few attempts have been made to investigate the rheological properties of amylose-lipid and starch-lipid systems either in solution or in gel state and this is due to the difficulty in avoiding retrogradation of amylose or precipitating of the complexes during their formation.

Osman and Dix (1960) studied the effect of oils, fats and surfactants on starch pastes using a Brabender amylograph. The oils and fats showed no difference in their effect on the gelatinisation or cooling of the starch pastes. Addition of surfactants to the starch-water-fat mixture usually resulted in a marked increase in the temperature at which maximum viscosity was attained. Moreover gels prepared from pastes containing any of the surfactants were much weaker than the gel containing no surfactants. These effects appeared to be

related to the length of the hydrocarbon chain and the number of hydrocarbon chains in the molecule of surfactants. The character of the hydrophilic portion of the molecule also exerted an effect. Goering, Jackson and De Haas (1975) studied the add back effect of lipids extracted from starches on the viscosity of heated defatted starch suspensions. The lipids were extracted with methanol (85%) for 72h. The defatted starches when heated in a Brabender amylograph showed negligible pasting peaks, greater cooking stability and lower setback. Addition of the methanolic extracts to the defatted starches essentially reproduced the curves obtained from the untreated starches. The addition of pure unsaturated fatty acids at the concentration found in the original starch essentially reproduced the original curves. An examination of the various fatty acids present in the lipid fraction indicated that linolenic acid was the most effective in modifying the viscosity curve. It was suggested, by the authors, that the pasting peak was not due to gelatinisation and breakdown of granules, but it was due to the breakdown of an amylose-fatty acid complex. Eliasson et al. (1981) examined the rheological behaviour of wheat starches with regard to differences in the distribution of surface lipids in the starch granules. The lipids present on the surface of the granules were extracted with isopropanol by shaking for 1h. The lipids in amylose-lipid complexes were extracted with water-saturated butanol by shaking for 1h. However, the extraction temperature was not mentioned. The viscosity of starch suspensions in water was measured with a Rotovisco RV3. The measurements were started at 70^{o}C to avoid sedimentation, and the temperature was increased at the rate of 1·5^{o}C/min up to 91 - 92^{o}C; after 10 min at this temperature cooling was started

at the same rate. The initiation of gelatinisation, the rise in the viscosity and the maximum viscosity were all displaced towards high values as the level of butanol extractable lipids increased. Nihara and Matsumoto (1981) attempted to measure the effect of lipids on the rheological properties of starch pastes during the gelation process. The starch was from wheat and it was used without defatting. The lipids used were fatty acids, monoglycerides, triglycerides and lecithin. Starch was mixed witha diethyl ether solution of a lipid, the ether removed by agitation at room temperature and the mixture heated in boiling water for 20 min. The final concentration of starch in the paste was 6%. The viscometer used was of the cone and plate type. Paste sample was placed on the plate of the viscometer, homogenised by shearing at a high shear rate (384 s^{-1}) for 3 min and allowed to relax for 2 min. The apparent viscosity of each sample was measured at a fixed rate 76·8 s^{-1} at 30°C for 3 min in order to obtain information about the sample's structural breakdown during shearing. Then the sample was allowed to relax for 27 min. The above two measurement steps were repeated for eight cycles, the whole ageing period for each sample being 210 min. The apparent viscosity of all the samples decreased greatly during the 3 min shearing due to thixotropic breakdown of the structure of the samples. The results suggested that the presence of a small amount of free fatty acid or monoglyceride might play a role in reducing the mechanical strength of the starch gel structure. According to the authors the formation of amylose-lipid helical complexes could not be explained on the basis of flow measurements of starch pastes in the presence of lipids. Harbitz (1983) studied the effects of sodium dodecyl sulphate (SDS) and lauric acid on

gelatinisation and gel formation of potato starch. The development of viscosity in the starch slurries during heat treatment was determined in a Brabender amylograph. The presence of SDS resulted in a dramatic increase in the initial viscosity compared to the viscosity of starch without SDS. Stress relaxation measurements were performed in a cone and plate instrument. Starch slurries mixed with SDS in water were heated up to 95°C for 15 min and after cooling the formed gels were measured at 20°C. The results showed that the addition of SDS to starch gels enhanced the formation of strong cross links in the gel network. Starch gels were prepared as mentioned above and kept for 24h before their apparent gel strengths were measured with an Instron Machine. The presence of SDS dramatically increased the gel strength of the starch gels; the effect of lauric acid was the same but less pronounced than that of SDS. The author concluded that when a complexing agent was present during gelatinisation of starches the leached amylose complexed with the agent immediately or at least during cooling of the paste. The helical areas of the amylose molecules resulting from the complexation may form strong "junctions" in the gel with the helices aligned along each other and probably stabilised by hydrogen bonds. Germani, Ciacco and Rodriguez-Amaya (1983) investigated the effect of lipids and type of starch on the mode and kinetics of retrogradation of concentrated maize starch gels. The lipids tested were triglycerides and hydrogenated oils and the gels were prepared by mixing starch and oil in water and heating the mixture at 95°C for 10 min. The strength of the gels was measured 30 min after preparation at 21°C and then at daily intervals up to 5 days with an Instron machine. The results, interpreted according to the Avrami theory, suggested that the mechanism

of crystallisation was instantaneous nucleation followed by a rod like growth of crystals regardless of the type of starch or lipid used. Lipids retarded retrogradation and the most effective in retarding retrogradation were found to be those with the shortest fatty acid chain length. Goshima et al. (1985) defatted potato starch and then added back to the starch suspension, the lipid extract, in an attempt to find out whether there was a difference in the amylographic pattern of the untreated starch from the treated one. From the results it was concluded that the characteristics of amylographic alteration were caused by the internal starch lipids together with the starch surface lipids. Capillary viscometry experiments conducted by Whittam, Ring and Orford (1986), showed that the addition of sodium caprate to dilute amylose solutions up to a ratio lipid:amylose 2:1 $^{w}/_{w}$ caused a drop in the specific viscosity of the amylose solution which was attributed to conformational transition of the amylose molecule, which is believed to exist as a flexible coil in neutral aqueous solutions. The same phenomenon was observed, but much less pronounced, when sodium caprate was added to amylopectin solutions. The authors also examined the rigidity modulus of a series of amylose gels to which fatty acid soaps were added with a chain length from C_{10} to C_{18} as well as monoglycerides in the same chain length range. The results showed that the addition of monoglycerides or fatty acids caused a reduction in the rigidity of the amylose gels which was concentration dependent i.e. the higher the concentration of the acid or the monoglyceride, the lower the rigidity modulus of the gel. Moreover, the hydrocarbon chain length did play a role. The decrease in the rigidity modulus was in the sequence $C_{14} < C_{12} < C_{10} < C_{16} < C_{18}$. They reported that monomyristin was the most

effective of the monoglycerides in lowering gel rigidity. The effect of added lipid on the firmness of starch gels was studied with a 30% w/w starch gel containing 0·7% w/w myristic acid. The initial shear modulus, measured after one day, was lower for the gel to which fatty acid was added compared with the control. During ageing the increase in firmness was again much lower and slower for the gel containing fatty acid. Bohlin, Eliasson and Mita (1986) performed stress relaxation experiments on native starch gels and potato gels with lipid (monolaurin) coated starch granules. The gels were prepared by heating starch-water suspensions to 90°C and then quickly transferring the gels to the plate of the cone and plate rheometer. It was found that lipid coating greatly increased the stiffness of the gels, which was observed as increased relaxation modulus G(t). Gels prepared with lipid coated granules behaved like viscoelastic solids as observed from the increased relaxation time ($T_{\frac{1}{2}}$) and the increased apparent residual modulus G . The increase in G(t) was attributed to the increased rigidity of the particles (granules) of the composite material. The increased rigidity of the particles was probably due to the restricted swelling of the granules because of the formation of amylose-lipid complexes which restricted the leaching of amylose from the granule.

1.8 Enzymic degradation of amylose-lipid complexes

A number of hydrolytic enzymes degrade amylose and amylopectin by the successive removal of low molecular weight products. Amylases are capable of catalysing the hydrolysis of the (1 → 4) glucoside bonds between the α-D-glucopyranose residues and are widely produced by

plants, bacteria and animals. Amylases have been classified according to different criteria: (Robyt, 1984) i) the configuration of the anomeric carbon atom of their products ii) the biological source iii) the type of attack on the polymer substrate i.e. endo- or exo-attack iv) whether they produce a rapid reduction in the viscosity of the substrate (liquefying) or a slow reduction (saccharifying), v) the type of product(s) produced e.g. D-glucose, maltose and maltotriose and vi) the nature of the protein structure. One of the more common classifications is the α- and β-designation, which is based on the anomeric configuration of the products released, thus α-amylases release products with the α-D-configuration and β-amylases release products with the β-D-configuration. In general, α-amylases are endoglucosidases attacking glucans somewhere away from the chain ends at an internal glucosidic bond and producing a rapid drop in the viscosity of the substrate. Generally, β-amylases attack glucans in an exo-fashion from the non-reducing end to produce a single type of low molecular weight product with the β-D-configuration. The products are β-maltose and β-limit dextrins. Limit dextrins result when the enzyme reaches a branch point in amylopectin; e.g. β-amylase cannot by-pass α-D-(1→6) linkages. Another exo-acting amylase is glucoamylase which releases β-D-glucopyranose from the non-reducing end of the starch chain. This enzyme differs from β-amylase in that it does not produce limit dextrins. It catalyses the hydrolysis of both the α-D-(1→4) and α-D-(1→6) linkages and can completely convert starch to D-glucose.

The debranching enzymes isoamylase and pullulanase can hydrolyse the α-D-(1→6) linkages of amylopectin. Several attempts have been

made to hydrolyse quantitatively starches which contained lipids as well as amylose complexed with lipids and there have been met with various degrees of success.

Van Lonkhuysen and Blankestijn (1976) measured the degree of digestibility of starch-monoglyceride complexes with bacterial α-amylase. The complexes were prepared by mixing starch suspensions with commercial monoglycerides either at room temperature or heated at 70°C for 10 min. The freeze-dried and ether extracted complexes were mixed with α-amylase solution and suspended in buffer solution (pH = 6•0) at 25°C. Aliquots of the mixture were taken at time intervals and the released glucose was measured spectrophotometrically. It was found that pure inclusion compounds of monoglycerides with amylose when kept in aqueous suspension could not be hydrolysed by α-amylase. Starch-monoglyceride complexes were broken down to approximately 50%. The time needed for the hydrolysis to reach a constant level was 20 min. Lorenz and Kulp (1983) found that if wheat starches were defatted, then their enzymic susceptibility was reduced. When the starches were heat treated at 24 - 27% water content and then defatted with methanol, their enzymic susceptibility decreased even further. Robinson, Weinert and Solms (1983) prepared starch-lipid complexes by treating starch and lipid mixtures in water at boiling point. The precipitated complexes were removed by centrifugation and freeze dried. Samples of the complexes were dissolved in NaOH, neutralised with HCl and digested with amyloglucosidase at 55°C for 30 min. The released glucose was determined spectrophotometrically. Other samples were firstly treated with thermostable α-amylase and then with amyloglucosidase. It was

found that the treatment of the complexes with amyloglucosidase resulted in 28 to 36% hydrolysis whereas the pretreatment with α-amylase followed by the treatment with amyloglucosidase increased the hydrolysis up to 97%. Holm *et al.* (1983) investigated the digestibility of amylose-lipid complexes *in vitro* and *in vivo*. Potato amylose was complexed with lysolecithin and oleic acid. The degradation of complexed amylose with porcine pancreatic α-amylase *in vitro* was studied as well as the *in vivo* absorption in the rat. Complexed amylose displayed a substantially reduced susceptibility to α-amylase *in vitro*. However, when adding a large excess of enzyme, the complex was completely hydrolysed after 3h. Amylose-lysolecithin complex disappeared from the gastrointestinal tract within 120 min. The complexed amylose was hydrolysed and adsorbed to the same extent as free amylose *in vivo* but somewhat slower. Eliasson and Krog (1985) investigated the effect of chain length on the resistance to the enzymic hydrolysis of amylose-monoglyceride complexes. They employed the combined action of bacterial α-amylase and amyloglucosidase to treat samples of complexes for up to 6h. It was found that the resistance to hydrolysis increased progressively with the increase in the chain length. The greatest resistance was shown by the monostearate complex ($C_{18:0}$) which was hydrolysed up to 45% relative to the hydrolysis of pure amylose. Monolaurate complex was hydrolysed as efficiently as pure amylose after 2h of incubation. With regard to unsaturated monoglyceride complexes it was found that all complexes were hydrolysed to a lesser extent than pure amylose. The monolinoleate ($C_{18:2}$, *cis-cis*) complex was hydrolysed faster than the monooleate ($C_{18:1}$, *cis*) and especially the monoelaidate complex ($C_{18:1}$, *trans*). The monoelaidate was found

to be even more resistant than the monostearate.

1.9 Technological importance of amylose-lipid complexes

The ability of monoglycerides to form complexes, with amylose is an important function in many starch containing foods. It is believed that monoglycerides are responsible for the crumb softening in bread and they act as antistaling agents by inhibiting amylose from leaching out of the starch during the baking of dough (Krog and Nybo-Jensen, 1970; Krog and Lauridsen, 1976; Schuster, 1984). Monoglycerides are also added in the production of dehydrated mashed potatoes in order to prevent stickiness after reconstitution (Hoover and Hadziyev, 1981). There is evidence that amylose-lipid complexes are formed during extrusion cooking of starch containing products when fatty acids or monoglycerides are added. It has been reported that the complexes could affect the texture of these products by decreasing the solubility of the soluble fraction (Mercier et al., 1979; Mercier et al., 1980; Launay and Lisch, 1983).

In the manufacture of cornflakes monoglycerides are also added at the step of the cooking process (steaming) of maize grits. Their role is to inhibit the leaching of amylose during heating, hence preventing the cooked grits from sticking together and forming large aggregates (lumps) which are difficult to handle in subsequent processing (Personal industrial experience).

AIMS OF THE PROJECT

1. Although the interaction of amylose with lipids, and other organic molecules, to form helical inclusion complexes has been known for over 40 years, the quantitative aspects of this interaction have been largely ignored. The terms 'amylose complexing index' and 'relative complexing efficiency' have been used by some investigators to describe and characterise the tendency of saturated and unsaturated monoglycerides to form complexes with amylose. Since these terms appear to be arbitrary a more fundamental approach is necessary. In principle, the longer the lipid or guest molecule, the larger the quantity of amylose expected to interact with this molecule. However, it is also understood that problems of availability of the guest molecule and steric considerations must play a role. One of the aims of the present work is, therefore, to study systematically the complexing of amylose with a number of lipids of increasing molecular weight in order to elucidate the nature of this interaction. The effect of double bonds and glycerol ester bonds in the guest molecule with regard to complex formation will also be investigated.

2. X-ray crystallography, the most commonly used technique for the study of the structure of crystalline complexes does not provide any data with regard to the stoichiometry of these complexes. Very recently, optical rotation, surface tension and NMR have been used for the elucidation of the transitional conformation of amylose in solution on the addition of salts of fatty acids. These methods suggest that under optimal conditions saturation of the amylose

helical molecule takes place when a sufficient supply of guest molecules is available. DSC is also a relatively recent technique applied to the complexes for the purpose of understanding the forces that hold the complexes together. The latter method appears to be most promising for the exploration of the energy relations of complexes prepared under rigorous conditions. Published data show very wide variations in the dissociation energy of allegedly identical complexes; the origin of this variation is not clear. DSC will be used in order to explore the structure of complexes, prepared under rigorous conditions, based on their thermal stability in an effort to explain the causes of variability reported in the literature.

3. Some of the rheological properties of amylose-, or starch-lipid complexes have been reported in a speculative rather than objective manner. Based on a number of rheological techniques the present study will be directed towards the measurement of the viscosity of dilute solutions and the elastic moduli of concentrated gels as a function of added lipids. Attempts will also be made to investigate the possibility of interactions of lipids with amylopectin.

4. The technological significance of the interaction of amylose with surfactants in food systems will be considered when appropriate.

2. EXPERIMENTAL

2.1 Materials

2.1.1 Potato amylose (A-9262), fatty acids, monoglycerides (purity 99%) and α-amylase (A-3403) were obtained from the Sigma Chemical Company. Potato starch (30262), β-amylase (39094) dimethylsulphoxide (10323) and MeOH-BF_3 (27419) were obtained from BDH. Waxy maize starch was a free sample from CPC(UK) Ltd. Amyloglucosidase (208469) was obtained from Boehringer-Mannheim and all other reagents were of analytical grade.

2.1.2 Maize starch (cornflour) was a commercial product and was defatted by extraction in a Soxhlet apparatus using anhydrous methanol for not less than 165 hours.

Vicia faba starch was isolated from dry faba beans purchased locally. The beans were cracked in a roller mill and immersed in water for 30 min to loosen the hulls which were removed manually. The dehulled beans were steeped in water, containing 0·5% sodium metabisulphite, at 37°C for 48h. The soft cotyledons were comminuted in a blender with excess water. Damage to starch granules was found to be insignificant on testing with Congo red. The slurry was passed through a stainless steel sieve with 152 μm aperture. The sieved slurry was centrifuged for 30 min at 2000 rpm. The sediment was redispersed in a small quantity of water and passed through a 75 μm sieve. The slurry

was then centrifuged repeatedly and the upper layer of the sediment, which was grey in colour and contained most of the protein, was skimmed off. Centrifugation was discontinued when no visible layer of impurities remained. The starch was filtered by suction on a Buchner funnel and washed with acetone to remove most of the water. The acetone was evaporated spontaneously at room temperature. The yield was approx. 30% of the original weight of the beans.

2.2 Instrumentation

2.2.1 Suspended level viscometers. A series of Ubbelohde capillary viscometers were used ranging from a custom made one with a flow time for water of about 180 s, to a commercial one with a flow time of 80 s.

2.2.2 Rheomat 115, Contraves Ltd., Switzerland. The viscometer used had concentric cylinder geometry (measuring system MS-D/115) suitable for measuring viscosities of solutions ranging from 1·5 mPa s to 150 mPa s. Shear rate was adjustable in 15 steps. Calibration was checked with a series of standard sucrose solutions. The instrument was equipped with a temperature controlled water bath.

2.2.3 Weissenberg rheogoniometer R16, Sangamo, England. The instrument was fitted with a cone-and-plate measuring system. The angle of the cone was $1{\cdot}6^{\circ}$ and the radius 3·75 cm. The constants of the torsion bars used were 1·02 Nm/rad and 0·5332 Nm/rad. The constant of the normal force spring was $3{\cdot}37 \times 10^{3}$ N/m. The instrument was regularly calibrated and checked with a series of standard silicone fluids ranging

from 0•2 to 0•5 Pa s. The rheometer was equipped with a temperature control unit and a U.V. recorder.

2.2.4 Viscoelastic analyser, Sangamo-Schlumberger, England. This was a constant stress rheometer. The system applied a preselected torsional shear stress to the sample providing information about the material while avoiding breakdown of the structure. The measuring section incorporated a compensated air bearing support giving frictionless coupling for the rotating measuring component. It was electronically controlled and was fitted with a water jacket for temperature control for the stationary component. The control console was a microprocessor preprogrammed for the torque to be applied in creep, continuous or sinusoidal oscillatory modes. The console had high level analogue outputs for direct recorder plotting of shear-strain, shear rate and sweep and digital display of parameters such as torque, angular velocity and programme status. The instrument was also equipped with a computerised temperature control water bath and an X-Y chart recorder. The measuring unit was fitted with cone-and-plate geometry. The angle of the cone was 2^{o} and the radius 2 cm. The calibration of the instrument was checked with standard silicone fluids and the air bearing friction was determined as described in appendix I.

2.2.5 The torsion balance used for surface and interfacial tension measurements was made by White Electrical Instrument Co. Ltd., England. The instrument was used as a du Nouy ring tensiometer fitted with a platinum ring having a circumference of 4 cm.

2.2.6 The gas liquid chromatograph model 104, Pye Ltd., England, was fitted with a flame ionisation detector. The injector, oven and detector temperature was fixed at 185°C. Glass columns (2·1 m long, 2 mm internal diameter) were packed with 15% EGSSX on Gaschrom P (100 - 120 mesh) or with 15% CP-Sil 84 on Chromosorb WHP (100 - 120 mesh).

2.2.7 The thermal analysis system was a model TA 300, Mettler Instrumente AG, Switzerland. It consisted of a TC 10 TA processor, the control and evaluation unit, the DSC 30 low temperature cell and a printer/plotter integrator. Calibration of the instrument was carried out regularly with pure indium as a standard.

2.2.8 X-ray diffractometer system, Philips APD 15, modified by the addition of a BBC/Torch microcomputer for the analysis and storage of data. The main components of the diffractometer were: PW 1050 Vertical Goniometer, PW 1730 X-ray Generator, PW 2230/20 Normal Focus Anode X-ray tube, PW 1390 Channel control unit, PW 1394 Motor control unit for driving the goniometers stepping motor and P851 Minicomputer. X-ray work was carried out by members of the Department of Applied Biochemistry and Food Science, University of Nottingham.

2.3 Methods

2.3.1 Extraction of amylose from starches: A dispersion of starch in water (1 g/100 ml) was prepared in a Pyrex bottle (250 ml capacity) fitted with a PTFE lined screw cap. The bottle was placed in a water bath at 70°C for 1h with continuous shaking. The hot dispersion was

centrifuged for 30 min at 2000xg. The supernatant was filtered through a Pyrex sintered glass filter (porosity 2) directly into a conical flask containing butan-1-ol (double the volume of the dispersion) and stirred continuously. The insoluble amylose-butanol complex was left overnight and recovered by centrifugation. The yield of amylose was 6•5 - 7•0% by weight of the original starch (dry basis). Amylose was obtained as an aqueous solution on the addition of water to the butanol complex and by heating for 60 min in a boiling water bath while passing a stream of nitrogen through the mixture.

2.3.2 Characterisation of amylose and starch samples: Moisture content was calculated as g of water lost per 100 g sample at 130°C in 1h. Protein content was determined by the Kjeldahl method and the calcium content by atomic absorption spectrophotometry. Amylose content was determined according to the method of Morrison and Laignelet (1983). Starch lipids were extracted by heating, in a boiling water bath, dispersions of starch samples in a mixture of propan-1-ol - water (3:1) as suggested by Morrison and Coventry (1985). The extracted lipids were determined by GLC after conversion into their methyl esters according to Morrison and Smith (1964). The peak temperature of gelatinisation was determined by differential scanning calorimetry (DSC).

For the determination of the β-amylolysis limit samples of amylose (1 ml, containing between 1 and 2 mg/ml) were transferred into Pyrex tubes (10 ml capacity, fitted with PTFE lined screw caps). A solution of β-amylase (1 ml, containing 2 mg/ml acetate buffer, pH 5•0) was added, followed by 8 ml of water. The tubes were incubated at 35°C for

not less than 3h. Aliquots (1 ml) were taken for the determination of maltose by the method of Nelson (1944). Blank determinations were performed on the amylose and enzyme solutions. The β-amylase solution used for this determination was ultrafiltered to remove maltose which was present as a contaminant. The β-amylolysis limit was calculated as: $\% \beta\text{-amylolysis} = \frac{\text{Reducing capacity (as maltose)}}{\text{Total carbohydrate (as maltose)}} \times 100$

Total carbohydrate was determined by the enzymic method described in section 2.3.3. Limiting viscosity number $[\eta]$ of amylose dissolved in 0•15M KOH (5 - 10 mg/ml) was obtained by capillary viscometry (Ubbelohde) according to Greenwood (1964). The viscosity average molecular weight for each sample was calculated according to the Mark-Houwink relation $[\eta] = 8{\cdot}36 \times 10^{-3} M_w^{0{\cdot}77}$ as reported by Banks and Greenwood (1975).

2.3.3 Preparation of amylose and amylopectin in solution Potato amylose or waxy maize starch (99•6 ± 0•2% amylopectin content) was dissolved in DMSO (3 g/100 ml) by continuous stirring for 24h at room temperature. The solution was centrifuged for 30 min at 2000xg to remove traces of suspended matter and the amylose or amylopectin, in the supernatant, was precipitated by the addition of two volumes of butan-1-ol under stirring. The precipitate was recovered by centrifugation after three successive washings with butanol and stored as such. Amylose or amylopectin was obtained as an aqueous solution on the addition of water to the butanol complex and by heating for 60 min in a boiling water bath while passing a stream of nitrogen through the mixture. The concentration of amylose or amylopectin was measured by the enzymic method of Karkalas (1985).

2.3.4 Preparation of complexes in solution To the aqueous solution of amylose or amylopectin, prepared as described in the previous section, 0·1M KOH was added immediately and diluted to 0·01M by the addition of water. The solution was filtered through a Pyrex sintered glass filter (porosity 2) and used within 1h. The potassium salts of fatty acids were obtained by the addition of a calculated volume of 0·1M KOH to a given mass of fatty acid and by heating in a boiling water bath to give a clear solution which was diluted with 0·01M KOH to a fatty acid concentration of 1 mg/ml. Complexes were prepared in 15 ml capacity glass bottles fitted with PTFE-lined screw caps. For this purpose, 4 ml amylose or amylopectin solution (~60 mg in 0·01M KOH) were transferred into each bottle followed by the addition of the required volume of fatty acid solution. The final volume was made up to 15 ml with 0·01M KOH. The samples were vortex mixed and allowed to stand overnight at room temperature before measurement of the viscosity. A control solution containing amylose or amylopectin only (~4 mg/ml) remained perfectly clear and no change in the viscosity could be detected within 24 hours. Most of the samples also remained clear, but a slight turbidity was observed in samples of amylose-fatty acid complexes with high levels of added fatty acids. Turbid samples were filtered through glass microfibre filters (Whatman GF/A).

2.3.5 Capillary viscometry: Viscosity was measured by means of Ubbelohde viscometers. The viscometer used for amylose complexes was specially made to give a flow time of about 180 s for the pure solvent, while that used for the amylopectin complexes was obtained commercially (k = 0·01 cs/s) with a flow time for the solvent of about 80 s.

Reproducibility of flow times was ensured by regular cleaning with chromic-sulphuric acid mixture. The temperature was controlled at $20 \pm 0{\cdot}1^{o}C$. Determinations were performed in triplicate.

2.3.6 Enzymic determination of complexes in solution: Alkaline samples (3 ml) of complexes, after the completion of the viscosity measurements, were transferred into glass bottles (capacity 15 ml, fitted with PTFE-lined screw caps) and neutralised with 3 ml of 0·01M HCl. Citrate buffer (6 ml, containing 460 mg citric acid monohydrate and 840 ml sodium citrate dihydrate in 100 ml water, pH 4·6) was added and the contents mixed thoroughly by vortex mixing. Aliquots (1 ml) were transferred immediately into screw cap tubes (10 ml capacity) and 1 ml amyloglucosidase solution (2 mg/ml citrate buffer) was added. After hydrolysis at $60^{o}C$ for 30 min, 8 ml water were added and the glucose determined by a glucose oxidase method (Karkalas, 1985).

2.3.7 Determination of residual amylose and amylopectin in solution: The samples, neutralised as described in the previous section, were allowed to stand at room temperature overnight then centrifuged at 2000xg for 15 min. The supernatant, from the amylose samples only, was filtered through a Millipore filter (RAW P01300, pore size 1·2 μm) and the residual amylose in the filtrate determined as described earlier. Amylopectin samples were not filtered.

2.3.8 Viscosity measurements of complexes in concentrated solution: A quantity of the amylose-butanol complex was dissolved in boiling water while a stream of nitrogen was passed through. Upon cooling,

0•5M KOH was added and the solution diluted to 0•15M KOH by the addition of water. The amylose solution was filtered through a Pyrex sintered glass filter (porosity 2). The potassium salts of fatty acids were prepared as described before (section 2.3.4). After the calculated volumes of amylose and fatty acid solutions were mixed in universal glass bottles fitted with screw caps (capacity 20 ml), the final volume of the samples was made up to 20 ml by the addition of 0•15M KOH (amylose concentration 10 - 20 mg/ml, determined enzymically as described before). The samples were heated at 60°C for 30 min before the viscosity measurements were taken. Amylopectin samples were prepared in a similar way but the concentration of KOH was 0•01M (amylopectin concentration about 15 mg/ml). The experiments were carried out within 30 min after preparation of the samples to avoid retrogradation of amylose. Measurements were taken in duplicate with the Rheomat 115 at 20 $\pm$ 0•1°C.

2.3.9 Viscosity measurements of amylose-amylopectin mixtures complexed with fatty acids in solution: The amylose and amylopectin solutions in 0•01M KOH were prepared as described before and their concentration was determined enzymically. The required volumes of amylose and amylopectin solutions were mixed together in universal glass bottles fitted with screw caps (capacity 20 ml), in the desired ratio of amylose/amylopectin and the complexes prepared as before. The final volume of the samples was 20 ml and the concentration of total glucan 10 - 20 mg/ml. The samples were heated for 30 min at 60°C before measurements were taken. The amylose stock solution was always kept at 60°C prior to its use to avoid retrogradation. Viscosity measurements were taken with the Rheomat 115 at 20 $\pm$ 0•1°C.

2.3.10 Preparation of starch in solution: Potato starch was dissolved in DMSO in a Pyrex bottle fitted with PTFE lined screw cap (capacity 250 ml) to a concentration of 3 g/100 ml. The solution was stirred for 5h then placed in an incubator at 37°C for 48h. The solution was centrifuged for 30 min at 2000xg to remove traces of suspended matter and to give a visually clear solution. The solution was then poured into butan-1-ol (two volumes) under vigorous stirring with a magnetic bar and the stirring was continued overnight. The resulting precipitate was purified by centrifugation after three successive washings with butan-1-ol and stored as such. The supernatant was checked for total glucan, both by the addition of iodine and enzymically. Both tests were negative. The starch was obtained as an aqueous solution on the addition of water to the butanol complex and by heating for 60 min. in a boiling water bath while passing a stream of nitrogen through the mixture.

2.3.11 Viscosity measurements of starch-fatty acid complexes: The complexes were prepared as before (2.3.4) but without filtration through sintered glass. The KOH concentration was 0·01M and the starch concentration of the samples ranged from 7 to 45 mg/ml. After preparation the samples were heated for 30 min at 60°C and allowed to stand overnight at room temperature before the viscometric measurements were taken at 20 ± 0·1°C. The Rheomat 115 was employed for low starch concentrations (up to 10 mg/ml), the viscoelastic analyser for higher concentrations (about 15 mg/ml) and the Weissenberg rheogoniometer for concentrations above 20 mg/ml.

2.3.12 Creep experiments

(i) Amylose-fatty acid complexes: Amylose and fatty acid potassium salts in 0·01M KOH solutions were prepared as previously described (2.3.3). Predetermined volumes of amylose and fatty acid solutions were mixed in bottles (capacity 15 ml fitted with PTFE lined screw caps) amd made up to 15 ml with 0·01M KOH. After vortex mixing 1 ml aliquots were transferred into 50 ml volumetric flasks, neutralised with 1 ml 0·01M HCl, 5 ml of citrate buffer (pH 4·6) were added and made up to 50 ml with water. The amylose concentration in each flask was determined enzymically as described in section 2.3.6. The amylose concentration in the samples ranged from 28 to 35 mg/ml. The samples were placed in a water bath at 60°C to avoid gelation. After 30 min heating, the sample containing only amylose, serving as the control, was taken out of the bath and left to cool spontaneously at ambient temperature for about 15 min. Then 1 ml sample was placed by means of a syringe, fitted with a 75 μm stainless steel strainer, onto the plate of the measuring unit of the viscoelastic analyser (cone and plate) which had already been adjusted. The sample was allowed to set for 90 min at 20 ± 0·1°C before the measurements commenced. To avoid evaporation, the edge of the sample between the cone and the plate was covered with a thin layer of liquid paraffin. The instrument was programmed in the creep mode. After the ageing time had elapsed the sample was stressed by applying a given torque. The deformation of the sample was recorded as a deflection in relation to the time of shearing. After the predetermined time of stressing was completed the stress was removed and the sample was allowed to relax. The experiment was repeated for at least 5 times, each time applying a different torque to test

whether the viscoelastic behaviour was linear, i.e. the ratio of stress to strain at any particular time was independent of the magnitude of the applied stress. This procedure was repeated for each of the samples. The chart speed of the recorder was set to 1 mm/sec and the input sensitivity was set to 0·05 V/cm full scale deflection (FSD). The gap setting of the measuring unit was 0·283 mm. The range of the angular displacement was $\pm$ 0·5 rad and the analogue output was $\pm$ 10V FSD.

(ii) Starch-fatty acid complexes: A quantity of granular starch was placed in a Pyrex bottle (capacity 100 ml) fitted with PTFE-lined screw cap. A calculated amount of water was added and the suspension was heated for 30 min at 95°C with constant stirring. After heating, the sample was allowed to cool spontaneously. Upon cooling 0·1M KOH was added and the final concentration of the alkali, after thorough mixing, was made to 0·01M KOH. Samples of the complexes were prepared as described before, by mixing predetermined volumes of gelatinised starch dispersion and fatty acid potassium salt solution and diluted with 0·01M KOH as required. The exact concentration of starch in the samples was determined according to Karkalas (1985). For this purpose aliquots of sample (1 ml) were neutralised with 0·01M HCl and diluted to 10 ml with deionised water in a 10 ml Pyrex tube fitted with PTFE lined screw cap, 0·2 ml α-amylase was added and the tube heated at 85°C for 15 min. After cooling the sample was diluted with water to 100 ml in a volumetric flask. The diluted sample (1 ml) was treated with 1 ml AMG (2 mg/ml citrate buffer) and the released glucose was determined by the glucose oxidase method. Starch concentration in the samples was in the range 30 - 40 mg/ml. The prepared complexes were placed in a water bath at 60°C. After 30 min the sample containing only

starch, serving as the control, was allowed to cool spontaneously for 30 min, before transferring 1 ml by means of a syringe onto the plate of the measuring unit of the viscoelastic analyser. Evaporation of water from the sample was avoided by placing a thin layer of liquid paraffin on the edge between the cone and the plate of the instrument. The sample was left to relax for 30 min before the creep experiments were carried out, as in the case of the amylose samples, at 20 $\pm$ 0·1^{o}C.

2.3.13 Dynamic experiments

(i) Starch-fatty acid complexes: The samples were prepared and measured in the manner described above (2.3.12.ii). Starch concentration in the samples ranged from 25 to 35 mg/ml. The Viscoelastic analyser was programmed for the torque to be applied in the oscillatory mode over a range of frequencies. A test was also carried out to ensure that the sample was stressed within its linear viscoelasticity limit. The signals from both the torque and the angular displacement were traced on a two pen recorder of which the chart speed was adjusted every time the frequency of oscillation was changed.

(ii) Amylose-fatty acid complexes: The samples were prepared and measured as described in section 2.3.12.i. The experiments were concerned with the ageing effect of the samples over a period of time, therefore only one frequency was employed which was chosen to be 0·314 rad/s. Measurements were taken at regular time intervals up to 160 min.

2.3.14 Surface tension experiments: Amylose and fatty acid solutions were prepared in 0·01M KOH. The complexes were obtained as described

in section 2.3.4. The measurement of the surface tension of the complexes took place at room temperature (~21°C). The surface tension of a series of samples, containing only the fatty acid in concentrations identical to those used for the preparation of the complexes, was measured. The surface tension of amylose in 0·01M KOH and of 0·01M KOH alone was also measured. Replicate readings were taken until a constant value was attained. The platinum ring was dipped in chromic-sulphuric acid mixture and thoroughly washed with water before measurements of the next sample were resumed.

2.3.15 Preparation of amylose-monoglyceride complexes: Alkaline degradation of monoglycerides of fatty acids was avoided by the preparation of liposomes in an aqueous medium according to Riisom et al. (1984). A 10% dispersion of monoglyceride was prepared by the addition of 1% aqueous potassium cholate. The dispersion was heated to 60°C for 15 min and sonicated for 5 min. The translucent and homogeneous dispersion was added to a freshly prepared aqueous solution of amylose, vortex mixed and heated to 60°C for 30 min, with the exception of monooleate which was heated to 40°C. After overnight precipitation the complexes were centrifuged and the filtered supernatant used for the determination of residual amylose (2.3.7).

2.3.16 Gas-liquid chromatography experiments: Amylose and fatty acid solutions were prepared in 0·01M KOH. For complex formation 1 ml amylose solution was added to 1 ml fatty acid solution and vortex mixed in a Pyrex tube (10 ml capacity, fitted with PTFE lined screw cap). The concentration of amylose was about 13 mg/ml, while that of

the fatty acids varied in the range of 0·1 to 1·2 mg/ml according to the molecular weight. Complexes were prepared in duplicate for each fatty acid concentration. A third tube containing fatty acid and 1 ml 0·01M KOH, but no amylose, served as a control. The mixtures were heated in a water bath at 60°C for 30 min, cooled, neutralised with 2 ml 0·01M HCl, adjusted to pH 4·6 with 1 ml citrate buffer and left at room temperature overnight. One of the replicates was treated with AMG solution in citrate buffer at 60°C for 2 h to hydrolyse the amylose completely and to release the fatty acid. Subsequently, the tubes containing the control, the complex and the AMG hydrolysed sample, for each level of added fatty acid, were used for the extraction of the fatty acids with diethyl ether (3 x 2 ml). To the ether extracts, heptadecanoic acid was added as an internal standard and the ether evaporated to dryness in a stream of nitrogen. The methyl esters of the fatty acids were prepared by the addition of 1 ml of methanol-boron trifluoride, according to Morrison and Smith (1964). Fatty acids were determined in a Pye 104 gas chromatograph. The temperature was set to 185°C. The carrier gas (nitrogen) and the hydrogen flow rates were 30 ml/min and the air flow rate was 500 ml/min.

2.3.17 DSC experiments with amylose-fatty acid complexes

(i) Phase transitions of the complexes: Complexes were prepared in an alkaline solution as described in section 2.3.4. Samples (15 ml - 4 mg/ml were heated for 30 min at 60°C and then allowed to cool spontaneously. Sample volumes (14 ml) were transferred into Pyrex conical flasks (50 ml capacity, fitted with PTFE-lined screw-caps), neutralised with 1·4 ml 0·1M HCl and adjusted to pH 4·6 with 15 ml

citrate buffer. The samples were left overnight at room temperature the precipitated complexes recovered by centrifugation and freeze-dried for 48h. In a different method of preparing amylose-fatty acid complexes, (40 mg/ml) and palmitic acid (2·4 mg/ml) were dissolved in DMSO. Samples of amylose (1 ml) were mixed with palmitic acid solution ranging from 0·7 to 2·0 ml, in Pyrex conical flasks (50 ml capacity with PTFE-lined screw-caps). In all samples the volume was made up to 3 ml by the addition of DMSO. To each flask 2 ml citrate buffer (pH 4·6) and 35 ml water were added. After vortex mixing the samples were heated for 15 min at 85°C and then rapidly cooled in running water for 5 min. The precipitated complexes were recovered by centrifugation and freeze-dried for 48 h. Freeze-dried complexes (2·0 - 3·0 mg, wet basis), prepared by either the KOH or the DMSO route were weighed into aluminium pans (capacity 25 µl), 15 ml of water were added and the contents thoroughly mixed into a slurry by means of a needle. The pans were then hermetically sealed. Each pan was placed into the DSC cell with a pierced aluminium pan as a reference to balance the heat capacity of the sample pan. The calibrated instrument was programmed to heat the pans from 5° to 135°C at a rate of 10°C/min and to cool immediately. The dissociation temperature could be read accurately from the thermogram, together with the dissociation enthalpy which was calculated automatically by integration of the area under the peak. The dry weight of each sample was determined enzymically as amylose. The pan was torn open by two pairs of tweezers and placed in a Pyrex tube (10 ml capacity fitted with PTFE-lined screw cap). Water (1 ml) was added and vortex mixed, then 1 ml of 2·0M KOH was added and vortexing was continued for exactly 5 min. Prolonged contact of alkali with the

aluminium pans was avoided. The mixture was neutralised by adding 2 ml HCl 1·0M and the pH was adjusted to 4·6 with 1 ml citrate buffer. After thorough mixing 1 ml was transferred into another Pyrex tube, 1 ml of AMG (2 mg/ml citrate buffer) was added and left for 30 min at 60°C. The glucose was determined by the glucose oxidase method. The amylose content of the DSC pan was calculated by multiplying the glucose by 0·9. The enthalpy was expressed as J/g amylose.

ii) Experiments on the thermal stability of the complexes: The complexes prepared according to the methods described above i.e. KOH and DMSO methods. The fatty acids used were lauric, palmitic and arachidic. Samples were prepared in duplicate. The volume of amylose solution (4 mg/ml) was 50 ml and the concentration of fatty acids about 0·24 mg/ml for lauric, 0·3 mg/ml for palmitic and 0·24 mg/ml for arachidic. One lot of the samples was kept in an oven at 135°C for 30 min, then transferred into another oven at 90°C for 16h. The other lot was directly placed at 90°C for 16h. All samples, after heating, were immediately cooled in running cold water. The samples were centrifuged and the supernatant extracted with diethyl ether (2 x 5 ml). To the ether extracts, heptadecanoic acid (0·09 mg/sample) was added as an internal standard and the ether evaporated to dryness in a stream of nitrogen. After treatment with methanol-boron trifluoride, the samples were analysed for free fatty acids by GLC (Morrison and Smith, 1964). The precipitates, recovered by centrifugation, were freeze-dried and analysed by DSC.

Replicate samples of amylose-(15 ml, 4 mg/ml) palmitic acid complexes were prepared *via* the KOH method. After neutralisation and centri-

fugation, the supernatants were decanted and 8 ml toluene added to each Pyrex tube (10 ml capacity, fitted with PTFE lined screw caps) containing the precipitated complexes. After vortex mixing the individual samples were heated to 80°, 90° and 100°C for 4h in an oven. After spontaneous cooling, the samples were centrifuged and the supernatants discarded. The precipitates were freeze-dried and examined by DSC.

Replicate samples of amylose palmitic acid complexes (15 ml, 4 mg/ml) were prepared *via* the KOH method. After neutralisation and centrifugation, the supernatants were discarded. The wet precipitates were mixed with 8 ml anhydrous methanol in Pyrex tubes (10 ml capacity, fitted with PTFE lined screw caps). The tubes were placed in shaking water baths at 50°, 60°, and 70°C for 1h. After spontaneous cooling, the tubes were centrifuged and the supernatant evaporated to dryness in a rotary evaporator. Heptadecanoic acid was added (0·09 mg/sample) as an internal standard and after methylation analysed for free palmitic acid by GLC. The precipitates were freeze-dried and examined by DSC.

Replicate samples of amylose-lauric acid complexes (50 ml, 4 mg/ml) were prepared *via* the KOH method. The clear solutions were heated in duplicate at 50°, 60° and 70°C for 30 min, in a water bath. At the same time, calculated volumes of 0·1M HCl and citrate buffer (pH 4·6) were kept at the same temperature. After equilibration, the samples were neutralised by HCl held at the same temperature, the pH adjusted by the addition of citrate buffer and the tubes kept in a shaking water bath for 30 min. After spontaneous cooling, the precipitates were recovered by centrifugation, freeze-dried and examined

by DSC. The supernatants were extracted with diethyl ether and were analysed for free lauric acid by GLC.

2.3.18 X-ray diffraction analysis of amylose-fatty acid complexes: Samples were prepared *via* the KOH method for capric, palmitic and arachidic acids and *via* the DMSO method for palmitic acid. After neutralisation and precipitation, the precipitates were freeze-dried.

Samples were placed in a sample holder of dimensions 10 x 20 x 5 mm so that the largest surface was flush with the face of the sample holder. This procedure was necessary because the parafocussing geometry of the goniometer relies on the specimen presenting a flat surface which is tangential to the focussing circle of the incident X-ray beam. Monochromatic copper K alpha radiation of wavelength 0•154178 nm was used to irradiate the sample. Data was collected over an angular range from 1•5 degrees 2ϑ to 31•3 degrees 2ϑ at 0•05 degree 2ϑ steps. The time of data collection per step was 2 seconds.

3. RESULTS AND DISCUSSION

3.1 Characterisation of amylose and starch samples

The characterisation of the starch samples used throughout the course of the rheological experiments is given in Table 1.

Table 1. Composition and properties of starch samples

Origin	Moisture g/100g	Protein g/100g (N x 6.25)	Apparent Amylose g/100g	Calcium mg/kg	Temperature[b] of gelatinisation (^{o}C)
Potato	16.0±0.1	0.12±0.01	23.5±0.6	460	62.8±0.8
Maize	11.3±0.05	0.34±0.01	21.8±0.2[a]	150	69.0±0.5[c]
Vicia faba	14.5±0.5	0.34±0.08	36.2±0.5	n.d.[d]	60.0±0.5
Waxy maize	11.4±0.4	0.31±0.01	0.6±0.04	n.d.[d]	n.d.[d]

a. Amylose content of defatted maize starch: 26.3±0.2 g/100g

b. Calculated as peak temperature of gelatinisation from DSC measurements.

c. Temperature of gelatinisation of defatted maize starch: 66.4±0.3^{o}C.

d. Not determined.

Vicia faba starch was isolated according to the method described in section 2.1.2. The starch granule damage was less then 0.2%. As

far as the calcium content is concerned, it is known that calcium forms insoluble salts with fatty acids and its determination was deemed necessary at this stage. Its implications will be discussed later. Fig. 4 shows the limiting viscosity number $[\eta]$ of amylose isolated from various sources and of commercial amylose, which was used throughout the course of the experimental work. Table 2, shows the properties of amyloses.

Table 2. Properties of amylose from various sources.

Origin	Amylose g/100g, dry basis	$[\eta]$ ml/mg	Viscosity average mol. weight[a]	β-amylolysis %
Potato Amylose (Sigma)	99.2±0.5	134.5	2.9×10^5	83.5±0.5
Potato Starch (BDH)	99.96±0.02	270.8	7.2×10^5	91.2±0.4
Maize	100.3±0.3	138.8	3.0×10^5	95.9±0.1
Vicia faba	100.5±0.5	158.5	3.6×10^5	100.2±0.2

a. Calculated from the Mark-Houwink relation $[\eta]=8.36\times10^{-3}M_w^{0.77}$ (Banks and Greenwood, 1975).

The β-amylolysis limit of the commercial (Sigma) amylose was close to the values reported by Banks and Greenwood (1975) and by Biliaderis, Grant and Vose (1981). However, the β-amylolysis limits of the isolated amyloses showed considerably higher values and particularly that from

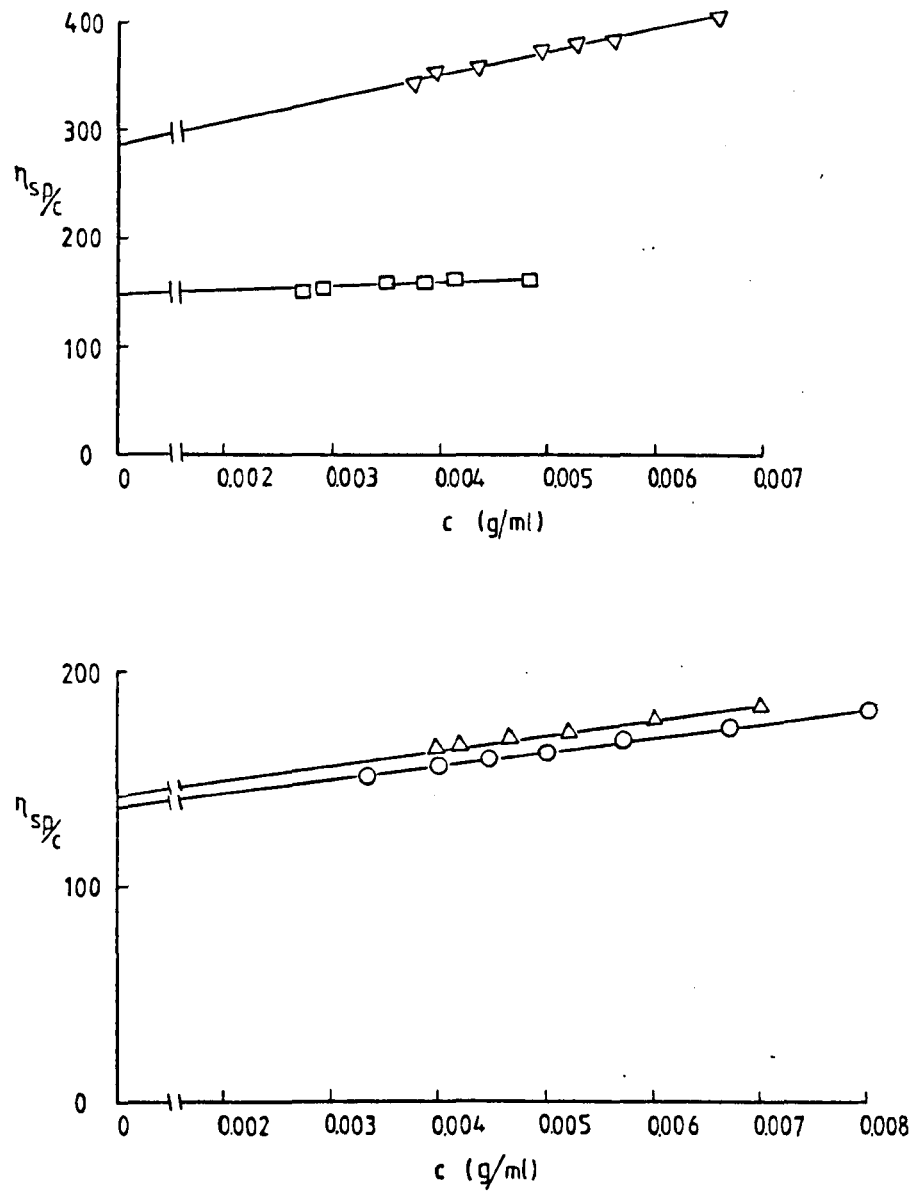

Fig. 4 Limiting viscosity number of amylose isolated from various sources. ○, potato (Sigma); △, maize; □, Vicia faba; ▽, potato (BDH)

Vicia faba in comparison with values reported by Biliaderis *et al.* (1981). The discrepancy is probably due to the method used in isolating amylose from starch. Biliaderis *et al.* (1979) heated the starch suspension to 98°C and this high temperature could cause the leaching of amylopectin as well, as has been shown by Banks and Greenwood (1975), whereas in the present study the heating temperature employed was 70°C which ensured that amylose almost exclusively was leached out from the starch granule. This is also perhaps the reason why Biliaderis reported higher limiting viscosity numbers. The values for lipid content shown in Table 3, for the non-defatted starches are in good agreement with those reported by Morrison and Laignelet (1983) and by Morrison and Coventry (1985).

Table 3. Lipid content of starch samples

Starch	Lipid[a]	Lipid composition (%)[c]				
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Non-defatted maize	634	35	2	12	48	3
Maize extracted for 150h with MEOH[b]	67	62	4	16	18	n.d.[d]
Maize extracted for 180h with MEOH[b]	11	78	22	n.d.[d]	n.d.[d]	n.d.[d]
Vicia faba	19	50	11	38	-	-

a. Lipid as mg fatty acid methyl esters (FAME)/100g dry weight. Results are means of triplicate determinations.
b. Soxhlet extraction
c. Composition as determined by GLC
d. Not detected.

The use of n-propanol-water mixture (3:1) for the extraction of lipids from starches was proposed by Morrison and Coventry (1985) in preference to the acid hydrolysis method because it gives undamaged lipids and it eliminates the possibility of artefacts. The results (Table 3) show that prolonged Soxhlet extraction with methanol (over 150h) is absolutely necessary to ensure the almost complete removal of lipids from starch granules for the purposes of rheological work. The gelatinisation of a small percentage of the granules is only a minor drawback of the method. This was checked enzymically and microscopically. Most of the researchers tend to overlook the need for prolonged extraction and normally employ 48 to 72h of extraction with methanol and assume that the cereal starches will become virtually fat-free. So far, it appears that only Kugimiya *et al.* (1980) and Bulpin *et al.* (1982) employed methanol extraction for 160h for the study of starch-fatty acid interactions. The former authors used as a criterion for the complete removal of lipids the fact that DSC thermograms of methanol treated starches did not show an endothermic peak which is attributed to amylose-lipid complexes. In this study, it was found that this is not the case, because even after 180h extraction there still was some lipid left; because of its low sensitivity DSC is not a suitable method for the detection of traces of lipids. In all rheological experiments the fat-free starch used was prepared by methanol extraction for not less than 170 hours. The *Vicia faba* starch was used in the rheological experiments without defatting.

3.2 Quantitative aspects of fatty acid requirements for saturating the amylose helix

The formation of helical complexes of fatty acids and their monoglycerides with amylose, under favourable conditions, is now well established. Nevertheless, relatively little work has been done on the solution properties of the complexes and particularly on the quantitative relations of fatty acids and amylose. The present study was initiated in order to elucidate aspects of the conformational change of amylose in alkaline solution (pH 12) on the addition of fatty acids and to explore the nature and composition of amylose-fatty acid (and monoglyceride) complexes precipitated at pH 4.6 after neutralisation of the alkali.

3.2.1 Viscometric measurements

The viscosity number, i.e. the specific viscosity (pH 12 at 20°C) as a function of the molar ratio (mol fatty acid/mol amylose, FA/AM) is shown in Figures 5 and 6. The molecular weight of amylose was taken as that of the anhydroglucose monomer ($C_6H_{10}O_5$) *viz.* 162. A sharp decrease in the viscosity number was observed on the addition of increasing quantities of fatty acids as potassium salts. With myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0) and behenic (22:0) acids an almost constant viscosity was reached and this suggests that the amylose helix was saturated with the fatty acid anions. The effect of chain length is also obvious since saturation occurred at a lower molar ratio as the chain length of

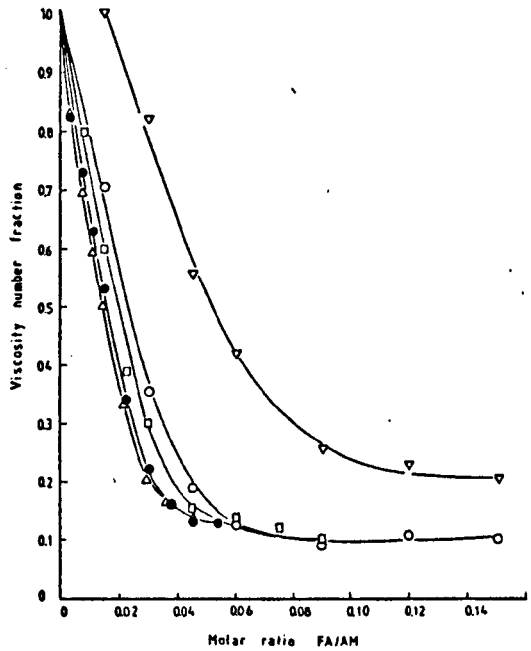

Fig. 5 Viscosity number of amylose (4 mg/ml) in 0·01M KOH with added fatty acid potassium salt; ▽ , lauric; ○ , myristic; □ , palmitic; ● , stearic; △ , arachidic acid

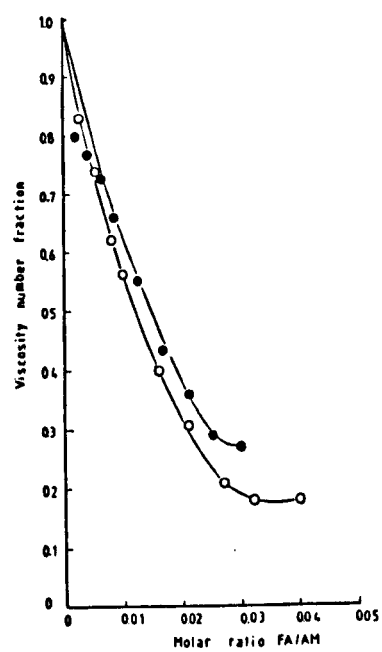

Fig. 6 Viscosity number as in Fig. 5 with: ○ , behenic; ● , lignoceric acid

the fatty acid increased. With lauric acid at pH 12 saturation was attained at a relatively high molar ratio. The viscosity number at saturation was also higher than that measured for the longer chain fatty acids, thus suggesting a high hydrodynamic volume. In all probability this observation would be attributed to ionisation of the hydroxyl groups of amylose. At pH 12 amylose possesses polyelectrolyte character, the induced negative charges repel one another, and the coil dimensions, and thus the limiting viscosity number increase (Banks and Greenwood, 1975). The electrostatic repulsion forces probably compete with the hydrophobic interactions which stabilise the laurate ions inside the helical cavity. Short chain fatty acids (with less than 10 carbon atoms) did not form detectable complexes at pH 12 because of the electrostatic repulsion forces and lack of sufficient hydrophobicity to counteract them. Lignoceric acid (24:0) interacted with amylose as expected but the saturation concentration for the helix was slightly below that for behenic acid. This anomaly was attributed to the fact that lignocerate ions form crystals or gels, even in very dilute solutions, which diminish the availability of free lignocerate ions for complexing with amylose. Although attempts were made to heat solutions containing amylose and very low concentrations of lignoceric acid ($2.7 \times 10^{-5} - 6.5 \times 10^{-5}$M) to 60°C for 1 h, the results did not show any improvement compared with those obtained at room temperature (Fig. 6).

The viscosity number for oleic (cis-unsaturated) and elaidic (trans-unsaturated) complexes is shown in Fig. 7. Elaidic acid was

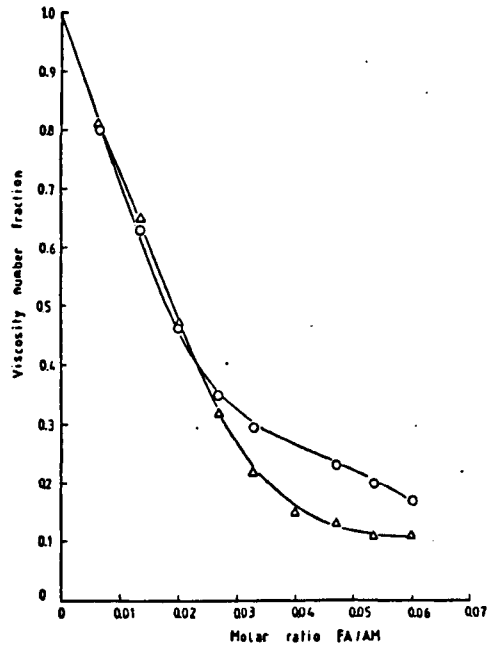

Fig. 7 Viscosity number as in Fig. 5 with: O , oleic; △ , elaidic acid

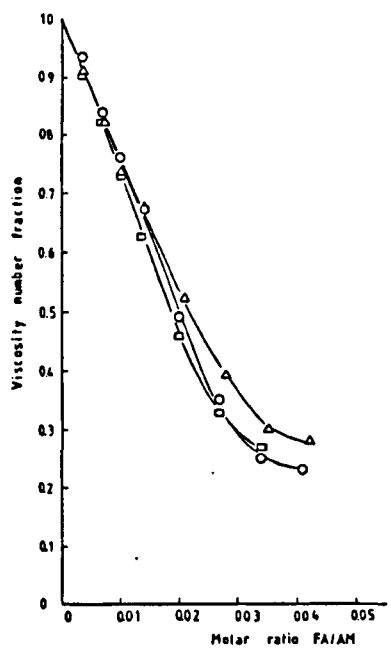

Fig. 8 Viscosity number as in Fig. 5 with: △ , linoleic; O , linolenic; □ , eicosenoic acid

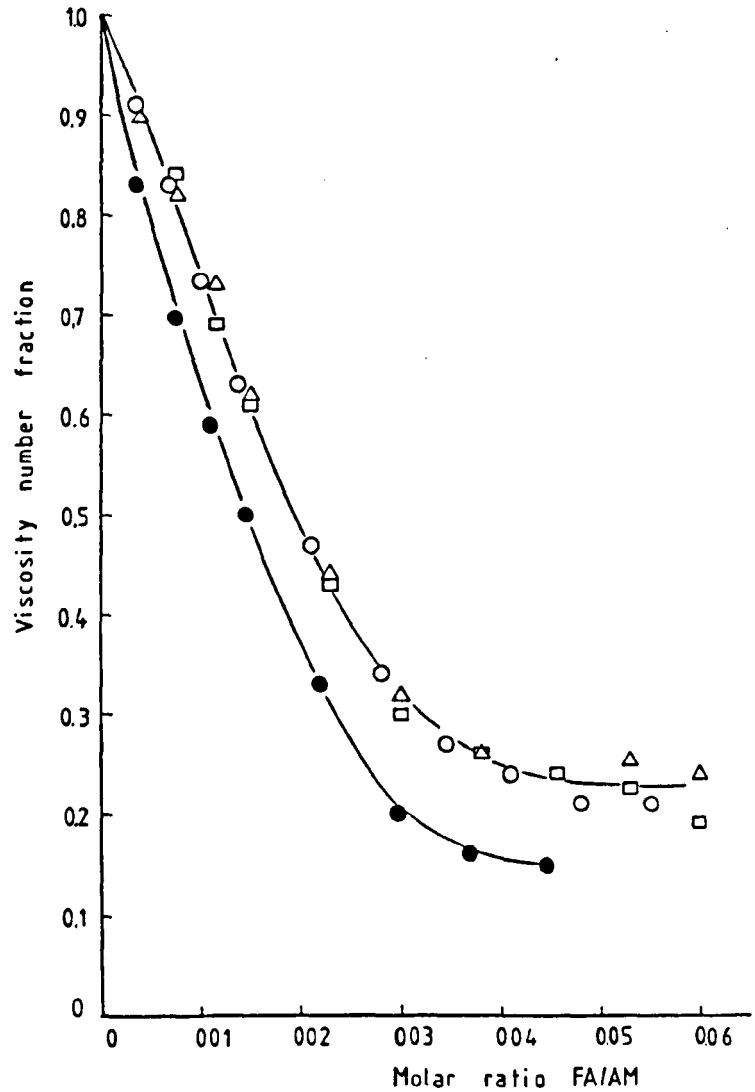

Fig. 9 Viscosity number as in Fig. 5 with: △, eicosadienoic (all cis); □, eicosatrienoic (all cis); ○, arachidonic (all cis); ●, arachidic acid

more effective than oleic acid at a high molar ratio probably because of the quasi-linearity of the molecule. In the case of oleic acid the molecule is curved and the complex presumably occupies a larger hydrodynamic volume compared with elaidic acid and this is reflected on the viscosity number. The effect of other unsaturated fatty acids is shown in Figures 8 and 9. All cis-unsaturated acids showed good complexing ability and arachidonic acid with 4 cis-double bonds showed only a small difference from its saturated counterpart. For comparison, Fig. 10 shows the configuration of fatty acids with cis-double bonds.

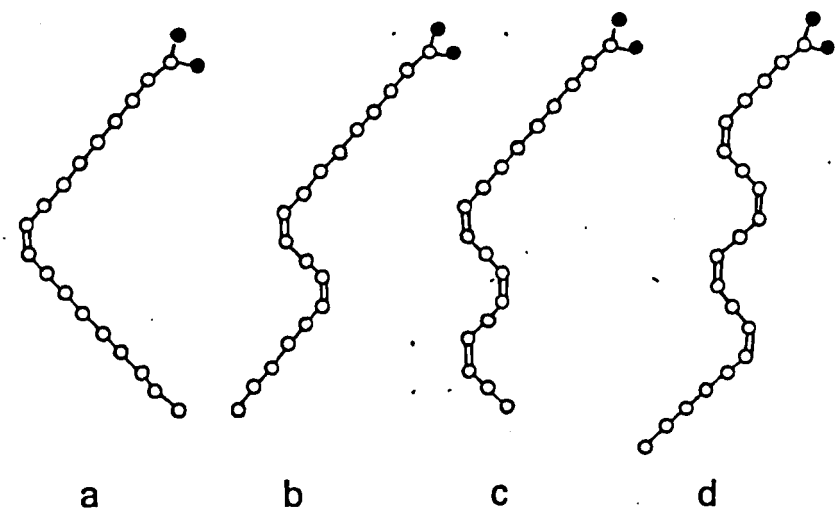

Fig. 10. Conformation of fatty acids with cis-double bonds. (a) 9-octadecenoic (oleic) acid; (b) 9,12-octadecadienoic (linoleic) acid; (c) 9,12,15-octadecatrienoic (linolenic) acid; (d) 5,8,11,14-eicosatetraenoic (arachidonic) acid (Small, 1986).

It is emphasised that the foregoing measurements were carried out at pH 12 with solutions that were visually perfectly clear and stable even after 48 h from the time of preparation. The only samples which were cloudy were those with fatty acid concentration in excess of

that needed for saturation of the amylose helix. The turbid samples, after filtration through GF/A filter, gave viscosity values identical with those given by fatty acids at concentrations required for saturation of the amylose helix. Moreover, the higher acid concentrations used exceeded the critical micelle concentration (CMC) of the individual acids, nevertheless the formation of micelles did not prevent complex formation. This was also confirmed by surface tension experiments (section 3.4).

Although the conformation of amylose in solution has been a controversial matter for years, at pH 12 it is known (Hollo and Szejtli, 1968) that hydrogen bonds which could stabilise a helical structure of amylose molecules do not exist. At this pH we must consider that amylose adopts a random coil conformation in order to explain the viscometric data. The dramatic decrease in viscosity, hence in hydrodynamic volume, could only be explained if we accept the conformational transition from a random coil to a helix, where the degree of linearity of the guest molecule does play a role. Experiments carried out in 0.1M KOH (pH 13.1) and in 0.01M KOH (pH 10.7) show that viscosity changes followed the same pattern as in 0.01M KOH although the viscosity values obtained for the 0.1M KOH concentration were higher due to the fact that the amylose chain is in the form of an expanded coil (Fig. 11). The effect of amylose concentration on complex formation is shown in Fig. 12, which indicates that saturation of the helix occurs at the same molar ratio regardless of the concentration of amylose employed. The effect of temperature on the viscosity number is shown in Fig. 13. At 65°C

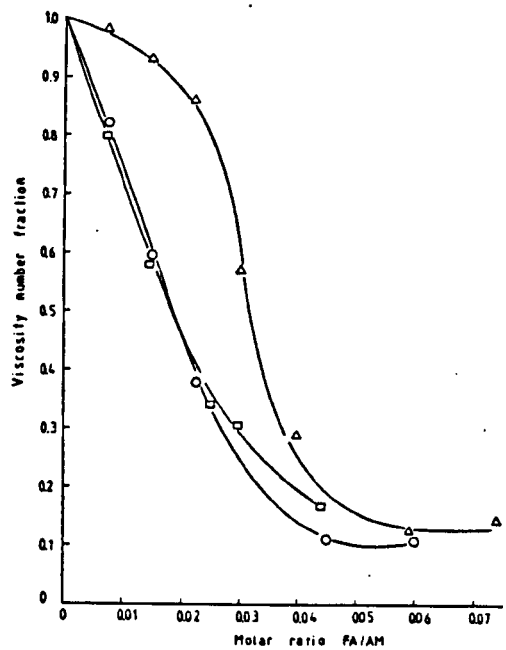

Fig. 11 Viscosity number of amylose-palmitic acid in: △ , 0·1M KOH (pH 13·1); ○ , 0·01M KOH (pH 12); □ , 0·001M KOH (pH 10·7)

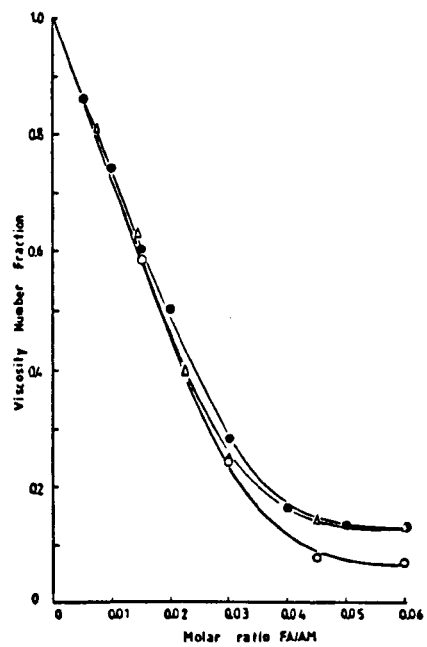

Fig. 12 Viscosity number of amylose-palmitic acid with amylose concentration: ● , 5·31 mg/ml; △ , 3·6 mg/ml; ○ , 1·8 mg/ml

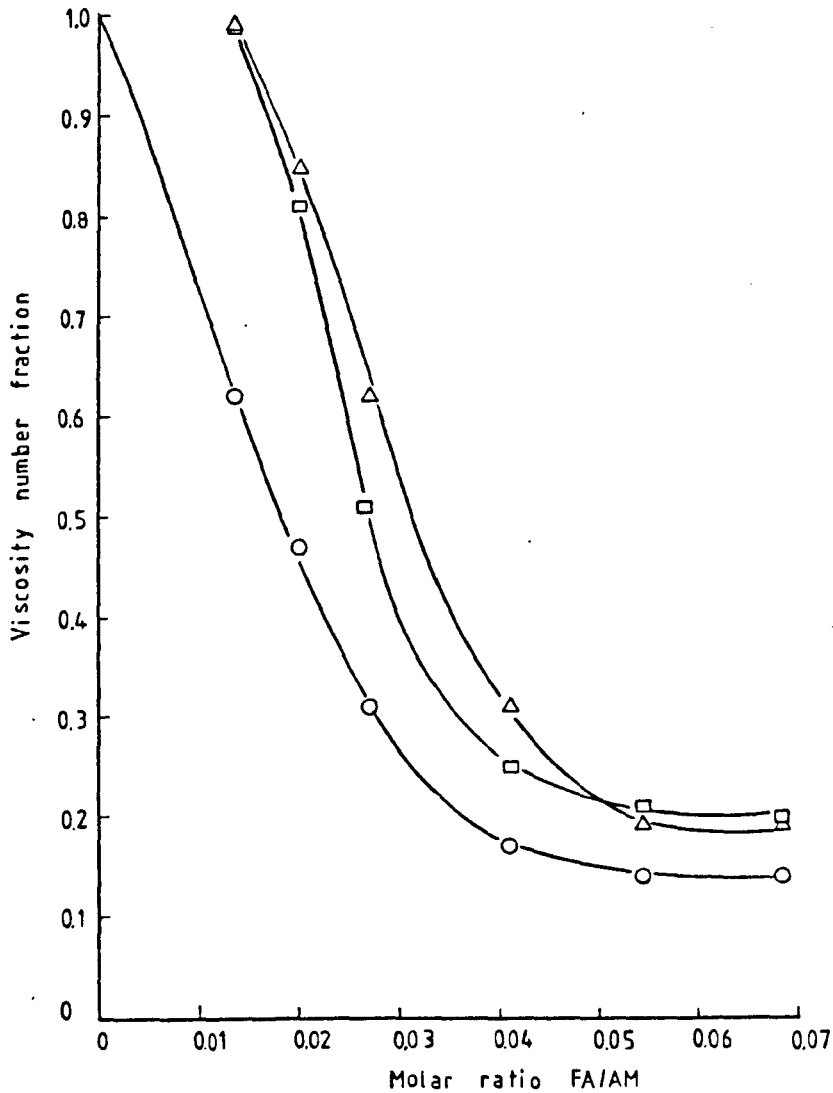

Fig. 13 Viscosity number of amylose-palmitic acid in 0·01M KOH:
○ , complexed at 20°C and viscosity measured at 20°C;
△ , complexed at 20°C and measured at 65°C;
□ , complexed at 65°C and measured at 65°C

the palmitate complex did not show signs of dissociation even at pH 12. Bulpin et al. (1986), using optical rotation, reported that a transition mid-point temperature of 63°C could be obtained for the dissociation and reformation of the amylose conformation when complexed with sodium myristate in aqueous solution.

The results from possible amylopectin-fatty acid interactions are shown in Fig. 14. There was a slight decrease in viscosity for the caprate complex which has also been reported by Whittam et al. (1986). No such decrease in the viscosity was observed with palmitate ions while with arachidate there was an initial decrease followed by an increase in the viscosity with increasing concentration. It is possible that caprate ion being of a small size could complex with the outer branches of the amylopectin molecule. The behaviour of the arachidate could be explained by assuming that the molecules being sufficiently long could complex with neighbouring branches of amylopectin molecules thus increasing their hydrodynamic volume.

3.2.2 Precipitation of amylose-lipid complexes

Insoluble precipitates of the complexes were obtained after neutralisation of the alkaline solutions used for viscosity measurements and adjustment of the pH to 4.6. Precipitated complexed amylose was calculated after determination of the residual amylose as described in section 2.3.7. As shown in Figures 15-19 at least 98% of the amylose was complexed. Similar results were obtained with the monoglycerides of fatty acids (Fig. 20). Capric (10:0) acid did not

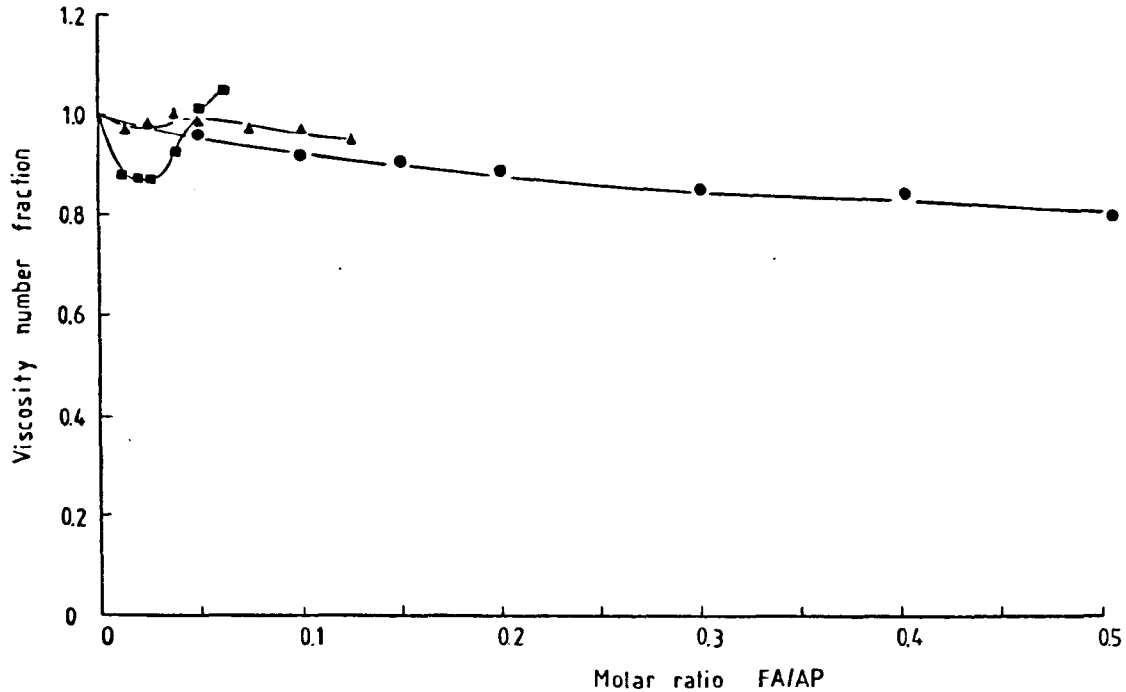

Fig. 14 Viscosity number of amylopectin (5•1 mg/ml) in 0•01M KOH with added fatty acid potassium salt: ● , capric; ▲ , palmitic; ■ , arachidic acid

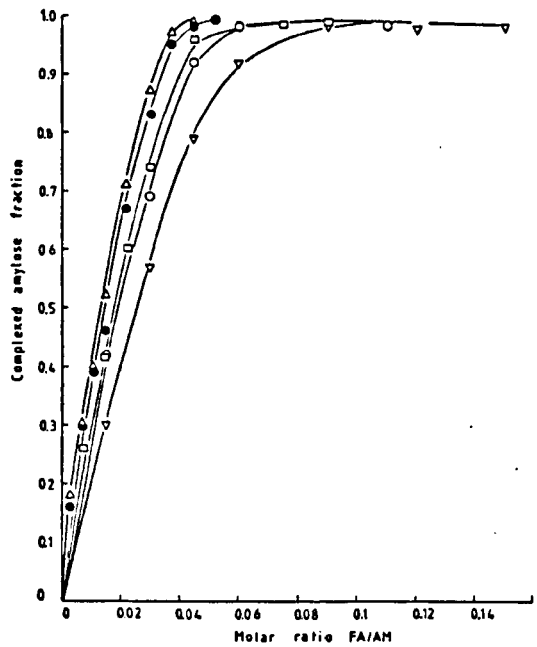

Fig. 15 Complexed amylose precipitated at pH 4·6 with:
▽, lauric; ○, myristic; □, palmitic; ●, stearic;
△, arachidic acid

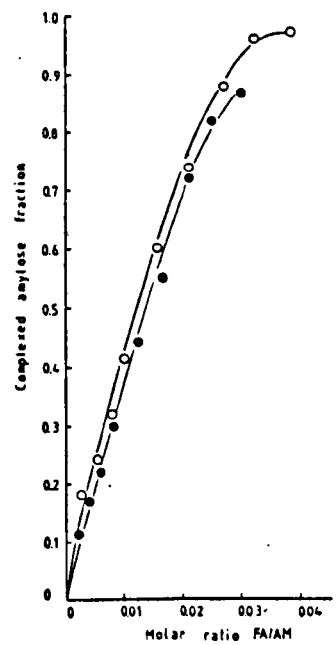

Fig. 16 Complexed amylose precipitated at pH 4·6 with:
○, behenic; ●, lignoceric acid

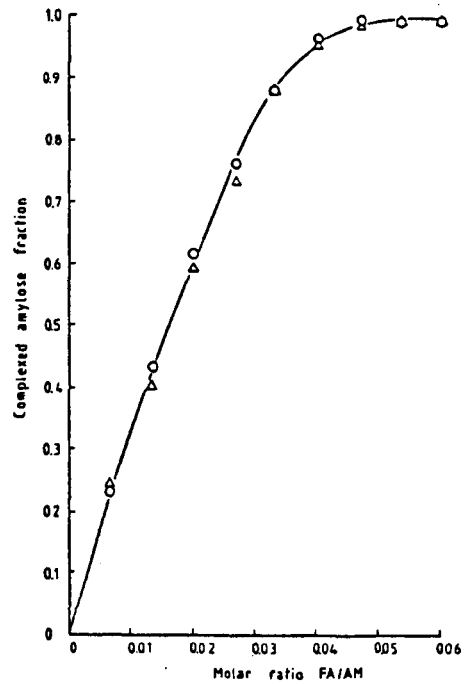

Fig. 17 Complexed amylose precipitated at pH 4·6 with:
○ , oleic; △ , elaidic acid

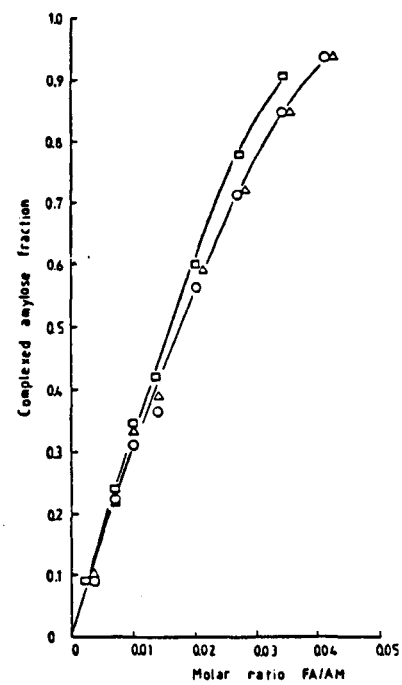

Fig. 18 Complexed amylose precipitated at pH 4·6 with:
○ , linoleic; △ , linolenic; □ , eicosenoic acid

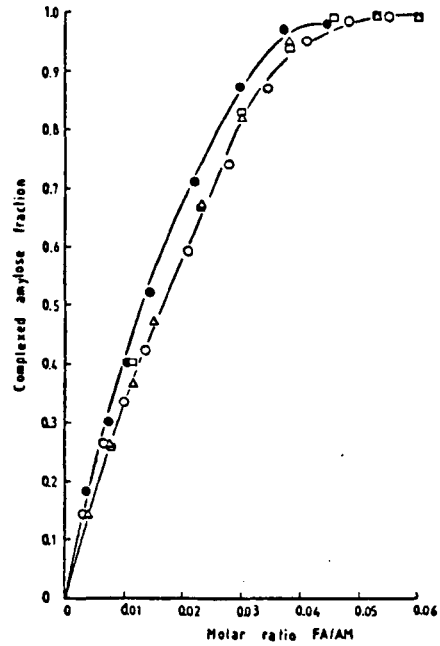

Fig. 19 Complexed amylose precipitated at pH 4·6 with:
△, eicosadienoic; □, eicosatrienoic; ○, arachidonic;
●, arachidic acid

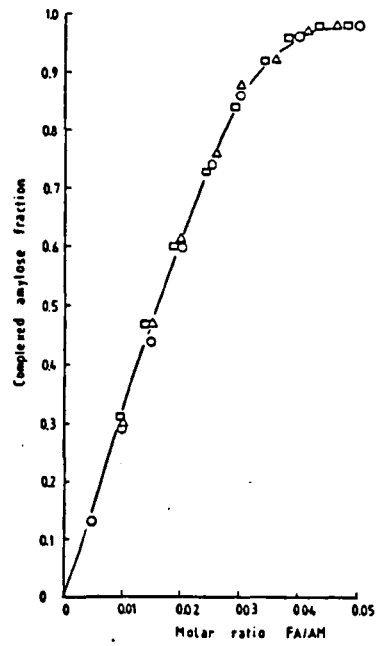

Fig. 20 Complexed amylose precipitated at pH 4·6 with liposomes:
○, 1-monopalmitoyl-rac-glycerol; △, 1-monostearoyl-rac-glycerol; □, 1-monooleoyl-rac-glycerol

complex at pH 12 but gave complete precipitation at pH 4.6 (Fig. 21). Caprylic (8:0) acid gave rise to complete precipitation at a high molar ratio as expected, while butyric (4:0) acid did not give a precipitate even at very high levels. As far as the effect of the KOH concentration is concerned, after neutralisation and precipitation of the complexes at pH 4.6, saturation of the helix occurred at exactly the same molar ratio regardless of the original pH (Fig. 22). The amylose concentration did not have any effect on the complexing ability at pH 4.6 as shown in Fig. 23. All three samples, each with a different amylose concentration, after neutralisation and precipitation, showed saturation at exactly the same molar ratio. On the other hand when amylopectin-fatty acid samples were neutralised and the pH adjusted to 4.6 crystalline aggregates of the free fatty acids were formed particularly at high levels of addition of fatty acids. Subsequent enzymic hydrolysis of the aqueous phase showed no change in the amylopectin concentration in comparison with the control containing only amylopectin. It was thus confirmed that amylopectin did not form inclusion complexes and that the formation of aggregates was due to the phase change of the fatty acid anions during neutralisation. According to Small (1986) during the titration of fatty acid soaps from pH 12 to pH 4, depending on the concentration and chain length, fatty acids form (at pH 12) micelles which, as the pH decreases, give rise to a mixture of micelles and acid soap-crystals. Eventually the system becomes increasingly turbid and at pH 5 two phases are formed *viz.* fatty acid crystals and water. This does not happen in the amylose-fatty acid soap systems, unless the fatty acid concentration is well

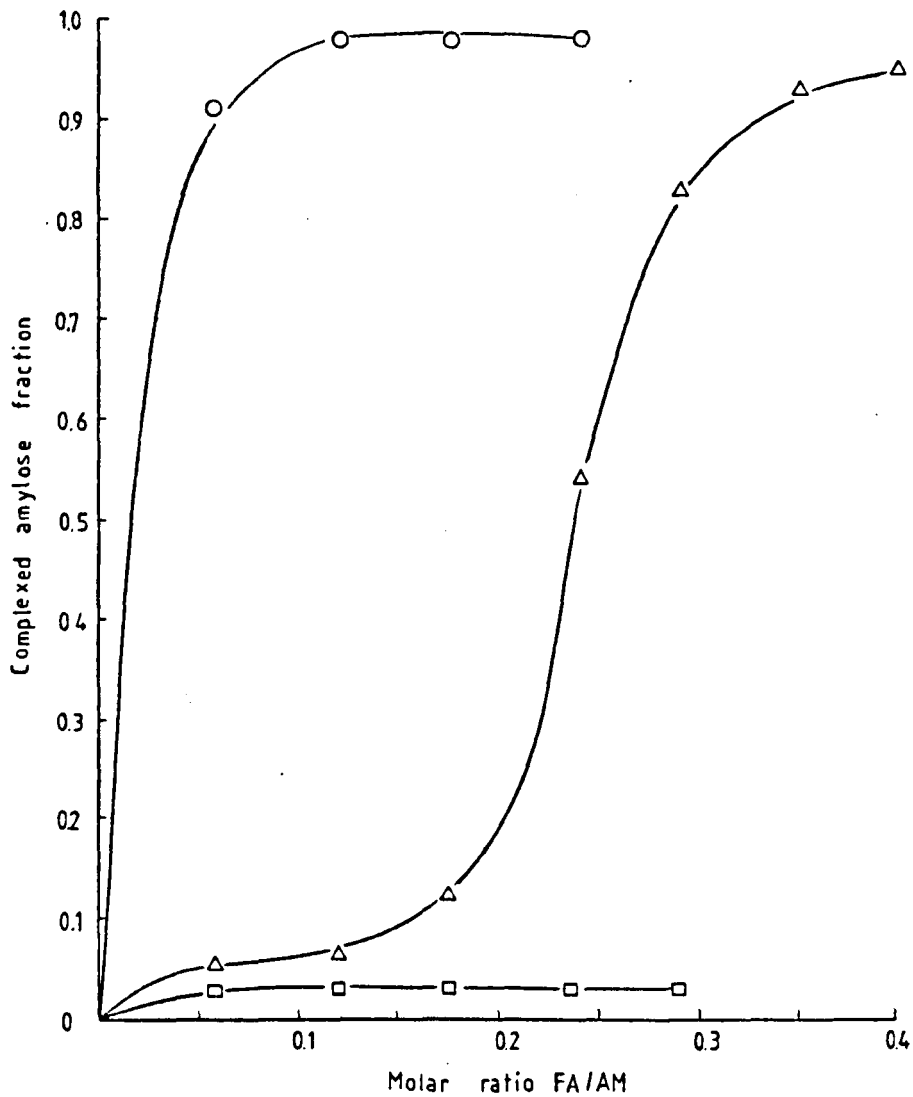

Fig. 21 Complexed amylose precipitated at pH 4•6 with:
○ , capric; △ , caprylic; □ , butyric acid

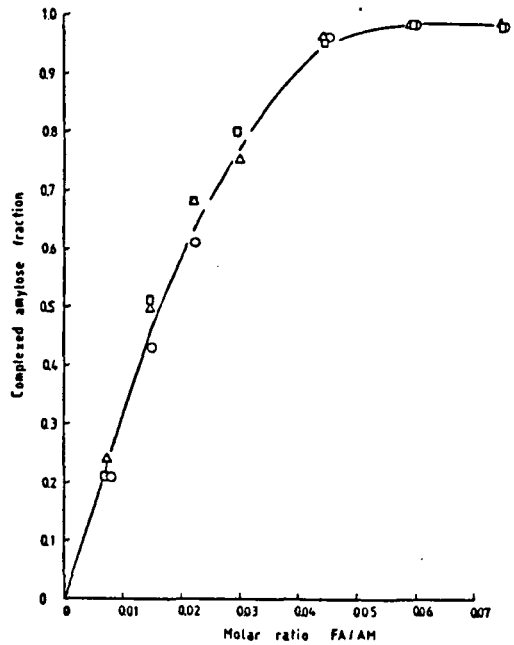

Fig. 22 Complexed amylose precipitated at pH 4·6 with palmitic acid from original KOH solutions: △ , 0·1M; ○ , 0·01M; □ , 0·001M

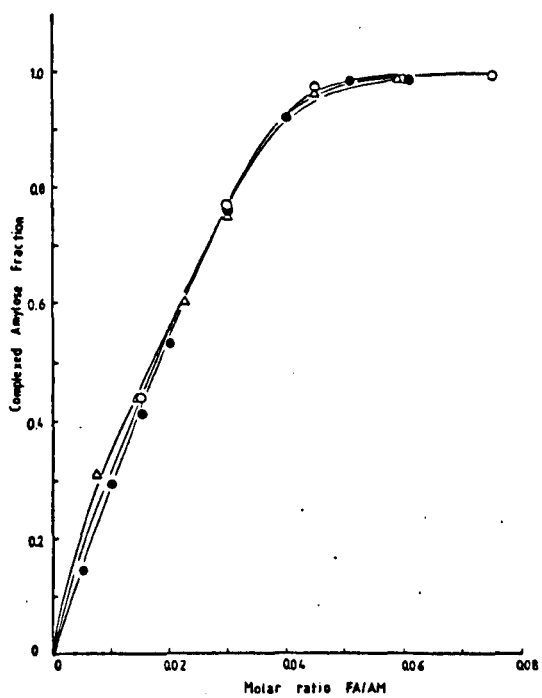

Fig. 23 Complexed amylose precipitated at pH 4·6 with palmitic acid from original KOH solutions. Amylose concentration: ○ , 1·8 mg/ml; △ , 3·6 mg/ml; ● , 5·31 mg/ml

in excess of that needed for saturation of the amylose helix and this was proved by GLC.

3.2.3 Gas-liquid chromatography

Fatty acids were determined by GLC after extraction with diethyl ether as explained in the experimental section 2.3.16. The results are shown in Table 4. Complexed fatty acids were calculated as the difference between added fatty acids (control) and ether extractable fatty acids (free). After hydrolysis with amyloglucosidase it was expected that the extracted fatty acids will be equal to the control. The incomplete recovery, after hydrolysis, is probably a reflection of the difficulty in extracting the fatty acids from the aqueous medium due to proteinaceous material by the enzyme. High levels of amyloglucosidase addition (10 mg/ml) resulted in low recoveries. It is possible that a fatty acid-protein interaction at the ether/water interface prevented the complete extraction of the acid. Replicate determinations of amylose indicated that a virtually complete hydrolysis of amylose lipid complexes was achieved by the method employed (Karkalas, 1985). Therefore, incomplete enzymic degradation of the complexes cannot explain the low recovery figures. It is emphasised that samples of amylose-lipid complexes 1 mg/ml suspended in buffer (pH 4.6) are completely hydrolysed by amyloglucosidase (1 mg/ml) within 30 min at 60^{o}C, in contrast to the general belief that enzymic degradation of the complexes is either incomplete or that it requires prolonged incubation time, over 6 hours, as described in section 1.8. On the other hand, an attempt

Table 4. Fatty acids extracted with diethyl ether from amylose complexes before and after hydrolysis with amyloglucosidase

Control[a]	Ether extractable (free)	Complexed (by difference)	Extracted after hydrolysis[b]	Recovery per cent by hydrolysis	Molar ratio[c]
Capric acid (mg)					
0.163	0.052	0.111	0.135	82.8	0.008
0.334	0.123	0.211	0.305	89.7	0.014
0.564	0.158	0.406	0.493	87.4	0.028
0.746	0.368	0.378	0.661	88.6	0.026
1.173	0.892	0.281	0.970	82.7	0.019
Lauric acid (mg)					
0.213	0.000	0.213	0.106	49.8	
0.390	0.000	0.390	0.186	47.7	0.024
0.580	0.222	0.558	0.360	62.1	0.034
0.780	0.043	0.737	0.451	57.9	0.045
1.170	0.316	0.854	0.920	78.6	0.052
Palmitic acid (mg)					
0.200	0.000	0.200	0.140	70.0	
0.408	0.000	0.408	0.292	71.6	
0.593	0.000	0.593	0.416	70.2	
0.790	0.000	0.790	0.556	70.4	0.037
1.152	0.330	0.820	1.040	90.3	0.038
Stearic acid (mg)					
0.132	0.000	0.132	0.095	72.0	
0.278	0.000	0.278	0.212	76.3	
0.476	0.000	0.476	0.321	67.4	
0.563	0.000	0.563	0.295	52.4	0.024
0.944	0.065	0.879	0.481	51.0	0.038
Arachidic acid (mg)					
0.105	0.000	0.105	0.050	47.6	
0.250	0.000	0.250	0.154	61.6	
0.410	0.000	0.410	0.274	66.8	
0.476	0.000	0.476	0.347	72.9	0.021
0.747	0.036	0.711	0.672	90.0	0.036

[a] Added fatty acid
[b] Includes free and complexed fatty acid
[c] Complexed fatty acid/amylose

to use carbon tetrachloride as a solvent gave rise to an emulsion and its use was discontinued. The results in Table 4 also suggest that complexed fatty acids with a long chain (16:0, 18:0 and 20:0) were protected within the helix and, therefore, could not be readily extracted with diethyl ether, although small quantities of these acids were extracted as saturation of the helix was approached. It is also noteworthy that short chain fatty acids (10:0 and 12:0) were easily extracted from the helix and this is in accord with their non-ideal behaviour.

3.2.4 Calculation of molar ratios at the saturation point

From X-ray diffraction studies of crystalline complexes of amylose with a variety of organic molecules, it is known that each turn of the amylose helix may consist of 6, 7 or 8 glucosyl residues (Takeo et al., 1973; Yamashita and Monobe, 1971) and that the number of residues depends on the size and shape of the guest molecule (French et al., 1963) as well as the degree of hydration (Bumb and Zaslow, 1967) of the complex. The majority of the authors appear to agree that the repeating distance, or pitch, between adjacent helical turns of the same coil is very nearly 0.8 nm. In the present study the chain length of fatty acids was calculated from published data (Pauling, 1960; C.R.C. Handbook, 1980) for bond angles. For the non-ionised acids, assuming no gaps between adjoining molecules, the number of glucosyl residues per single fatty acid was also calculated. Little is known regarding the exact arrangement of the fatty acids inside the amylose helix. It is possible that at pH

4.6 the molecules occupy the central cavity as paired dimers linked by hydrogen bonding at their carboxylic groups as Mikus et al. (1946) suggested and that the aliphatic chains are stabilised by hydrophobic interactions in the interior of the helix. However, this arrangement is unlikely at pH 12 because of the fatty acid salts. Table 5 gives the length of fatty acids, the number of glucosyl residues for each fatty acid chain and the corresponding saturation molar ratio of fatty acid/amylose (FA/AM). The structure of palmitic acid and 1-monopalmitoylglycerol is shown in Fig. 24 together with information for the calculation of the chain length.

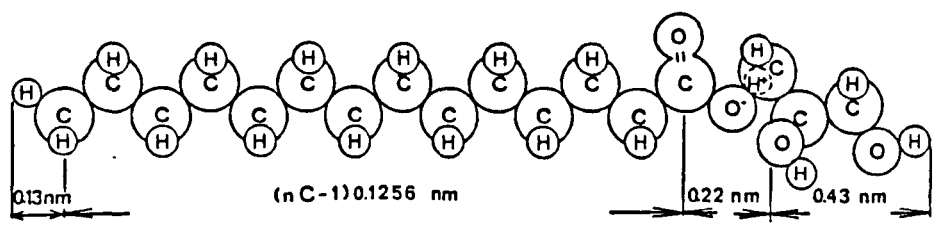

Fig. 24. Model structure of palmitic and 1-palmitoyl-rac-glycerol.
Hydrocarbon chain length = (nC-1) 0.154 cos 35.35° = (nC-1) 0.1256.
Total length of non-ionised fatty acid: L = (nC-1) 0.1256 + 0.13 + 0.22 nm.
Length of 1-palmitoyl-glycerol: L = (nC-1) 0.1256 + 0.13 + 0.22 + 0.43 nm.

It is noteworthy that since the minimum number of glucosyl residues per helical turn is known to be 6, the highest possible molar ratio (FA/AM) is as shown in Table 5 for various acids. It is, of course, assumed that the helical cavity is saturated by the linearly arranged fatty acid molecules and that no fatty acid molecules occupy the interstitial spaces between the folding lengths of the helices on the formation of crystalline complexes. For stearic acid, for instance, 9.5 mg acid are required to saturate

Table 5. Calculated fatty acid chain length, number of glucosyl residues per fatty acid and saturation molar ratio of fatty acid/amylose.

Fatty acid	Carbon atoms	Length (nm)	Glucosyl residues per fatty acid			Molar ratio (FA/AM)		
			6[a]	7[a]	8[a]	6[a]	7[a]	8[a]
Capric	10	1.48	11.1	13.0	14.8	0.090	0.077	0.058
Lauric	12	1.73	13.0	15.1	17.3	0.077	0.066	0.058
Myristic	14	1.98	14.9	17.3	19.8	0.067	0.058	0.051
Palmitic	16	2.23	16.7	19.5	22.3	0.060	0.051	0.045
Stearic	18	2.49	18.7	21.8	24.9	0.054	0.046	0.040
Arachidic	20	2.74	20.6	24.0	27.4	0.049	0.042	0.036
Behenic	22	2.99	22.4	26.2	29.9	0.045	0.038	0.033

[a]Numbers correspond to 6, 7 and 8 glucosyl residues per helical turn, with a repeating distance (pitch) of 0.8 nm between turns.

100 mg amylose. A higher figure would suggest accumulation of the fatty acid in the interstices. The results for the viscosity number of amylose with added fatty acids at pH 12 suggest that the amylose helix must be saturated by the complexed fatty acid anions since there is no further reduction in the viscosity number on the addition of excess fatty acid. The same effect is seen even more clearly at pH 4.6 when the insoluble complexes are precipitated from solution. Table 6 shows that for each fatty acid complexed at pH 12 and pH 4.6 saturation of the amylose helix occurs at a molar ratio FM/AM which, within the experimental error, is in very good agreement with calculated value for 7 glucosyl residues per turn. The occurrence of 7-membered helices has been advocated by other investigators who used different experimental techniques (Yamashita and Hirai, 1966; Bumb and Zaslow, 1967; Jane and Robyt, 1984). Takeo et al. (1973) reported 7 glucosyl residues per turn for wet precipitates of amylose fatty acid complexes and 6-glucosyl units per turn for complexes dried at 100°C. Fig 25 shows the computer drawn model of an inclusion complex with the amylose helix having 7-glucosyl residues per turn.

On the other hand, it could be argued that each helical turn consists of 6 glucosyl units and that the fatty acid molecules inside the helix are separated by a short gap, thus occupying 6/7 or about 86% of the available length. It should also be borne in mind that for six-membered helices the molar ratio at saturation is expected to be smaller if the van der Waals dimensions of the fatty acid molecules are taken into account (Mikus et al., 1946). In

Table 6. Experimental molar ratios of lipid/amylose for saturated amylose helices and calculated values based on the lipid chain length

Lipid	pH 12[a]	pH 4.6[b]	GLC[c]	Calculated[d]
Capric	-	0.100	0.029	0.077
Lauric	-	0.068	0.052	0.066
Myristic	0.060	0.058		0.058
Palmitic	0.055	0.052	0.038	0.051
Stearic	0.046	0.047	0.038	0.045
Arachidic	0.042	0.042	0.036	0.040
Behenic	0.040	0.038		0.036
Oleic (cis)	0.050	0.044		0.046
Elaidic (trans)	0.048	0.044		0.046
Linoleic (cis)	0.041	0.044		
Linolenic (all cis)	0.041	0.044		
Eicosenoic (cis)	-	0.042		
Eicosadienoic (all cis)	0.048	0.045		
Eicosatrienoic (all cis)	0.048	0.045		
Arachidonic (all cis)	0.048	0.048		
Monopalmitoyl glycerol	-	0.046		0.043
Monostearoyl glycerol	-	0.046		0.039
Monooleoyl glycerol	-	0.046		

[a] Capillary viscometry data
[b] By determination of precipitated amylose
[c] By extraction with diethyl ether and gas-liquid chromatography
[d] Molar ratio based on 7 glucosyl residues per helical turn

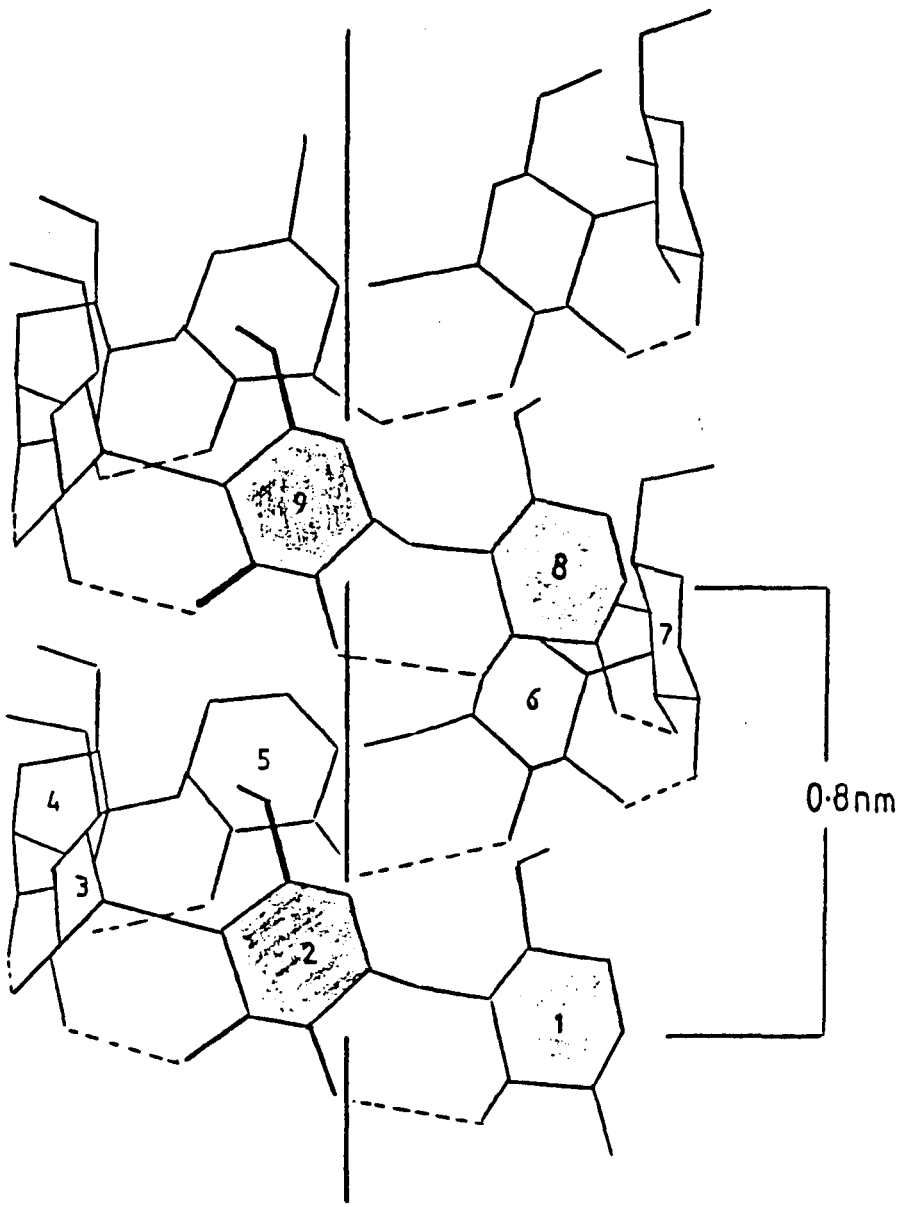

Fig. 25. Amylose helix showing pitch (0.8 nm) and seven residues per turn. (French and Murphy, 1977).

addition, the uncertainty in the estimation of the saturation molar ratio from the graphs should be considered. Bulpin et al. (1986) found, in agreement with the present work, that short chain fatty acids (below 10:0) did not give rise to saturation. They attributed this to adsorption of the acids onto the amylose. It is suggested here that this is due rather to electrostatic repulsions of the anions. On the other hand, Whittam et al. (1986) reported that sodium caprate did complex with amylose, in neutral aqueous solution, up to a lipid:amylose ratio 1:1 by weight. This ratio seems to be very high and it has also been used by Takeo et al. (1973) to precipitate amylose with short chain fatty acids e.g. (2:0 - 4:0). It is very likely that at this ratio coacervation might also occur apart from complex formation. The terms "amylose complexing index" and "relative complexing efficiency" have been used by Krog (1971) and by Riisom et al. (1984) to characterise the interaction of saturated and unsaturated monoglycerides with amylose. To eliminate the effects of different mesomorphic states, the monoglycerides were used by the latter authors, as in the present work, in the form of liposomes which are the most active physical form of monoglycerides. The results shown in Fig. 20, indicate that, within the experimental error, there is no difference between the complexing ability of monostearate, monopalmitate and monooleate and that the saturation molar ratio is 0.046. Recalculation of the data of Riisom et al. (1984) for monopalmitate and monooleate gives molar ratios of 0.033 and 0.024 respectively and therefore incomplete filling of the helix is indicated. Meuser, van Langerich and Stender (1984) used the term "starch-lipid complexing index" to express their results from

extrusion cooking of starch-lipid mixtures. According to their findings, myristic or monomyristate (14:0) showed the highest complexing ability with starch which has also been reported by Krog (1971). Although the results of the present work by no means support this view it is possible that, given the conditions these authors employed in their experiments, myristate derivatives could occur in the most favourable form, compared with other lipids, due to the intermediate size of the chain length. Aspects of the quantitative interactions between fatty acids and amylose, particularly with regard to the saturation of the amylose helix, have been described in a recent paper (Karkalas and Raphaelides, 1986).

3.3 Rheology

The rheological study of amylose solutions or of gelatinised starch suspensions poses many problems due to the retrogradation of amylose or to the structural breakdown of the starch granules during shearing. Dilatancy exhibited by very concentrated starch suspensions is an additional problem. In the present study, in order to prevent retrogradation during the viscometric measurements, amylose solutions in 0.15M KOH were used. It was found that this concentration of alkali gave stable solutions of amylose (up to 20 mg/ml) for at least one hour at 20°C. Besides, Banks and Greenwood (1975) have proved that alkaline degradation of amylose in 0.15M KOH did not occur within the time scale of their experiments. Amylopectin samples on the other hand were prepared in 0.01M KOH, since amylopectin retrogrades very slowly and it can remain stable for

weeks. Samples containing mixtures of amylose and amylopectin at various ratios were also prepared in 0.01M KOH, where the amylose concentration was sufficiently low (less than 15 mg/ml) and its precipitation was hindered by the presence of amylopectin.

In order to study the effect of the addition of fatty acids on the rheological properties of starch without the interference of the granule, non-granular starch was used. Non-granular starch was prepared essentially as described by Banks and Greenwood (1975) by dissolving it firstly in DMSO and then precipitating by the addition of excess butanol. The starch solutions were prepared as described in section 2.3.10. Moreover, Dickinson and Stainsby (1982) reported that starch dissolved in DMSO after continuous stirring for one hour completely separates into amylose and amylopectin and this implies that the granular structure is essentially lost. The starch solutions also contained 0.01M KOH to ensure that no precipitation would occur during the course of the experiments either due to retrogradation or due to interaction with added fatty acids with the amylose present. All rheological measurements were carried out at $20^{o}C \pm 0.1^{o}C$.

3.3.1 Viscosity measurements of amylose or amylopectin-fatty acid solutions

The viscosity, i.e. the ratio of tangential stress and shear rate in steady shear flow, as a function of the shear rate (measured in the Rheomat) is shown in Figures 26 - 28. On the addition of palmitic acid the viscosity decreased (Fig. 26) as the molar ratio FA/AM increased. Myristic and stearic acids showed

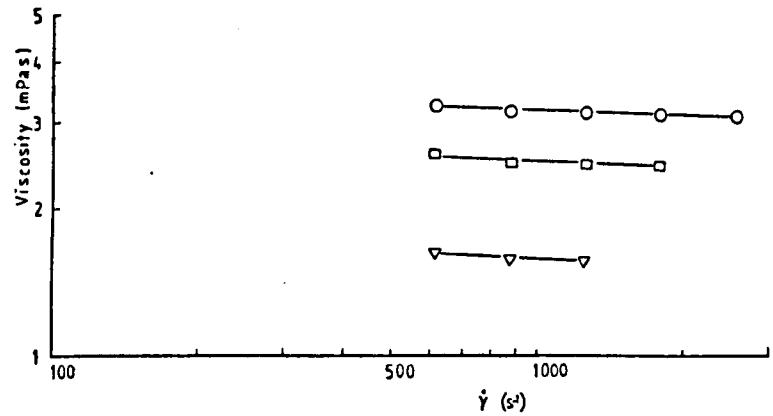

Fig. 26 Viscosity of amylose (10 mg/ml) in 0·15M KOH as a function of shear rate, with added fatty acid potassium salt: ○ , amylose (control); □ , palmitic acid (0·04); ▽ , palmitic acid (0·06). Molar ratio FA/AM in parentheses.

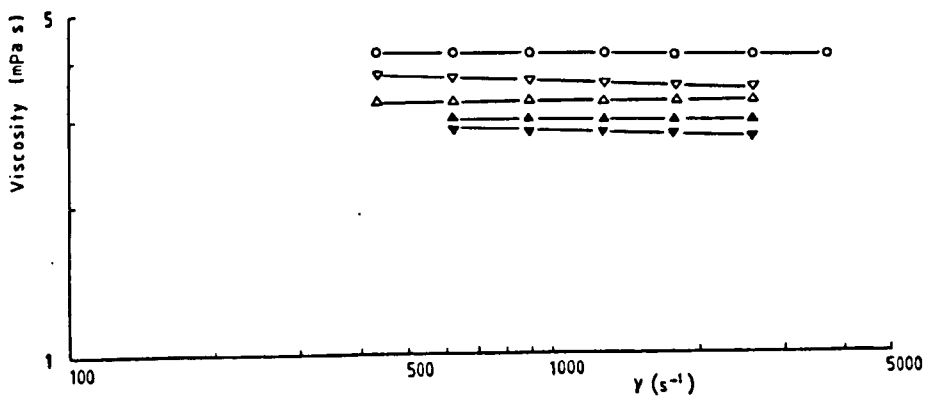

Fig. 27 Viscosity of amylose (13·4 mg/ml) in 0·15M KOH as a function of shear rate, with added fatty acid potassium salt: ○ , amylose (control); △ , myristic acid (0·050); ▲ , myristic acid (0·090); ▽ , stearic acid (0·015); ▼ , stearic acid (0·030). Molar ratio FA/AM in parentheses.

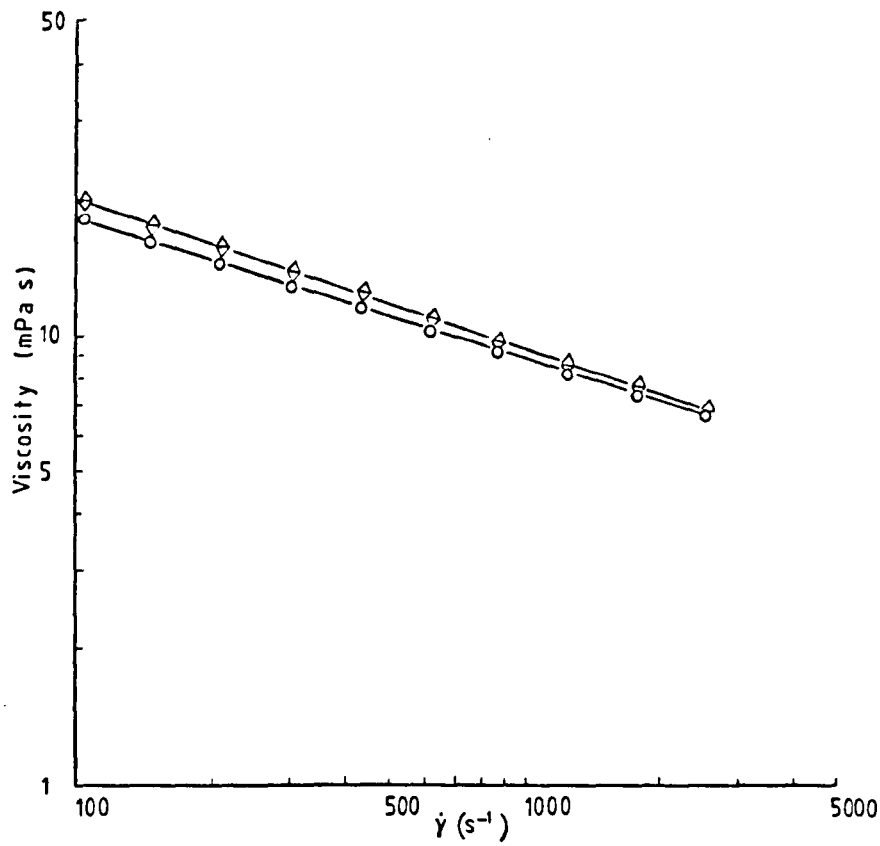

Fig. 28 Viscosity of amylopectin (13·6 mg/ml) in 0·01M KOH as a function of shear rate, with added stearic acid potassium salt: ○ , amylopectin (control); △ , (0·012); ▽ , (0·024). Molar ratio FA/AP in parentheses

similar effects (Fig. 27) and in accord with the results obtained by capillary viscometry (section 3.2.1). It is noteworthy that, with stearic acid, the pseudoplastic nature of the solution of amylose increased. This could be attributed to the increased rigidity of the helical complex due to the length of the fatty acid. By contrast, the addition of stearic acid to amylopectin gave rise to a very small increase in the viscosity (Fig. 28) which was largely independent of the fatty acid concentration. However, the pure amylopectin solution was strongly pseudoplastic and this could be explained as due to elongation and alignment of the highly branched molecules (i.e. the racemose structure) with increasing shear rate.

In general, while at a molar ratio of $\frac{FA}{AM}$ about 0.04 the solutions were virtually clear, a slight turbidity was observed at a molar ratio of 0.06. At higher molar ratios an increase in the viscosity was observed presumably because of aggregation of the fatty acid anions on the complexed amylose. Banks and Greenwood (1971b) observed a similar phenomenon with amylose-iodine complexes.

3.3.2 Effect of the addition of fatty acids on the viscosity of amylose and amylopectin mixtures

Fig. 29 shows the effect of adding various concentrations of stearic acid in 0.01M KOH solutions containing mixtures of amylose (3.4 mg/ml) and amylopectin (4.7 mg/ml) in which the ratio amylopectin to amylose ($\frac{AP}{AM}$) was 1.38. Addition of fatty acid at molar ratios up to 0.094 (i.e. $\frac{\text{fatty acid}}{\text{amylose}}$) caused a reduction in viscosity and an

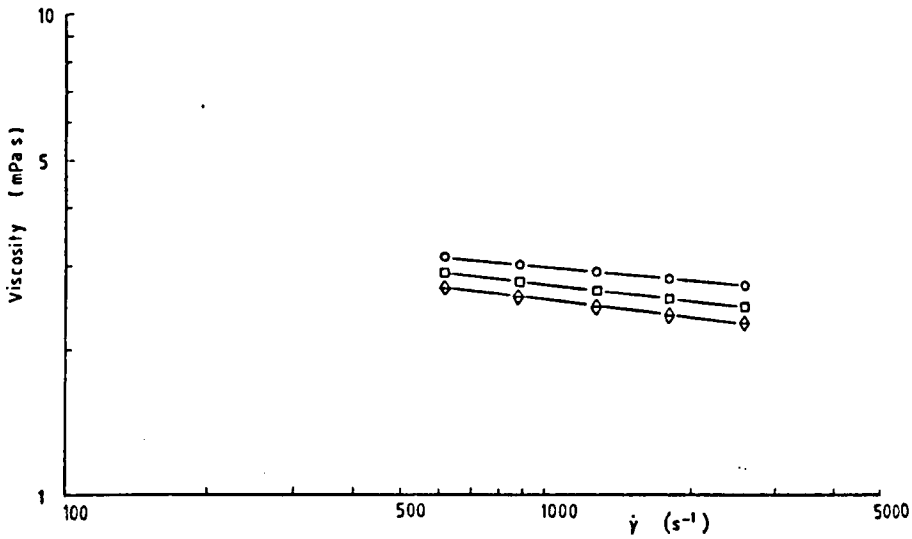

Fig. 29 Viscosity of amylopectin (4•7 mg/ml) and amylose (3•4 mg/ml) mixture in 0•01M KOH as a function of shear rate with added stearic acid potassium salt: ○ , control; □ , (0•047); △ , (0•094); ▽ , (0•188). Molar ratio FA/AM in parentheses.

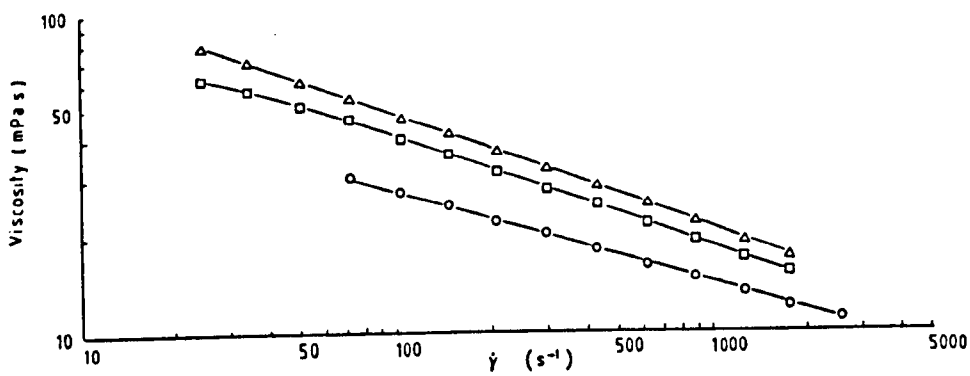

Fig. 30 Viscosity of amylopectin (18•7 mg/ml) and amylose (3•4 mg/ml) mixture in 0•01M KOH as a function of shear rate with added stearic acid potassium salt: ○ , control; □ , (0•05); △ , (0•1). Molar ratio FA/AM in parentheses.

increase in shear thinning; further addition of acid did not cause further change in the viscosity. On the other hand, when the amylopectin concentration was increased 4 times (amylose:3.4 mg/ml, amylopectin = 18.7 mg/ml, $\frac{AP}{AM}$ = 5.5), then the addition of the same quantities of stearic acid as in the previous experiment (0.3 - 0.6 mg/ml) showed a reversed behaviour (Fig. 30). There was an increase in the viscosity depending on the quantity of stearic acid added. This was investigated further with samples where the ratio of amylopectin to amylose was different whereas the concentrations of total glucan (18.4 mg/ml) and stearic acid (0.3 mg/ml) were kept the same. The results are shown in Fig. 31 (legend on Table 7). The sample containing amylopectin and stearic acid (◑) had a higher viscosity than that with amylopectin alone (○) When the ratio of amylopectin to amylose ($\frac{AP}{AM}$) was 4, the addition of stearic acid caused an increase in viscosity (■) but this increase was less pronounced than that shown by the sample containing only amylopectin and stearic acid (□). It is noteworthy that even if it were assumed that the stearate ions do not interact with amylose but form micelles instead, they cannot contribute significantly towards the increase of the viscosity of the sample. At this particular concentration (0.3 mg/ml) the viscosity of potassium stearate solution, in the absence of amylose, was indistinguishable from that of water. When the ratio $\frac{AP}{AM}$ was 1.5 the difference in viscosity was considerably reduced but again the addition of the stearic acid slightly increased the viscosity (▲ , △) When the ratio $\frac{AP}{AM}$ was 0.67 the addition of stearic acid (▼ , ▽) caused a reduction in viscosity in comparison with the sample

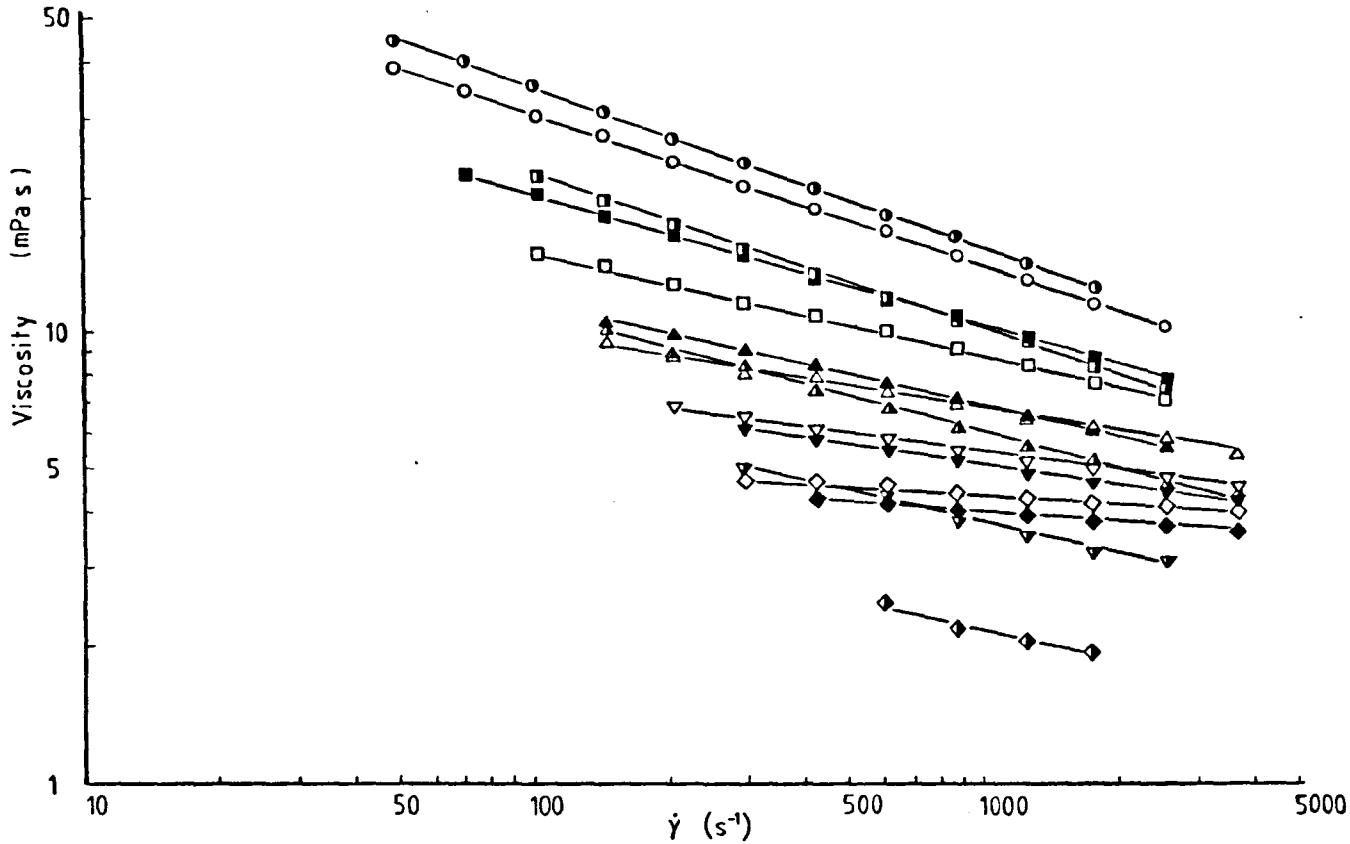

Fig. 31 Viscosity of amylopectin (AP) and amylose (AM) solutions in 0·01M KOH with and without stearic acid as a function of shear rate measured by means of the Rheomat at 20°C. For explanation of the symbols see Table 7.

Table 7. Amylopectin (AP) and amylose (AM) solutions in 0.01M KOH with, and without stearic acid, used for the determination of viscosity by means of the Rheomat.

Symbol[a]	AP (mg/ml)	AM (mg/ml)	Ratio AP/AM	Stearic acid (mg/ml)	Molar ratio Stearic acid/AM
○	18.40	-	-	-	-
□	14.72	3.68	4.00	-	-
△	11.05	7.36	1.50	-	-
▽	7.36	11.04	0.67	-	-
◇	3.68	14.72	0.25	-	-
◑	18.44	-	-	0.30	-
◧	14.72	-	-	0.30	-
◭	11.05	-	-	0.30	-
◬	7.36	-	-	0.30	-
◈	3.68	-	-	0.30	-
■	14.72	3.68	4.00	0.30	0.0465
▲	11.05	7.36	1.50	0.30	0.0230
▼	7.36	11.04	0.67	0.30	0.0155
◆	3.68	14.72	0.25	0.30	0.0120

[a] See Figure 31.

containing only amylose and amylopectin (▽) moreover the viscosity of the sample containing amylopectin and stearic acid decreased dramatically (▼). This reversion was even more pronounced when the ratio $\frac{AP}{AM}$ became 0.25. The results show that there is a significant influence of amylopectin on the interaction of amylose and stearic acid, which is particularly prominent at $\frac{AP}{AM}$ ratios close to those occurring in native starches (i.e. approx 3). The fatty acid concentration appears to be of lesser importance as shown in Fig. 29 and 30. Another interesting aspect is the shear thinning which is very pronounced in samples containing amylopectin and stearic acid or samples with high ratios $\frac{AP}{AM}$ and stearic acid. All the flow curves in Fig. 31 are linear and this implies that they obey the "power law" equation $\tau = K\dot{\gamma}^{\alpha}$ or $\eta = K\dot{\gamma}^{\alpha-1}$, where τ is the shear stress, $\dot{\gamma}$ is the shear rate, η is the apparent viscosity and K and α are constants (Evans and Haisman, 1980; Doublier, 1981). Data from the application of linear regression to the flow curves of Fig. 31 is given in Table 8. The dependence of parameter α, sometimes known as the flow behaviour index, on the amylopectin concentration is shown in Fig. 32.

The effect of fatty acid on the flow properties of amylopectin and amylopectin-amylose mixtures is clearly illustrated. At a low concentration of amylopectin (3.68 - 7.36 mg/ml) the addition of fatty acid caused an increase in the pseudoplasticity. The effect was not so pronounced when amylose was added, presumably because the acid interacted preferentially with the amylose while the amylopectin was little affected. Although it is doubtful whether true complex

Table 8. Linear regression of flow curves from Figure 31, based on equation $\eta = K \dot{\gamma}^{\alpha-1}$

Flow curve (symbol in Fig. 31)	Correlation coefficient	Consistency coefficient K	$\alpha - 1$	Flow behaviour index α
○	-0.9999	2.15	-0.33	0.67
□	-0.9995	1.64	-0.23	0.77
△	-0.9990	1.35	-0.17	0.82
▽	-0.9900	1.14	-0.13	0.86
◇	-0.9900	0.83	-0.06	0.94
◐	-0.9998	2.25	-0.35	0.65
◧	-0.9990	1.99	-0.32	0.67
◭	-0.9980	1.58	-0.27	0.73
▽	-0.9970	1.20	-0.21	0.78
◈	-0.9700	0.99	-0.21	0.78
■	-0.9999	1.90	-0.29	0.70
▲	-0.9990	1.50	-0.22	0.78
▼	-0.9970	1.14	-0.14	0.85
◆	-0.9850	0.90	-0.09	0.90

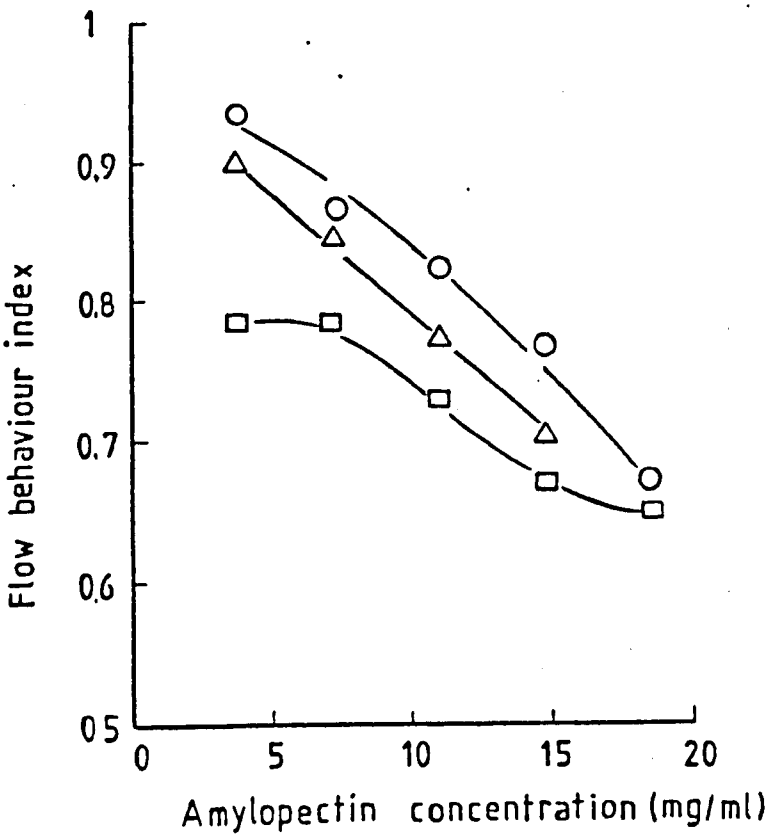

Fig. 32 The dependence of flow behaviour index on the concentration of amylopectin: ○, amylopectin + amylose; □, amylopectin + fatty acid; △, amylopectin + amylose + fatty acid.

formation can occur between amylopectin molecules and stearate anions, nevertheless the assumption of the existence of some kind of association provides an explanation for the considerable shear thinning observed; this is perhaps due to the disruption of the structure of amylopectin molecules bridged together by interactions with stearate ions.

3.3.3 Viscosity measurements of potato starch and fatty acid solutions

Potassium salts of palmitic, stearic and arachidic acids were used in these series of experiments where the effect of the fatty acid chain length on the flow properties of starch-fatty acid systems was investigated. In all the experiments the concentrations of the fatty acids were kept constant and only the concentration of starch was varied. At very low concentrations of starch (Fig. 33) all fatty acids behaved exactly in the same way as previously discussed at low glucan concentrations (Fig. 29). The maximum molar ratio, i.e. fatty acid/amylose used for all the acids was lower than that required to attain full saturation of the helix (molar ratios $\frac{FA}{AM}$: $C_{16=0}$ 0.037, $C_{18=0}$ 0.033, $C_{20=0}$ 0.030). The amylose represented 23.5% of the dry weight of starch. As the concentration of the starch increased the effect of the chain length became more pronounced. In Fig. 34 the molar ratios are close to those required to saturate the amylose helices assuming that no interaction occurs between amylopectin and fatty acids. The shear thinning is again significant in all samples compared with that of the control (without fatty acid). Figures 35 and 36 show that, with increasing concentration of starch, the viscosity

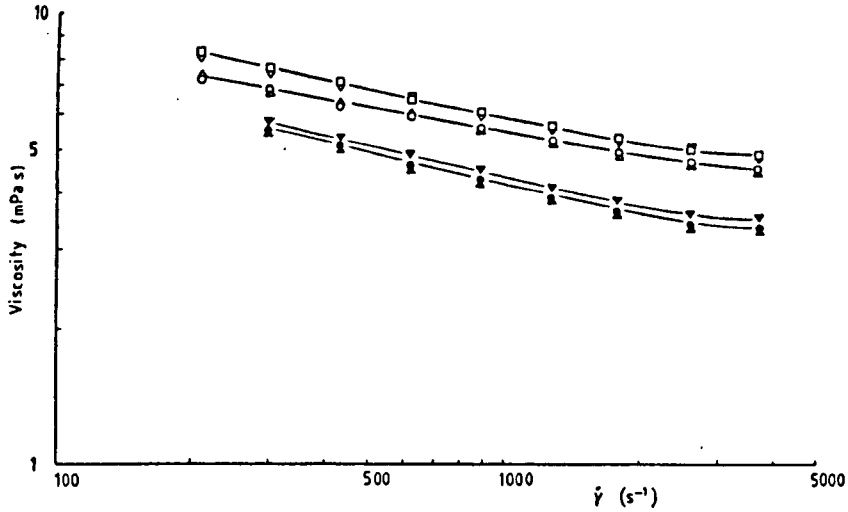

Fig. 33 Viscosity of potato starch (7•3 mg/ml) in 0•01M KOH as a function of shear rate with added fatty acid potassium salt: □ , control: ○ , palmitic (0•018); ● , palmitic (0•037); △ , stearic (0•016); ▲ , stearic (0•033); ▽ , arachidic (0•015); ▼ , arachidic (0•03). Molar ratio FA/AM in parentheses is based on the amylose content of the starch.

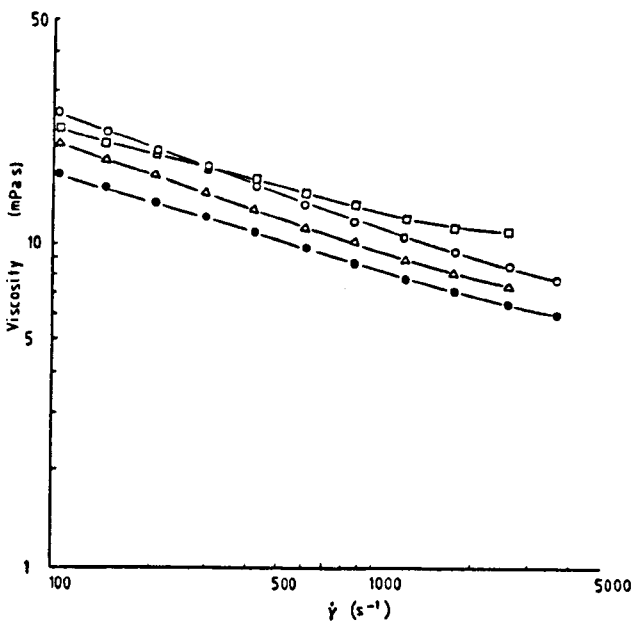

Fig. 34 Viscosity of potato starch (11•7 mg/ml) in 0•01M KOH as a function of shear rate with added fatty acid potassium salt: □ , control; ● , palmitic (0•046); △ , stearic (0•041); ○ , arachidic (0•038). Molar ratio FA/AM in parentheses is based on the amylose content of the starch.

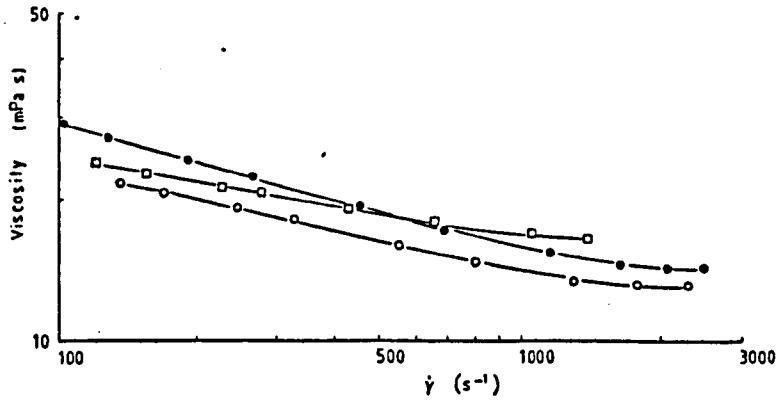

Fig. 35 Viscosity of potato starch (14·3 mg/ml) in 0·01M KOH as a function of shear rate with added palmitic acid K salt: □, control; ○, (0·038); ●, (0·075). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

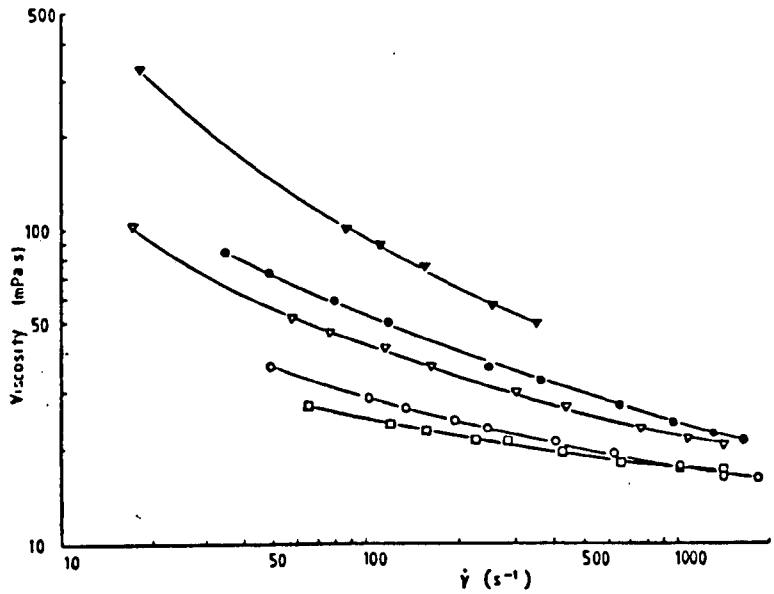

Fig. 36 Viscosity of potato starch (14·3 mg/ml) in 0·01M KOH as a function of shear rate with added fatty acid K salt: □, control; ○, stearic (0·034); ●, stearic (0·068); ▽, arachidic (0·031); ▼, arachidic (0·062). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

of even the shortest chain length fatty acid used (palmitic) increased significantly. Besides, the samples which contained an increased concentration of arachidic acid (Fig. 36, ▼—▼) showed incipient gelation. It should be pointed out that Figures 26-34 show results obtained with the Rheomat viscometer, while the results in Figures 35 and 36 were obtained with the Viscoelastic analyser operating in the continuous shear mode. The reason was that the upper limit of viscosity values which could be measured by the Rheomat, with the particular geometry used, was 150 mPa s. With arachidic acid (Fig. 36) this limit was exceeded and the Viscoelastic analyser was used instead. Nevertheless, samples from the last series of experiments, i.e. Figures 35 and 36 were measured with both instruments and the difference in the two sets of results was found to be approximately 5% which is considered satisfactory.

3.3.4 Effect of starch and fatty acid concentration on the flow behaviour of starch-fatty acid systems

The instrument used for these sets of experiments was the Weissenberg rheogoniometer which is capable of working at very low shear rates approx. $0.01 s^{-1}$. The starch concentrations used were high enough (20-45 mg/ml) to give viscosity values over 100 mPa s whereby the precision of the instrument was improved. It has to be noted that the instrument's precision was compared to that of the Viscoelastic analyser using standard silicone fluids. The difference between the two instruments was about 8% which is generally considered satisfactory. The results in Figures 37-42 show the

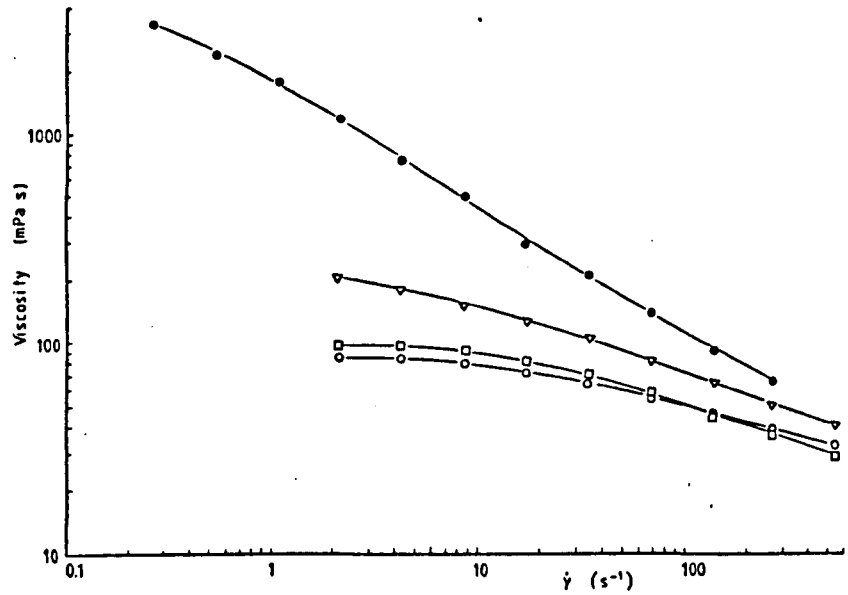

Fig. 37 Viscosity of potato starch (24 mg/ml) in 0·01M KOH as a function of shear rate, with added palmitic acid K salt: □ , control; ○ , (0·0255); ▽ , (0·045); ● , (0·09). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

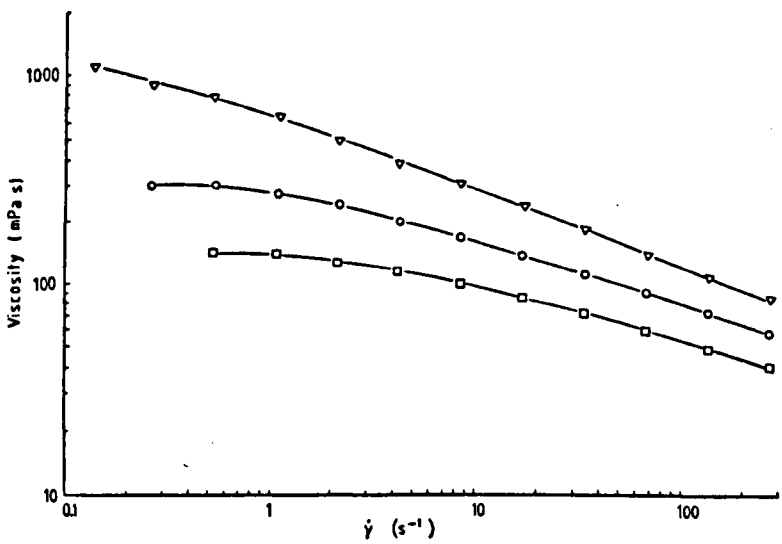

Fig. 38 Viscosity of potato starch (26 mg/ml) in 0·01M KOH as a function of shear rate, with added stearic acid K salt: □ , control; ○ , (0·0187); ▽ , (0·037). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

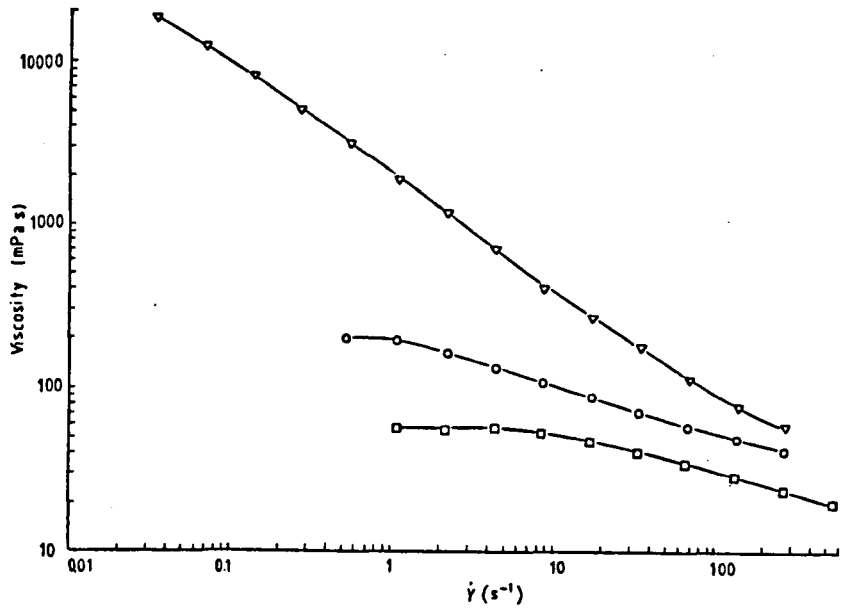

Fig. 39 Viscosity of potato starch (20 mg/ml) in 0·01M KOH as a function of shear rate, with added arachidic acid K salt: □, control; ○, (0·022); ▽, (0·044). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

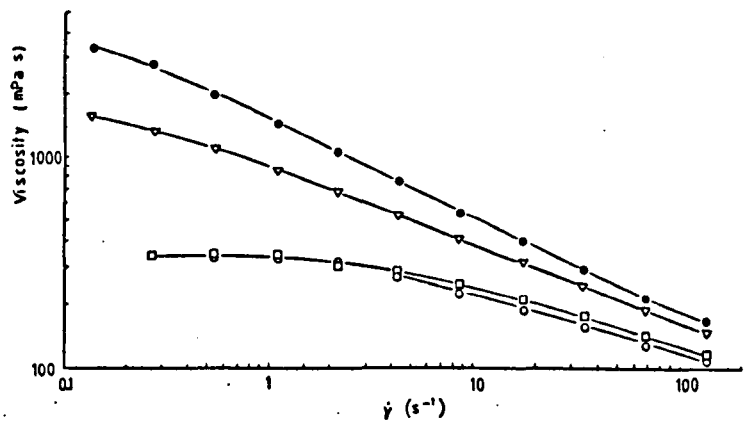

Fig. 40 Viscosity of potato starch (41 mg/ml) in 0·01M KOH as a function of shear rate, with added palmitic acid K salt: □, control; ○, (0·013); ▽, (0·026); ●, (0·039). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

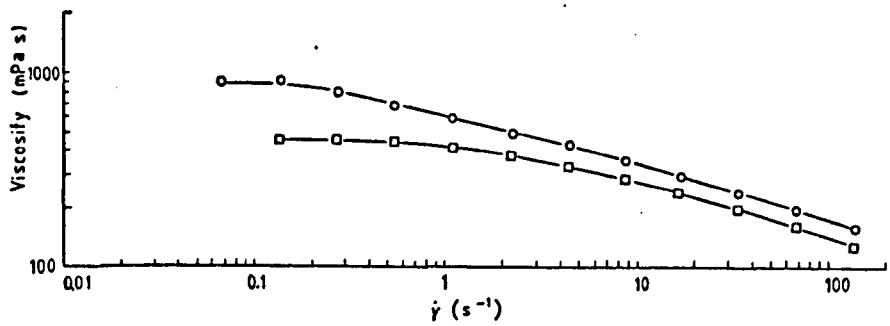

Fig. 41 Viscosity of potato starch (45 mg/ml) in 0·01M KOH as a function of shear rate, with added stearic acid K salt: □, control; ○, (0·01). Molar ratio, in parentheses, is based on the amylose content of the starch.

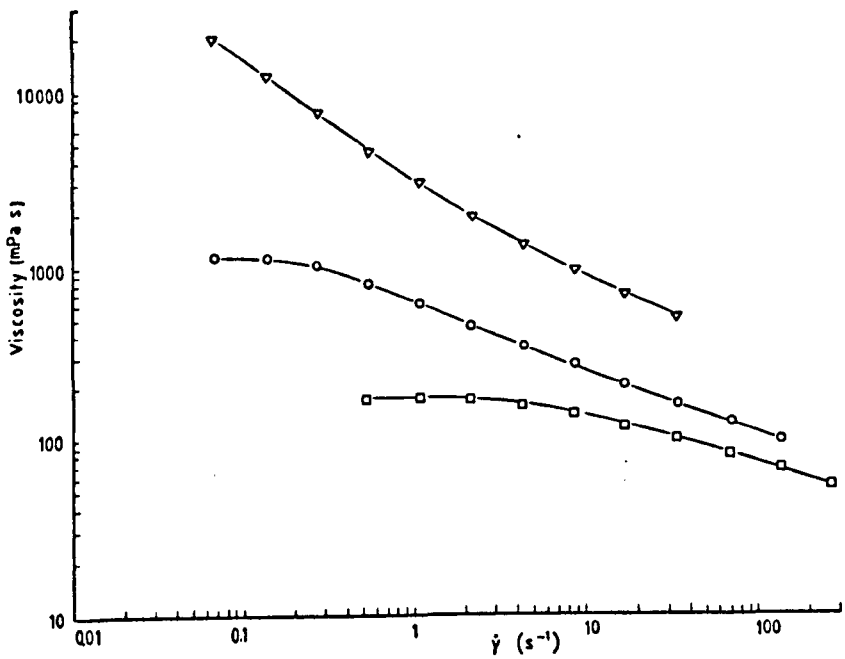

Fig. 42 Viscosity of potato starch (29 mg/ml) in 0·01M KOH as a function of shear rate, with added arachidic acid K salt: □, control; ○, (0·015); ▽, (0·03). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

effect of the fatty acid chain length with increasing starch concentration. Particularly for stearic and arachidic acids, at relatively high concentrations (0.4 mg/ml and over), caused incipient gelation of the starch solution or according to Ferry (1980) the solution became cross-linked. This was also assessed from the flow behaviour, when during shearing the samples showed structural break-down, whereas, both the control samples and samples with low fatty acid concentration did show a Newtonian region in their flow and no time dependence, i.e. no thixotropy. In order to avoid gelation, samples with a starch concentration over 30 mg/ml (with arachidic acid), and fatty acid concentrations over 0.4 mg/ml (in the case of stearic and arachidic acids) were not examined.

The rheological experiments on the amylose or starch-fatty acid systems showed that the addition of fatty acid caused changes in the flow behaviour of the systems. If the concentration of the acid added was near or above the concentration needed to saturate the amylose helices, then the viscosity of the solution increased depending, of course, on the concentration of both amylose and amylopectin. Although the amylose concentration used in all the experiments was lower than that needed to cause "physical entanglement" which is about C* = 1.5% by weight (Miles et al., 1985), however due to the polyelectrolyte character of amylose at pH 12 it is possible that the helical molecules are stretched and form partially flexible elongated molecules (Ferry, 1980) which already may overlap with each other at fairly low concentrations (Tanford, 1961; de Gennes, 1979). These helices can form "weak" gels which consist of an entanglement network formed when the system reaches a

certain critical concentration C_o which is totally different from the C* of random coil overlap concentration. The C_o could be less than C* sometimes by as much as 100 times (Ross-Murphy, 1984). These entanglements are not permanent and during shearing they break and reform depending on the relaxation period. Another contribution to the rise in viscosity could be from a form of interaction between acid ions and amylopectin branches which result into some degree of bridging particularly pronounced at high starch concentrations. This was apparent even when the fatty acid concentration was below that needed for saturating the amylose helices. In this case there should be some degree of competition between the two macromolecules in attracting the acid anions.

Recently, Evans (1986) reported that the addition of an anionic detergent, sodium dodecyl sulphate (SDS) or a cationic one, cetyl trimethyl ammonium bromide (CTAB), to gelatinised wheat starch or waxy maize starch suspensions in water caused an increase in the viscosity of the suspensions. When the author added 0·2M NaCl to the suspensions then the viscosity of the starch suspensions containing detergent was reduced to approximately the value attained by the starch suspension without detergent.

3.3.5 Normal force measurements of starch-fatty acid interactions in solution

Shearing flow tends to extend flexible macromolecules in the direction of flow. This deformation disturbs the equilibrium

conformation and stresses develop which are normal to the plane of shear. Normal stresses represent the elastic or stored energy which is the result of the molecule's internal encounter mechanism in response to the externally applied shear stress (Rha, 1979). When the stress is removed these normal or recoverable stresses return the molecule, at least partly, to its original conformation.

According to classical fluid mechanics these normal stresses are all equal to the local pressure and hence normal stress differences are zero. For non-Newtonian fluids, such as concentrated polymer solutions, at high shear rates these normal stress differences do not vanish. This situation arises because a concentrated polymer solution is a system of entangled long chains in random conformation. In the first Newtonian region (low shear rates), true viscous flow can occur because the chain segments are freely movable and because there is sufficient time for the loose entanglements to unmake and remake themselves without affecting the random ground state structure. In the pseudoplastic region (at higher shear rates), the original conformation will transform itself in such a way that the molecules will become increasingly aligned in the direction of flow. If the reattainment of the original conformation takes a relatively long time, then we have thixotropy or retarded elasticity. But at even higher shear rates, the entanglements will have no chance to unravel, consequently they will tend to tighten up frictionally and distortion of chemical bond angles and elongation of bond distances occur. This will exert a hydrostatic pressure on the containing surfaces of the liquid which can then be measured as a "normal force".

In the present work, the solutions examined were fairly dilute and although non-Newtonian, their elasticity was not particularly high, so it was decided to take into account the secondary flow which develops radially along the moving plate of the rheometer and is due to centrifugal force which becomes particularly prominent at high shear rates. This secondary flow gives an apparent negative normal pressure which depends on the velocity of the measuring plates of the rheometer, their diameter, and the viscosity and density of the test material (Walters, 1975). In the present study, the negative normal force developed by the solvent (aqueous 0.01M KOH) was measured over a wide range of shear rates (Fig 43) and the values obtained were added to the values of the positive normal force of the samples at each particular shear rate (King, 1964). The sum represents the corrected first normal stress difference ($P_{11}-P_{22}$) which is shown in Figures 44-47, as a function of shear rate. Since normal force is an indication of the elasticity exhibited by a material, it is obvious that the addition of fatty acids, particularly with long chain, e.g. arachidic, increased the elasticity of the starch samples in comparison with the control samples. This increase in elasticity, due to the addition of long chain acid molecules, was also observed in the case of concentrated amylose solutions and gelatinised starch suspensions as will be discussed in section 3.3.6.

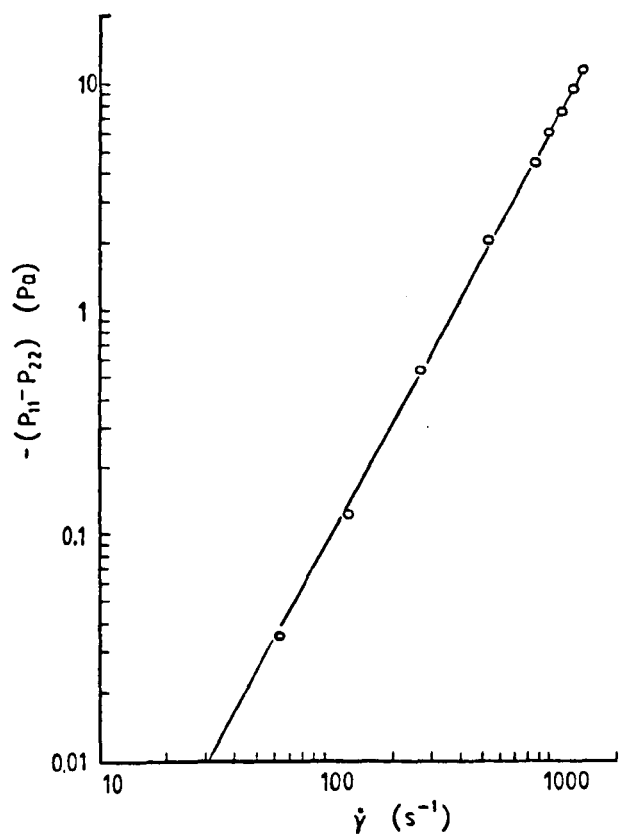

Fig. 43 .Negative normal force of 0•01M KOH at 20°C as a function of shear rate; r = 0•9999 and slope = 1•881

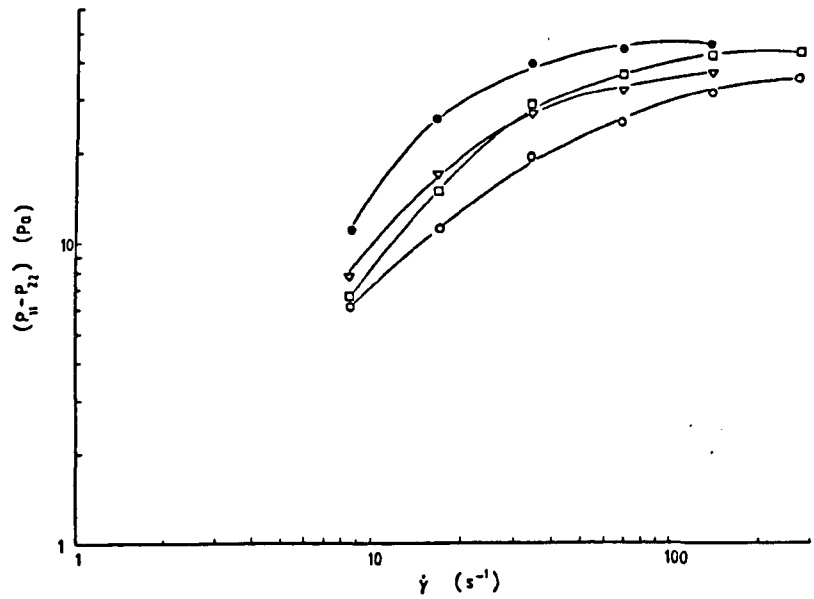

Fig. 44 Normal force as a function of shear rate for potato starch (41 mg/ml) in 0·01M KOH with added palmitic acid potassium salt: □ , control; ○ , (0·013); ▽ , (0·026); ● , (0·039). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

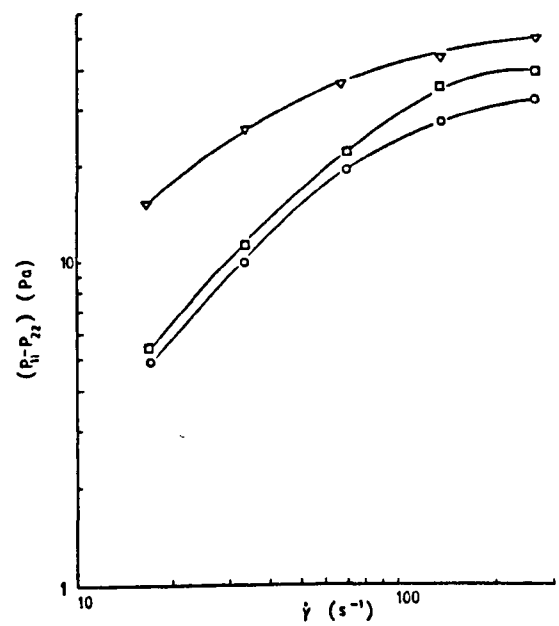

Fig. 45 Normal force as a function of shear rate for potato starch (26 mg/ml) in 0·01M KOH with added stearic acid potassium salt; □ , control; ○ , (0·0187); ▽ , (0·037). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

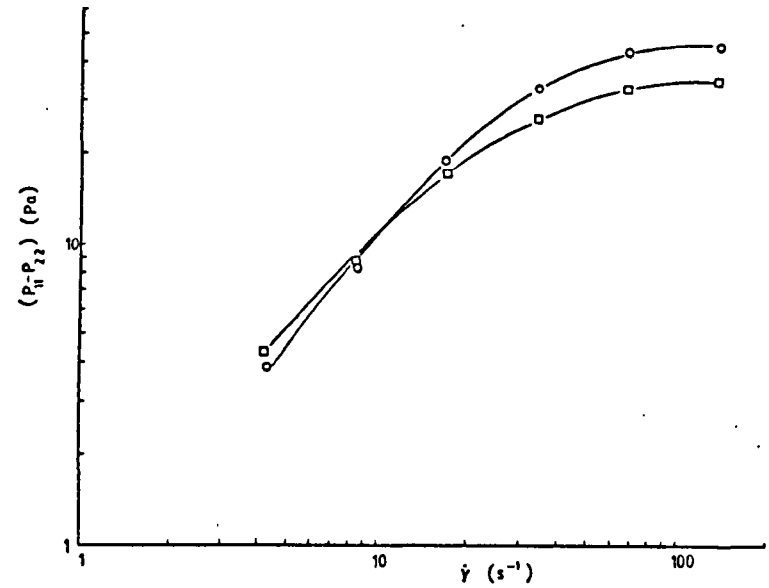

Fig. 46 Normal force as a function of shear rate for potato starch (45 mg/ml) in 0·01M KOH with added stearic acid potassium salt: □ , control; ○ , (0·01). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

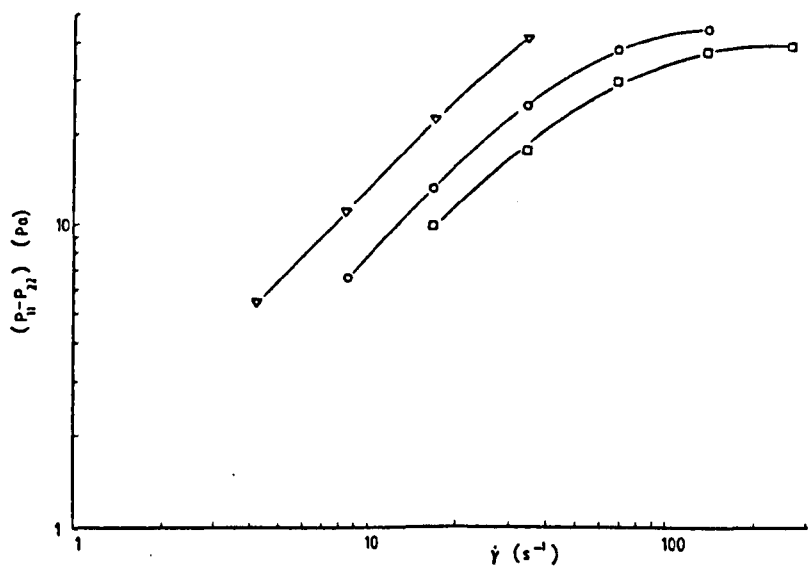

Fig. 47 Normal force as a function of shear rate for potato starch (29 mg/ml) in 0·01M KOH with added arachidic acid potassium salt: □ , control; ○ , (0·015); ▽ , (0·03). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

3.3.6 Creep experiments

Creep experiments were carried out on samples of amylose-fatty acid, and maize and Vicia faba starch-fatty acid complexes in 0·01M KOH. Samples were kept at 60°C prior to their examination in order to avoid retrogradation or gelation before placing in the measuring unit of the Viscoelastic analyser. Amylose, or starch, did not undergo alkaline degradation under these conditions, as shown in Fig. 48, since the viscosity number, and hence the molecular weight, remained constant for at least 7 h. For the preparation of complexes myristic, palmitic, stearic, oleic and elaidic acids were used. Fatty acids with shorter chain length were excluded because hydrodynamic studies (seetion 3.2.1) indicated that their complexing ability was diminished probably due to electrostatic repulsion between the fatty acid anions and partial ionisation of amylose at pH 12. Fatty acids with a chain length longer than stearic were also excluded because of the tendency of their potassium salt to form gels in 0·01M KOH which interfered with the measurements. The use of potato starch was discontinued because, on the addition of a fatty acid, the formation of insoluble white floccules was observed in an otherwise completely clear and homogeneous gel. This was attributed to the high calcium content of the potato starch (Karkalas, 1967) which gave insoluble calcium soap with the fatty acid. White floccules did not form with maize and faba bean starch, both of which are known to contain very low levels of calcium.

Gelatinised granules were found to exert a reinforcing effect on

the relatively weak gels (3·5 g/100ml) which became very fragile on the addition of the potassium salts of fatty acids at the concentration required for the saturation of the amylose helix.

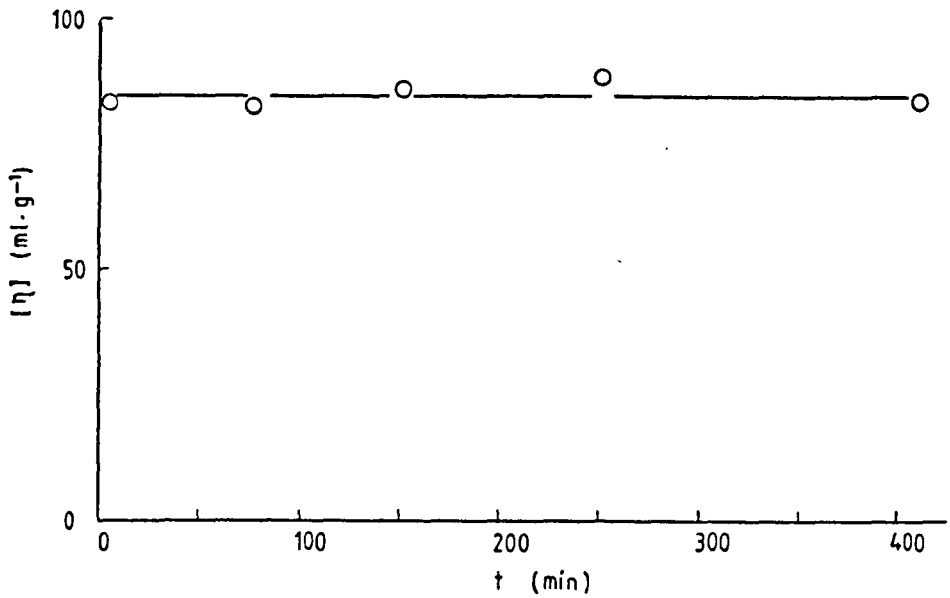

Fig. 48 Limiting viscosity number of amylose in 0·01M KOH, kept at 60°C, as a function of time.

Controlled tests were performed with solutions containing potassium palmitate and stearate, but no amylose, at concentrations up to those required to saturate the amylose at its highest level used in creep experiments. It was found that these solutions remained clear and that their viscosity did not differ appreciably from that of water.

Amylose gels were allowed to set for 90 min. at 20°C before the application of stress, since preliminary experiments indicated that gels containing amylose (3·2 g/ml) attained 75% of their final rigidity within this period of time. According to Welsh *et al.* (1982) the rigidity modulus of amylose (3 g/100 ml) in 1M NaCl reached a constant

value within 100 min. Miles et al. (1985) reported similar results with aqueous gels of amylose from pea starch (3·2 g/100 ml). Gelatinised starch suspensions containing fatty acids were kept for 30 min. before measurement to allow for the dissipation of stress. However, samples containing starch only, i.e. controls, remained liquid even after 24 h. This is presumably due to the low amylose concentration (1 g/100 ml) compared with the total starch concentration (3·5 g/100 ml). It also provides evidence that the rigidity of the gels is mainly due to the amylose matrix with the gelatinised granules participating in the reinforcement of the matrix (Ring and Stainsby, 1982).

During the creep experiments the time of constant shear was 180 s because preliminary work suggested that this was sufficient for the samples to reach steady state compliance or steady flow. At least five different stress values were applied per sample to ensure that the measurement was taken within the linear viscoelastic limit. As a rule, the maximum limit of strain was 0·25 for most samples. Typical stress-strain curves for starch and amylose with added stearic acid are shown in Fig. 49. It should be emphasised that all gels remained perfectly homogeneous throughout the course of the experiments.

3.3.6i Effect of the fatty acid concentration and chain length on the mechanical properties of amylose-fatty acid complexes

In each set of experiments a sample containing amylose only was used as a control in order to eliminate variations due to the

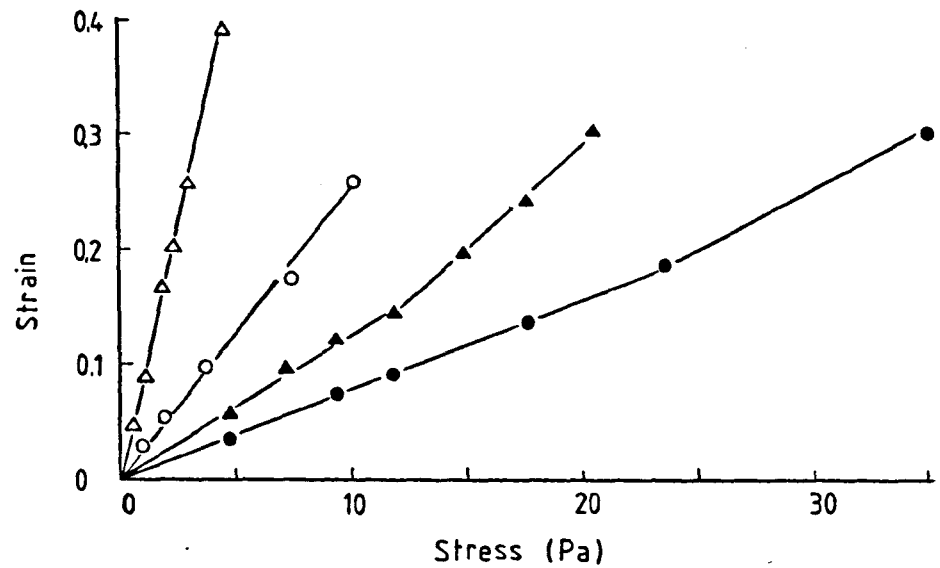

Fig. 49 Instantaneous strain plotted against applied stress for: ○, potato amylose (31·5 mg/ml); ● , potato amylose (31·5 mg/ml)-stearic acid complex (MR = 0·025); △ , maize starch (35 mg/ml); ▲ , maize starch (35 mg/ml)-stearic acid complex (MR = 0·028). All samples in 0·01M KOH; MR = molar ratio FA/AM.

concentration of amylose. Although the controls, on aging, formed gels whose rigidity was dependent on the concentration of amylose, their gel structure differed from that of neutral aqueous amylose gels as reported by other workers, e.g. Miles et al. (1985), who showed that crystallinity developed on aging. Alkaline gels of amylose became opaque but, after three weeks, did not show any crystallinity on X-ray analysis. According to Banks and Greenwood (1975) at pH 12, amylose behaves as a weak acid, hydrogen bonds are ruptured and the stability of the gel network is attained by non-covalent forces, e.g. van der Waals. Polyelectrolyte molecules at low ionic strength tend to expand and interfere with each other's freedom of motion, and electrostatic repulsions prevail (Tanford, 1961). The stabilising factor of the network could be a limited degree of ionic bonding between the ionised glucosyl residues and the potassium ions (Banks and Greenwood, 1975).

$$\begin{matrix} O^- & & O^- & & O^- \\ | & & | & & | \\ R & & R & & R \end{matrix} \quad \cdots K^+ \cdots \quad \cdots K^+ \cdots$$

(R = ionised glucosyl residues)

In the presence of myristate ions, it appears, that amylose assumes a loose helical conformation leading to weakening of the alkaline gel network. On saturation of the amylose helix with potassium myristate the repulsions between adjacent helices seem to be sufficiently strong to inhibit gel formation completely. Evidence is provided by the increase in creep complience of the amylose gels due to myristate ions as shown in Fig. 50. A similar effect was observed with palmitate ions at low concentration, although as the palmitate concentration increased the rigidity of the gel increased

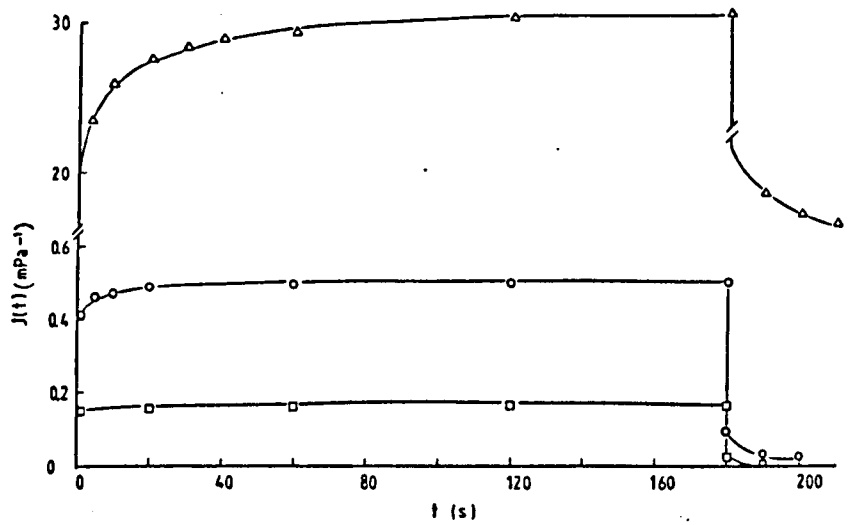

Fig. 50 Creep compliance as a function of time for potato amylose (34 mg/ml) in 0·01M KOH with added myristic acid K salt: □ , control; O , (0·0075); △ , (0·015). Molar ratio FA/AM, in parentheses. (—) Experimental data; (□ , O , △) points calculated from equation (1).

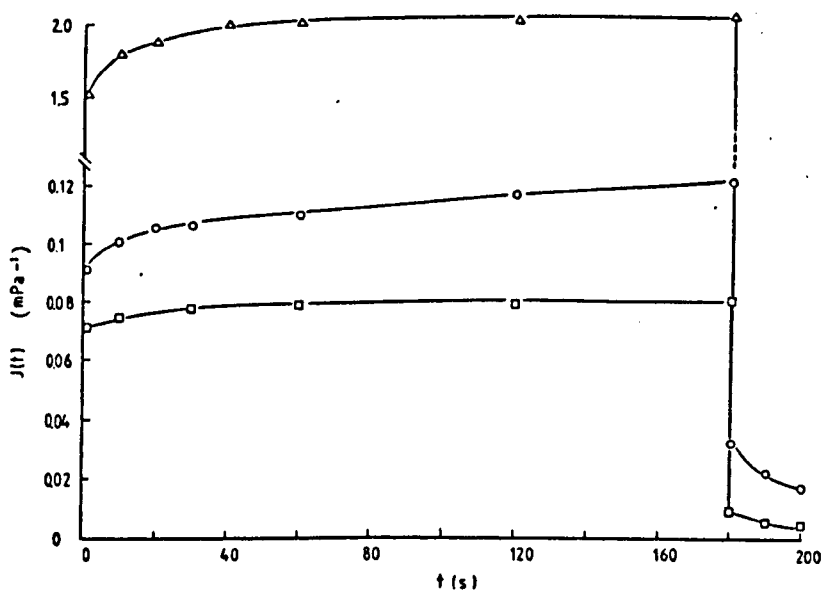

Fig. 51 Creep compliance as a function of time for potato amylose (40 mg/ml) in 0·01M KOH with added palmitic acid K salt: □ , control; △ , (0·015); O , (0·022). Molar ratio FA/AM in parentheses. (—) Experimental data; (□ , △ , O) points calculated from equation (1).

(Fig. 51). It is possible that the formation of amylose gels in 0•01M KOH in the presence of fatty acid anions is governed by two different mechanisms. At low fatty acid concentration few helices are formed and the gel is weakened since fewer junction zones exist. When the concentration of the fatty acid is increased, helix formation becomes dominant and the stiffness of the latter contributes to the rigidity of the gel. However, the chain length of the fatty acid is also expected to play a role, bearing in mind the results of the hydrodynamic experiments. For instance, with palmitic, stearic, elaidic and oleic acids, at concentrations leading to 60-70% saturation of the amylose helix, gels were found to be much more rigid compared with the control (Figs. 51-54). Indeed, with oleic acid the gel was ten times more rigid than the control. Oleic acid also gave more rigid gels than elaidic (Fig. 55). It seems, therefore, that electrostatic repulsions between helices due to the fatty acid anions should also be taken into account. Since fewer fatty acid molecules are required for saturation of the amylose helix as the chain length of the fatty acid increases, the repulsion forces between helices are expected to diminish thus resulting in more rigid gels.

It is also noteworthy that when oleic acid was added, at a level sufficient to saturate the amylose helix, gel formation was instantaneous and all attempts to transfer the gel into the measuring device of the viscoelastic analyser failed because of breakage of the gel.

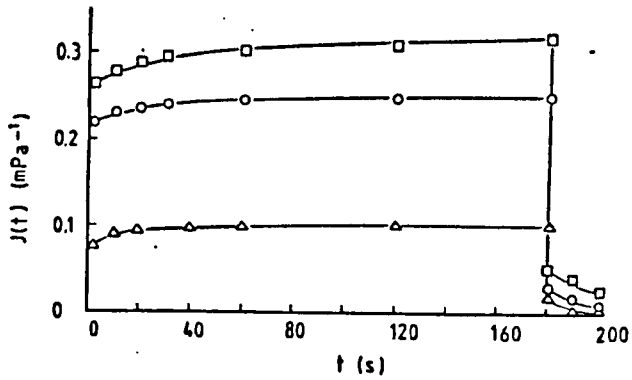

Fig. 52 Creep compliance as a function of time for potato amylose (31·5 mg/ml) in 0·01M KOH with added stearic acid K salt: □ , control; ○ , (0·017); △ , (0·025). Molar ratio FA/AM in parentheses. (—) Experimental data; (□ , ○ , △) points calculated from equation (1).

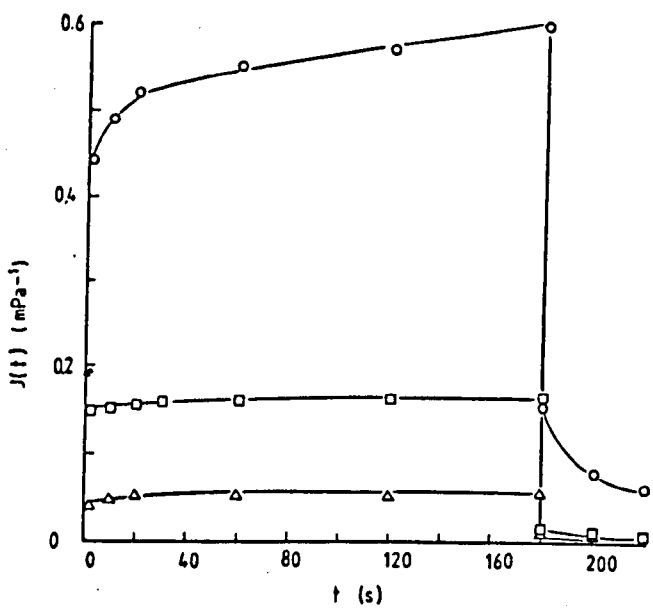

Fig. 53 Creep compliance as a function of time for potato amylose (34 mg/ml) in 0·01M KOH with added elaidic acid K salt: □ , control; ○ , (0·017); △ , (0·026). Molar ratio FA/AM in parentheses. (—) Experimental data; (□ , ○ , △) points calculated from equation (1).

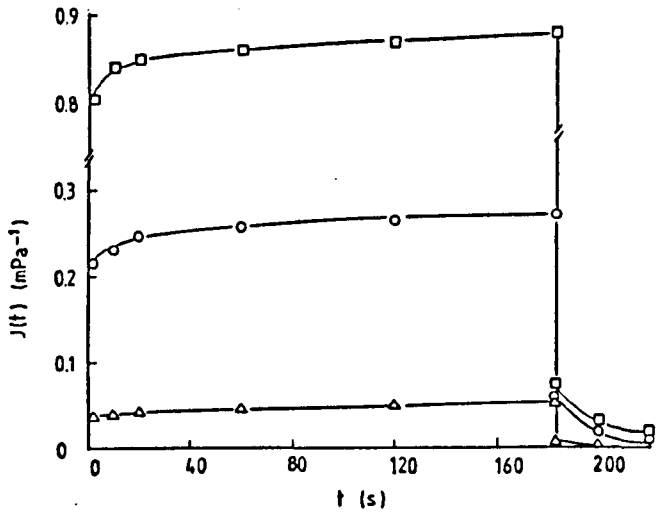

Fig. 54 Creep compliance as a function of time for potato amylose (26 mg/ml) in 0·01M KOH with added oleic acid K salt: □, control; ○, (0·022); △, (0·034). Molar ratio FA/AM in parentheses. (—) Experimental data; (□, ○, △) points calculated from equation (1).

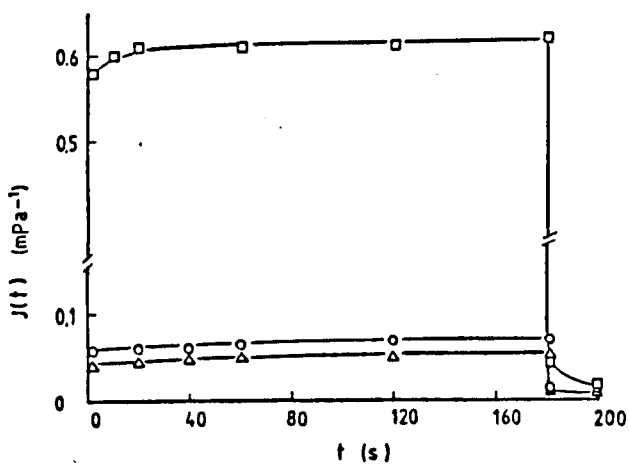

Fig. 55 Creep compliance as a function of time for potato amylose (29 mg/ml) in 0·01M KOH with added fatty acid K salt: □, control; ○, elaidic (0·03); △, oleic (0·03). Molar ratio FA/AM in parentheses. (—) Experimental data; (□, ○, △) points calculated from equation (1).

Alkaline gels of amylose became opaque with time, although the addition of fatty acids resulted in transparent gels. However, gels became less transparent (translucent) as the concentration of the fatty acid increased. It is conceivable that folding of the helices gave rise to aggregates which were responsible for the translucency of the gels. The rigidity of the gels was a function of the chain length as shown clearly in Fig. 56. The folding of helices has been demonstrated by Yamashita and Hirai (1966), by Bittinger and Husemann (1968), and by Jane and Robyt (1984).

In the early stages of this study it was found that amylose (3 g/100 ml) in 0•01M KOH did not gel when the temperature was kept at 35°C, at least within the time scale of the experiments, whereas samples containing palmitic or stearic acid at sufficiently high molar ratio formed gels even at 60°C. The rigidity of these gels was lower than that at 20°C by a factor of 5-10 and was dependent on the fatty acid concentration. When the gels were heated above 95°C they were liquefied immediately, but gelation was restored on spontaneous cooling at about 70°C, and the rigidity increased dramatically on further reduction of the temperature. As shown in section 3.5 amylose-fatty acid complexes dissociate at approximately 90-100°C. This dissociation is the result of transition from helix to coil and it is clearly responsible for the loss of gel stability.

The viscoelastic parameters of the gels were obtained (Table 9) by the graphical method of Inokuchi (1955). The creep curves could be fitted to a model (Fig. 57) which is a combination of a Maxwell

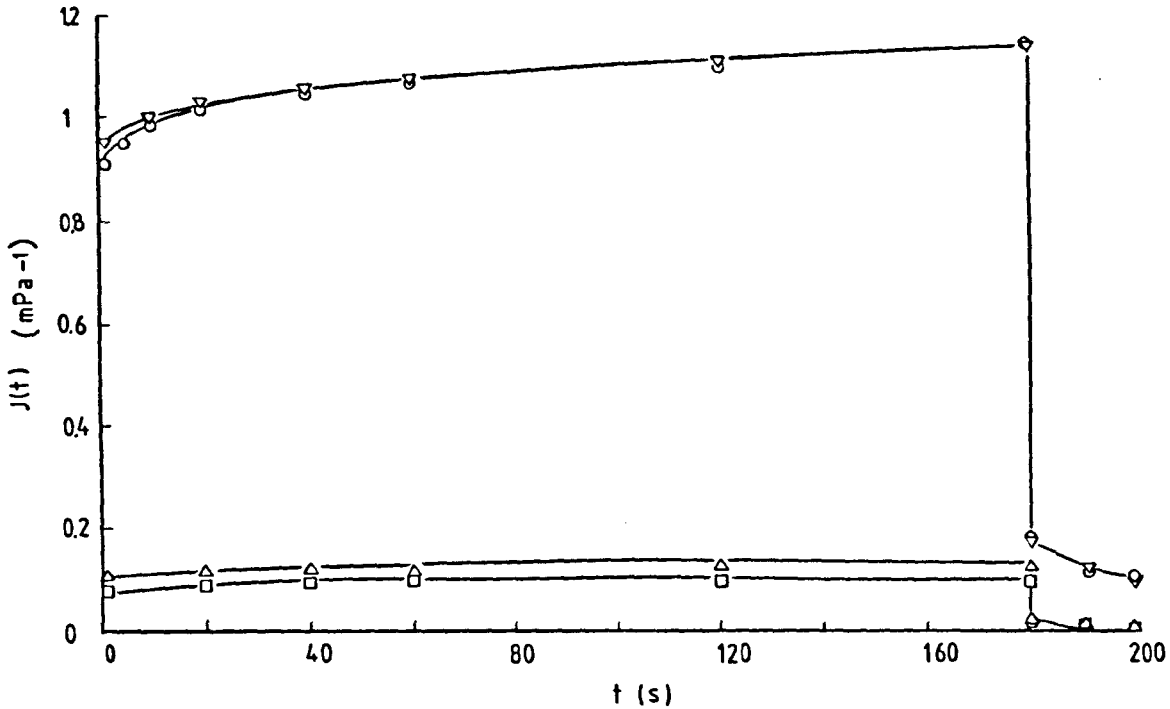

Fig. 56 Creep compliance as a function of time for potato amylose (39 mg/ml) in 0·01M KOH with added fatty acid K salt: □, control; ▽, myristic (0·01); ○, palmitic (0·01); △, stearic (0·01). Molar ratio FA/AM in parentheses. (—) Experimental data; (□, ▽, ○, △) points calculated from equation (1).

Table 9 Rheological parameters of amylose-fatty acid complexes calculated from creep compliance time responses

Sample	Parameters			
	$J_o(mPa^{-1})$	$J_r(mPa^{-1})$	$\tau(s)$	$\eta_N(Pa\ s)$
Amylose (control) (39·0 mg/ml)	0·0816	0·021	30	$2·5 \times 10^6$
" + myristic (MR[a] = 0·01)	0·940	0·105	9	$1·5 \times 10^4$
" + palmitic (MR = 0·01)	0·917	0·120	11	$1·76 \times 10^4$
" + stearic (MR = 0·01)	0·102	0·0234	12	$8·0 \times 10^5$
Amylose (control) (34·0 mg/ml)	0·150	0·012	10	$1·0 \times 10^6$
" + myristic (MR = 0·0075)	0·416	0·066	8	$6·0 \times 10^4$
" + " (MR = 0·0150)	20·00	8·700	13	$6·8 \times 10^2$
Amylose (control) (40 mg/ml)	0·071	0·0072	14	$6·0 \times 10^5$
" + palmitic (MR = 0·015)	1·530	0·390	17	$4·5 \times 10^3$
" + " (MR = 0·022)	0·091	0·0135	11	$1·0 \times 10^5$

Table 9 continued

Sample	Parameters			
	$J_o(mPa^{-1})$	$J_r(mPa^{-1})$	$\tau(s)$	$\eta_N(Pa\ s)$
Amylose (control) (31•5 mg/ml)	0•267	0•0295	13	$8•0 \times 10^4$
" + stearic (MR = 0•017)	0•220	0•0239	10	$3•0 \times 10^5$
" + " (MR = 0•025)	0•076	0•0196	9	$2•5 \times 10^5$
Amylose (control) (29•0 mg/ml)	0•580	0•022	9	$7•5 \times 10^4$
" + elaidic (MR = 0•03)	0•056	0•0069	16	$2•5 \times 10^5$
" + oleic (MR = 0•03)	0•040	0•0076	13	$4•0 \times 10^5$
Amylose (control) (26•0 mg/ml)	0•800	0•050	10	$5•8 \times 10^4$
" + oleic (MR = 0•022)	0•215	0•033	11	$6•2 \times 10^4$
" + " (MR = 0•034)	0•033	0•0142	20	$4•0 \times 10^5$
Amylose (control) (34•0 mg/ml)	0•147	0•0139	18	$3•5 \times 10^5$
" + elaidic (MR = 0•017)	0•440	0•084	10	$2•3 \times 10^4$
" + " (MR = 0•026)	0•0398	0•012	10	$2•5 \times 10^6$

a. MR = molar ratio FA/AM

and a Kelvin-Voigt element.

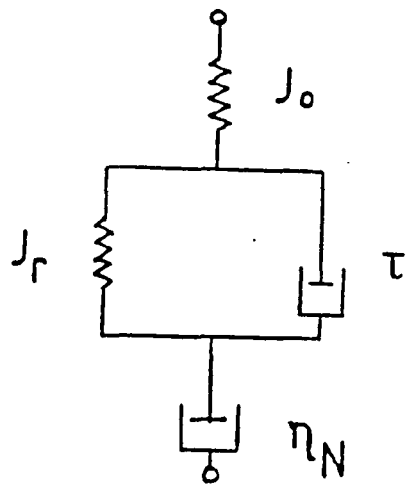

Fig. 57 Mechanical analogue of equation (1)

The equation which describes this model is:

$$J(t) = J_o + J_r(1 - e^{-t/\tau}) + t/\eta_N \qquad (1)$$

where $J(t)$ is the measured compliance

J_o is the instantaneous elastic compliance of the Maxwell spring

J_r is the compliance associated with retarded elastic behaviour and corresponds to the Voigt spring

τ is the retardation time associated with the Voigt dashpot and is the time taken for the delayed strain to reach approximately $^5/_8$ of its final value (Whorlow, 1980).

η_N is the Newtonian viscosity of the Maxwell dashpot

Applying the Boltzmann superposition principle (Ferry, 1980, Mitchell

and Blanshard, 1976) the validity of the model, assumed to explain the creep behaviour, was tested using the equation:

$$J_R(t) = J(t) - J(t - t_1) \qquad (2)$$

where $J_R(t)$ is creep recovery compliance

t_1 is the time at which the stress was removed.

It was found that the calculated and measured recoveries agreed well for all the creep curves. It is possible that the retarded elastic compliance, where the bonds are broken and reformed, can be attributed to van der Waals attraction forces. In the case of electrostatic interactions the energy could be about 20 kJ/mole, depending on the ionic strength of the medium (Ross-Murphy, 1984), whereas van der Waals forces are less than 4kJ/mole.

Recently, Whittam et al. (1986) carried out measurements of the shear moduli on amylose-fatty acid and amylose-monoglyceride gels. Although they did not describe the method of preparation of the gels it is obvious that they mixed hot aqueous neutral amylose solutions with lipids. They claimed that gelation occurred at lipid concentrations up to 0·5% $^{w}/_{w}$, while the amylose concentration was 3·8% $^{w}/_{w}$, in both cases of the gel. They also reported that beyond the 0·5% $^{w}/_{w}$ concentration precipitation rather than gelation occurred. They used lipids with chains containing from 10 - 18 carbon atoms. If we consider monopalmitate at 0·5% $^{w}/_{w}$ concentration, this corresponds to a molar ratio of approx. 0·065. In the present work it was found that the molar ratio for saturation of amylose in aqueous solution is 0·046 while the theoretically calculated value is 0·043 for the monopalmitate (section 3.2.4). Moreover, it was found that even at amylose

concentrations 10 times lower than those used by Whittam et al. (1986), and by employing a much lower monoglyceride concentration than required for saturation, the complex always precipitated immediately because it is insoluble in water. This has also been reported by Banks and Greenwood (1975) for the amylose-butanol complexes. It is, therefore questionable whether Whittam did not observe phase separation of the complexes especially at lipid concentrations 50% higher than those needed for saturation of the amylose. Nevertheless these authors found that the decrease in rigidity was greater with increasing lipid concentration and myristic acid and monomyristate caused the greatest reduction in gel rigidity, whereas the trend for the other lipids used was somewhat inconclusive. They postulated that glyceryl monomyristate and myristic acid are the most effective in interacting with amylose in accord with the claims of Krog and his co-workers (1971, 1984). However, as it has been demonstrated in section 3.2.4, fatty acids, and their monoglycerides, show the same ability in interacting with amylose under favourable conditions.

Nevertheless the interactions which take place during the formation of gels on the addition of lipids to an alkaline solution of amylose are by no means clear. Because of the multicomponent nature of the system and the conformational transitions of amylose during complexing the mechanisms proposed in this section must be considered as very tentative.

3.3.6.ii Effect of fatty acid concentration on the development of rigidity of amylose-fatty acid gels as a function of time

The development of the rigidity modulus of the gels is shown in Fig. 58 from which the effect of the fatty acid concentration is clear, although the highest molar ratio used was only about 50-55% of that required for saturation of the amylose helix. Nevertheless the increase in rigidity is not only dramatic but also very fast. The first measurement was taken after 20 min. because it was thought that the sample should be allowed to relax; however, rigidity developed within the initial few minutes. Attempts were made to prepare concentrated amylose solutions (up to 8% by weight) and to add oleic acid at concentrations close to the molar ratio required for saturation in order to monitor the development of rigidity over long periods of time (e.g. days), with the Instron Universal Machine. The potassium salt of oleic acid was chosen because, at the high concentrations used, it behaved as an isotropic liquid and did not form liquid crystals or a gel. However, as soon as the amylose solution was mixed with the potassium oleate solution a gel was formed which was impossible to handle because of its extreme fragility. This confirms the view that complexes of fatty acids above a certain concentration, and chain length, form true gels. During stressing over their yield point the gels break and do not reform as is the case with reversible networks i.e. weak gels or concentrated solutions.

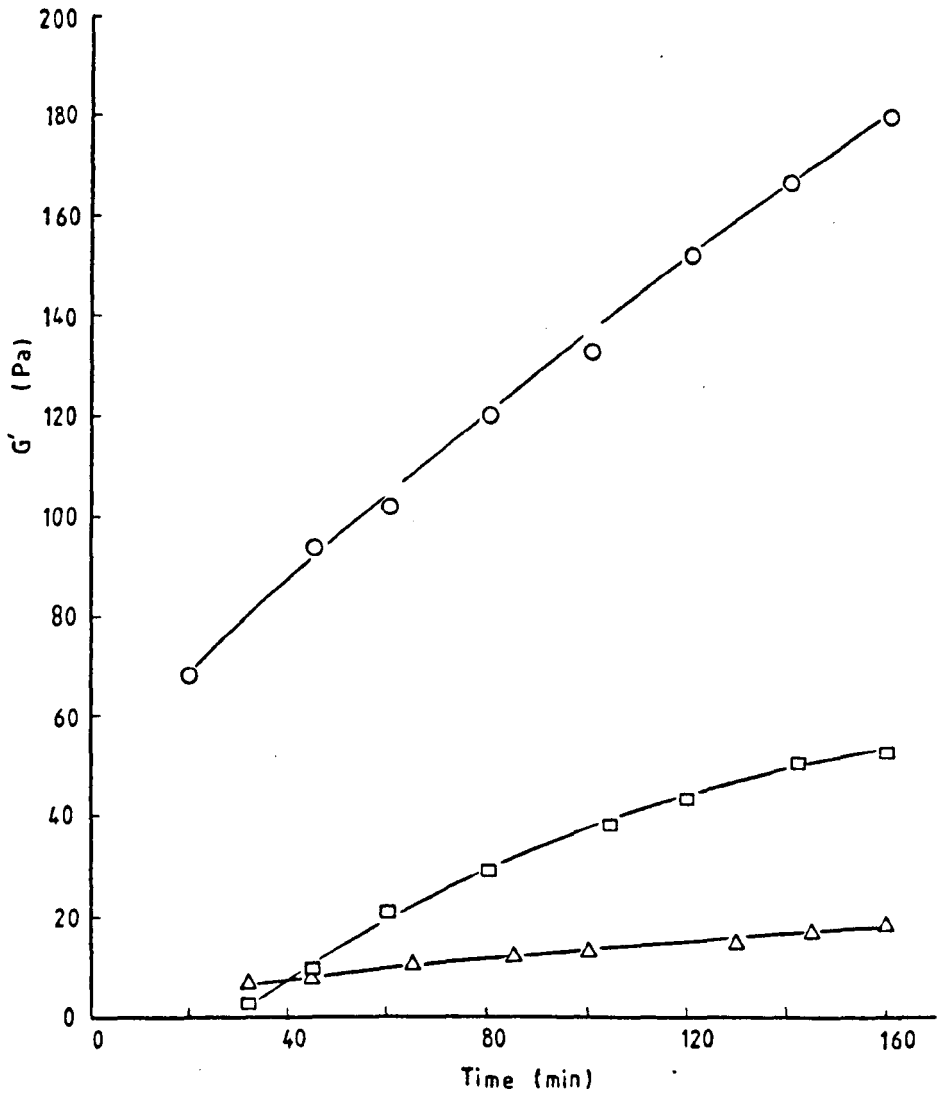

Fig. 58 Development of shear storage modulus G', at 0·314 rad/s, with time for potato amylose solution (32 mg/ml) in 0·01M KOH with added stearic acid K salt: □, amylose control; △, (0·016); ○, (0·025). Molar ratio FA/AM in parentheses.

3.3.6.iii Effect of fatty acid concentration and chain length on the mechanical properties of starch-fatty acid complexes

The two types of starch used differed in their amylose content, maize containing 26•5% amylose and faba bean starch containing 36•0%. The results are shown in Figures 59 - 69. By applying the graphical method of Inokuchi (1955) it was found that also in the case of starches equation (1) is applicable. The calculated rheological parameters are given in Tables 10 and 11.

The trends shown are in agreement with those for amylose complexes and the effect of the granules is particularly pronounced in the case of palmitic and myristic acid complexes where the increase in compliance is less dramatic than that observed with the pure amylose complexes.

It is possible that the granules interact with the fatty acid molecules to form bridges with adjacent granules. The strength of these bonds should depend on the chain length of the fatty acid. Especially the addition of stearic acid caused true gelation as will be shown in the next section (3.3.7). The results obtained from the creep experiments are in agreement with those obtained from the viscometric experiments described in sections 3.3.3 and 3.3.4 and clearly point out to the fact that amylopectin participates in the formation of a structure, although it is doubtful whether it forms complexes. Few attempts have been made previously to study the starch-lipid interactions rheologically. Nihara and Matsumoto (1981)

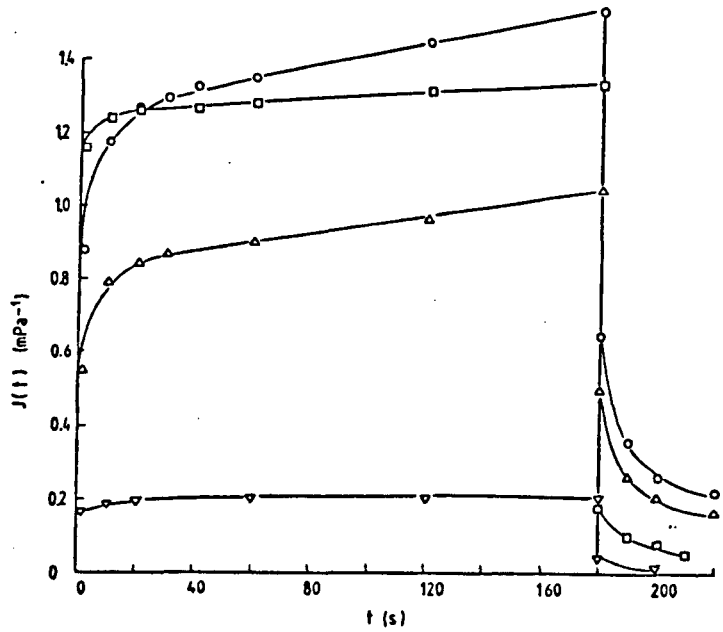

Fig. 59 Creep compliance as a function of time for maize starch (33 mg/ml) in 0·01M KOH with added fatty acid K salt: □, control; ○ , myristic (0·038); △ , palmitic (0·038); ▽, stearic (0·038). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch (—), Experimental data; (□, ○, △, ▽) points calculated from equation (1).

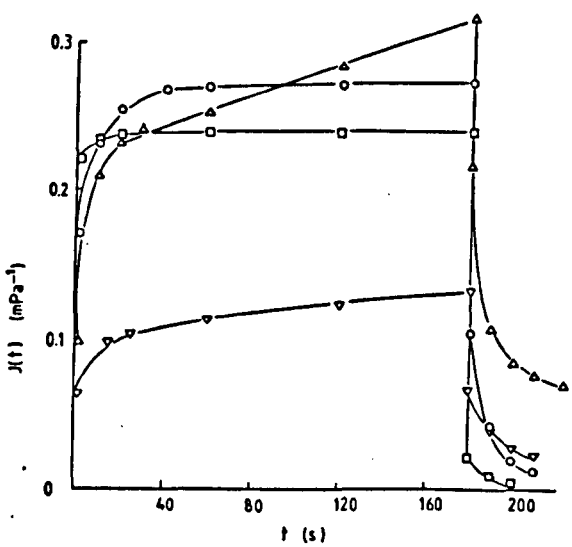

Fig. 60 Creep compliance as a function of time for faba bean starch (38 mg/ml) in 0·01M KOH with added fatty acid K salt: □ , control; ○ , myristic (0·039); △ , palmitic (0·039); ▽ , stearic (0·039). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △, ▽) points calculated from equation (1).

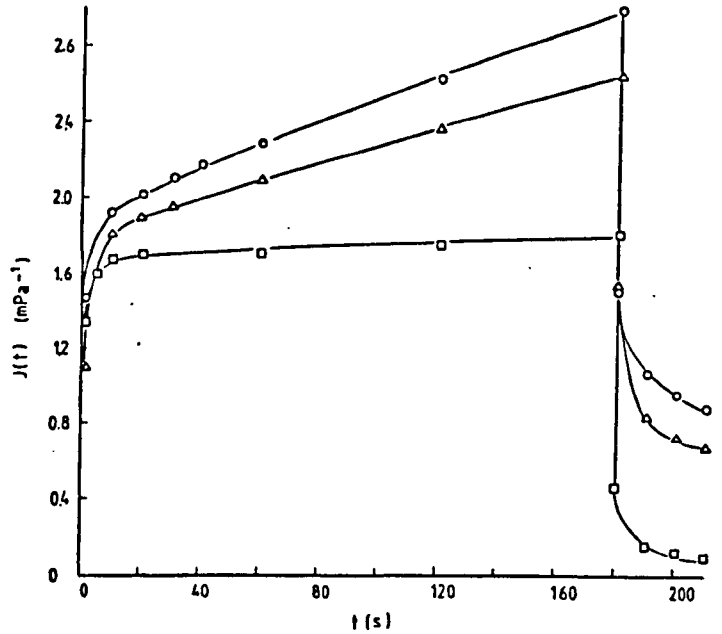

Fig. 61 Creep compliance as a function of time for maize starch (31 mg/ml) in 0·01M KOH with added myristic acid K salt: □, control; ○, (0·02); △, (0·04). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △) points calculated from equation (1).

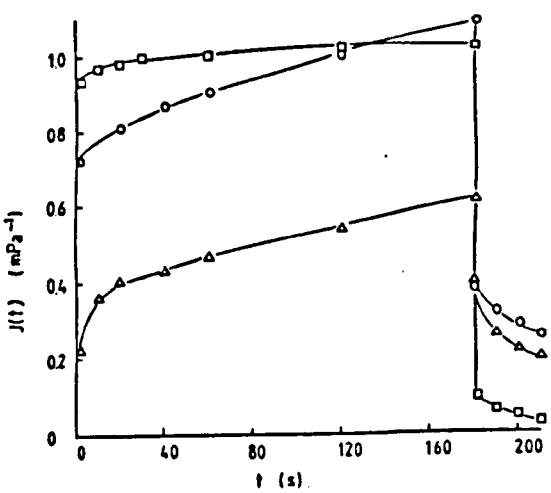

Fig. 62 Creep compliance as a function of time for maize starch (36 mg/ml) in 0·01M KOH with added palmitic acid K salt: □, control; ○, (0·015); △, (0·046). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △) points calculated from equation (1).

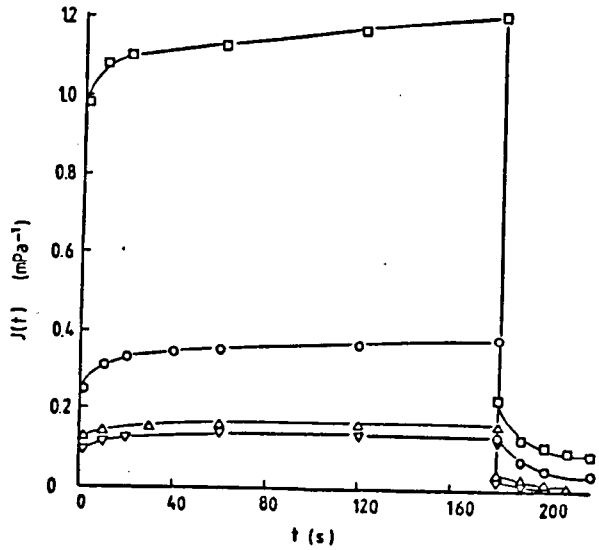

Fig. 63 Creep compliance as a function of time for maize starch (35 mg/ml) in 0·01M KOH with added stearic acid K salt: □, control; ○, (0·014); △, (0·028); ▽, (0·043). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △, ▽) points calculated from equation (1).

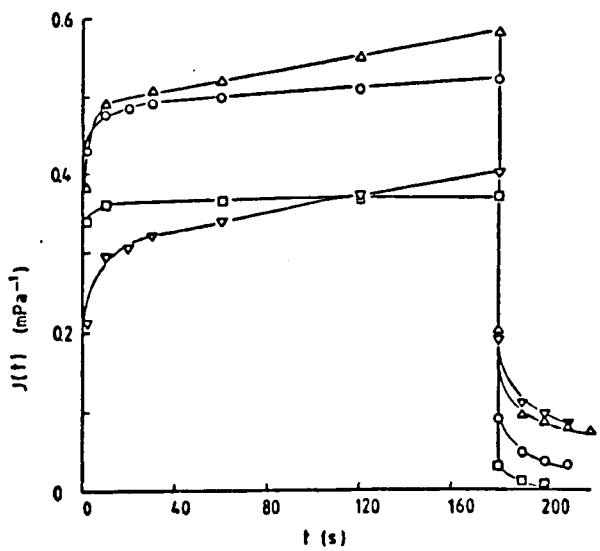

Fig. 64 Creep compliance as a function of time for faba bean starch (31 mg/ml) in 0·01M KOH with added myristic acid K salt: □, control; ○, (0·022); △, (0·044); ▽, (0·066); Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △, ▽) points calculated from equation (1).

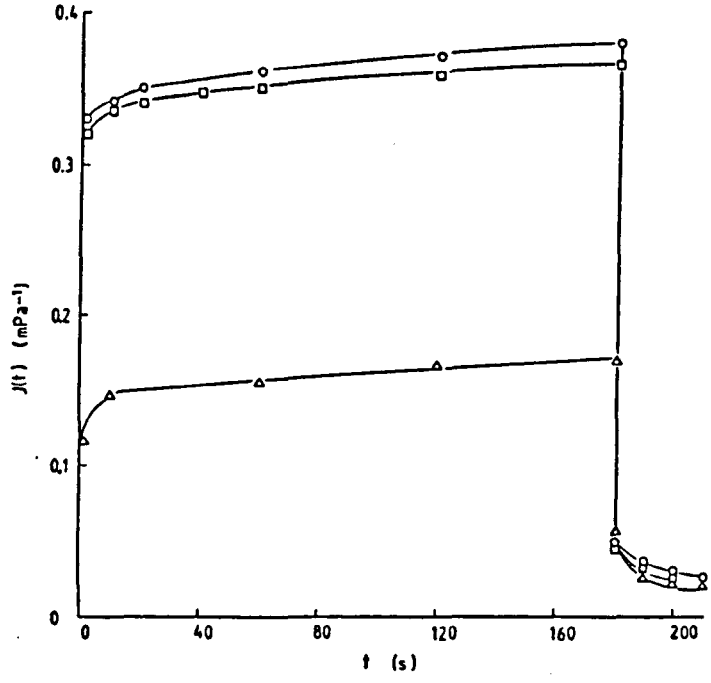

Fig. 65 Creep compliance as a function of time for faba bean starch (31 mg/ml) in 0·01M KOH with added palmitic acid K salt: □, control, ○, (0·022); △, (0·066); Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △) points calculated from equation (1).

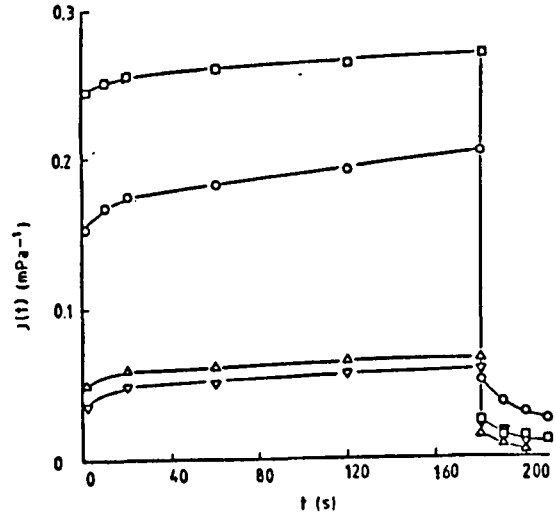

Fig. 66 Creep compliance as a function of time for faba bean starch (37·3 mg/ml) in 0·01M KOH with added stearic acid K salt: □, control; ○, (0·018); △, (0·036); ▽, (0·054). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △, ▽) points calculated from equation (1).

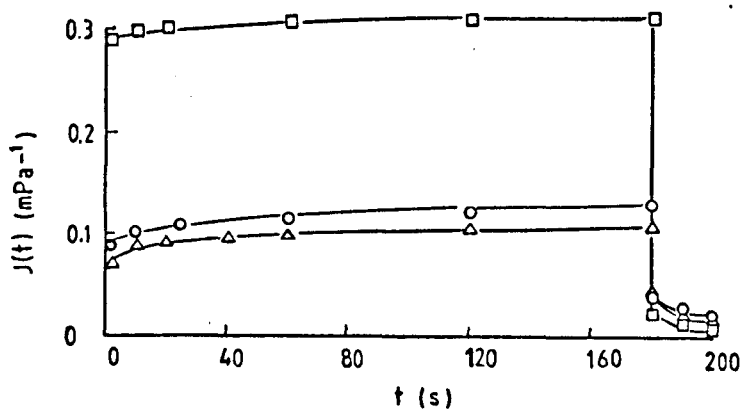

Fig. 67 Creep compliance as a function of time for faba bean starch (33·6 mg/ml) in 0·01M KOH with added fatty acid K salt: □, control; ○, elaidic (0·043); △, oleic (0·043). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (——) Experimental data; (□, ○, △) points calculated from equation (1).

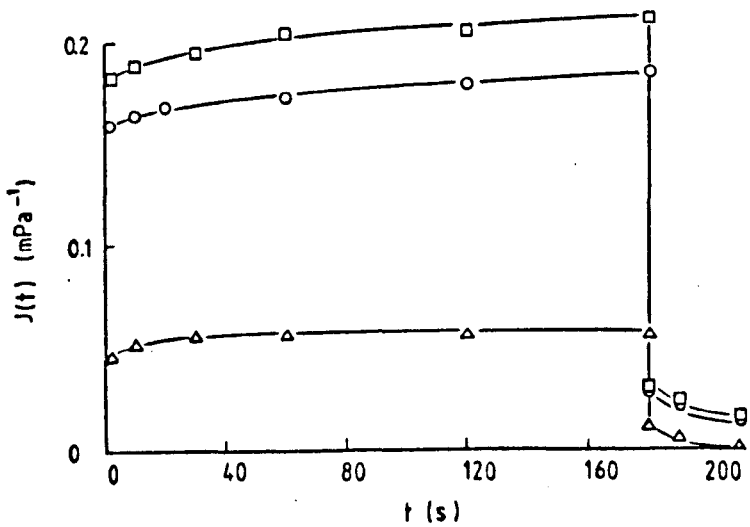

Fig. 68 Creep compliance as a function of time for faba bean starch (41 mg/ml) in 0·01M KOH with added oleic acid K salt: □, control; ○, (0·0165); △, (0·033). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (——) Experimental data: (□, ○, △) points calculated from equation (1).

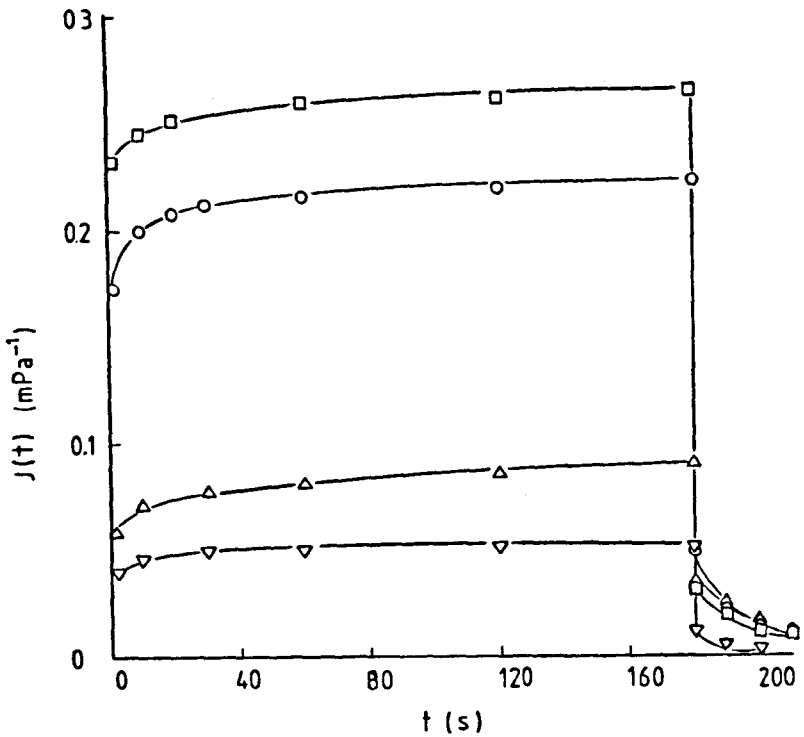

Fig. 69 Creep compliance as a function of time for faba bean starch (37 mg/ml) in 0·01M KOH with added elaidic acid K salt: □, control; ○, (0·018); △, (0·036); ▽, (0·054). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch. (—) Experimental data; (□, ○, △, ▽) points calculated from equation (1).

Table 10 Rheological parameters of maize starch (defatted)-fatty acid complexes calculated from creep compliance time responses (Amylose content: 26•3% $^{w}/_{w}$)

Sample	Parameters			
	J_o(mPa^{-1})	J_r(mPa^{-1})	τ(s)	η_N(Pa s)
Maize starch (control) (33 mg/ml)	1•160	0•078	5	$1•4 \times 10^4$
" " + myristic (MR[a] = 0•038)	0•890	0•36	5	$6•3 \times 10^3$
" " + palmitic (MR = 0•038)	0•550	0•27	10	$7•4 \times 10^3$
" " + stearic (MR = 0•038)	0•163	0•04	7	$1•2 \times 10^6$
Maize starch (control) (31 mg/ml)	1•345	0•27	5	$8•5 \times 10^3$
" " + myristic (MR = 0•02)	1•470	0•40	9	$1•5 \times 10^3$
" " + " (MR = 0•04)	1•100	0•70	4	$2•1 \times 10^3$
Maize starch (control) (36 mg/ml)	0•937	0•065	9	$6•0 \times 10^4$
" " + palmitic (MR = 0•015)	0•714	0•083	15	$5•7 \times 10^3$
" " + " (MR = 0•046)	0•220	0•145	6	$7•5 \times 10^3$
Maize starch (control) (35 mg/ml)	0•980	0•110	9	$1•5 \times 10^4$
" " + stearic (MR = 0•014)	0•245	0•086	10	$3•1 \times 10^4$
" " + " (MR = 0•028)	0•122	0•036	18	$8•0 \times 10^5$
" " + " (MR = 0•043)	0•100	0•035	6	$2•0 \times 10^5$

a: MR = molar ratio FA/AM expressed as mol fatty acid/mol of amylose content of maize starch

Table 11 Rheological parameters of Vicia faba starch-fatty acid complexes calculated from creep compliance time responses (Amylose content = 36·0% w/w)

Sample	Parameters J_o (mPa^{-1})	J_r (mPa^{-1})	τ (s)	η_N (Pa s)
Vicia faba (control) (38·0 mg/ml)	0·222	0·015	11	$3·6 \times 10^5$
" " + myristic (MR^a = 0·039)	0·170	0·098	9	$2·8 \times 10^5$
" " + palmitic (MR = 0·039)	0·098	0·123	7	$1·8 \times 10^4$
" " + stearic (MR = 0·039)	0·065	0·041	10	$6·6 \times 10^4$
Vicia faba (control) (31·0 mg/ml)	0·340	0·022	4	$2·0 \times 10^5$
" " + myristic (MR = 0·022)	0·430	0·058	12	$4·8 \times 10^4$
" " + " (MR = 0·044)	0·380	0·100	6	$1·7 \times 10^4$
" " + " (MR = 0·066)	0·214	0·093	12	$1·8 \times 10^4$
Vicia faba (control) (31·0 mg/ml)	0·320	0·021	12	$6·5 \times 10^4$
" " + palmitic (MR = 0·022)	0·330	0·020	13	$5·9 \times 10^4$
" " + " (MR = 0·066)	0·116	0·042	5	$1·2 \times 10^5$
Vicia faba (control) (37·3 mg/ml)	0·2450	0·010	9	$1·2 \times 10^5$
" " + stearic (MR = 0·018)	0·1530	0·019	9	$5·4 \times 10^4$
" " + " (MR = 0·036)	0·0490	0·095	10	$2·4 \times 10^5$
" " + " (MR = 0·054)	0·0367	0·012	11	$1·8 \times 10^5$

Table 11 continued

Sample	Parameters			
	$J_o(mPa^{-1})$	$J_r(mPa^{-1})$	$\tau(s)$	$\eta_N(Pa\ s)$
Vicia faba (control) (33•6 mg/ml)	0•290	0•014	13	$2•8 \times 10^5$
" " + elaidic (MR = 0•043)	0•089	0•020	12	$7•8 \times 10^4$
" " + oleic (MR = 0•043)	0•073	0•022	11	$2•2 \times 10^5$
Vicia faba (control) (37•0 mg/ml)	0•232	0•0260	12	$2•7 \times 10^5$
" " + elaidic (MR = 0•018)	0•173	0•0370	11	$1•4 \times 10^5$
" " + " (MR = 0•036)	0•057	0•0166	9	$9•3 \times 10^4$
" " + " (MR = 0•054)	0•040	0•0096	12	$4•8 \times 10^6$
Vicia faba (control) (41•0 mg/ml)	0•1835	0•0100	11	$9•2 \times 10^4$
" " + oleic (MR = 0•0165)	0•1590	0•00874	13	$1•0 \times 10^5$
" " + " (MR = 0•033)	0•0460	0•0087	10	$1•8 \times 10^6$

a: MR = molar ratio FA/AM expressed as mol fatty acid/mol of amylose content of faba starch

prepared complexes by mixing non-defatted wheat starch with lipids ranging from lecithin, fatty acids and monoglycerides, to triglycerides; the gelatinisation of starch was carried out in the presence of lipids. Although these authors used dispersions containing 6% $^{w}/_{w}$ starch in water, which should have formed strong gels, it appears that the gels were broken during shearing in the rheometer (cone and plate). The authors used a high shear rate (385 s^{-1}) in an attempt to homogenise the samples, which they reported to be heterodisperse, probably due to the precipitation of the formed complexes. They studied the thixotropic nature of the already broken gels by shearing them after regular relaxation time intervals and observing the further structural breakdown. Harbitz (1983) used sodium dodecyl sulphate (SDS) and its analogous fatty acid, lauric as complexing agents, to form potato starch-complex gels. The gels were prepared by heating the starch in the presence of the complexing agent and, after aging for 24 h, their strength was measured with an Instron machine. He reported that the strength of the gel containing SDS increased dramatically. The addition of lauric acid also enhanced the gel strength but to a lesser extent. In the author's opinion the difference shown by the two complexing agents was due to the better solubility of the SDS and hence its availability to form complexes with the leached amylose. He attributed the increase in gel strength to the formation of strong junctions in the gel network due to the alignment of the amylose helices parallel to each other, which were probably stabilised by hydrogen bonding. Whittam et al. (1986) prepared pea starch (30% $^{w}/_{w}$)-sodium myristate gels, but they did not describe the method of preparation. They studied the development of the modulus of rigidity during

the aging of the gels and reported that the gel of the complex was weaker than that of the starch (control) and that the development of the rigidity was much less pronounced than that of the control. Attempts were made during the present work to study the aging of starch gels, in the presence of fatty acids, by means of the Instron machine. As in the case of pure amylose, as soon as the gelatinised starch dispersions (7% $^{w}/_{w}$) were mixed with fatty acid potassium salt solutions, gels were formed almost instantaneously and broke during the transfer into moulds. Subsequent measurements of gel strength gave inconsistent results and the experiment was discontinued.

3.3.7 Dynamic experiments with starch-fatty acid systems

The dynamic viscoelastic parameters of the fatty-acid systems were measured by means of the Viscoelastic analyser operating in the oscillatory mode. Efforts were made to ensure that the recorded strain (displacement) was kept low (less than 0·05) in order to avoid breaking the network system. The phase lag of the input and output motions was determined from the twin pen recorder traces by the procedure of Walters (1975). The storage G' and loss moduli G'' were calculated using the equations:

$$G' = \frac{F_o \cos\varphi}{X_o b} + \frac{m\omega^2}{b} \quad (3) \text{ and } G'' = \frac{F_o \sin\varphi}{X_o b} \quad (4)$$

(Whorlow, 1980)

where F_o is the maximum (peak) sinusoidal stress applied

X_o is the maximum (peak) sinusoidal displacement recorded

φ is the phase lag between stress and strain

ω is the frequency of oscillation in rad/s

b is the geometrical factor of the measuring unit employed (cone and plate), specified by the formula $b = \frac{2\pi R^3}{3\vartheta}$ where,

R is the cone radius and ϑ is the cone-plate angle in radians.

The term $\frac{m\omega^2}{b}$ is related to the moment of inertia of the system (driving motor and cone). The mass of the moving system is generally kept small so that this term is small. However, the term could become significant at high frequencies and the G' values obtained should be corrected by taking into account this term. The method used to calculate the inertia effect of the moving system and consequently the term $\frac{m\omega^2}{b}$ is described in appendix 2. The inertia effect due to the mass of the sample was assumed to be insignificant and negligible. Figures 70-77 show the mechanical spectrum (frequency-dependence of the dynamic moduli G' and G'') of the starch-fatty acid system tested. It is characteristic that the control samples showed the behaviour of concentrated solutions with G' being greater than G'' at low frequencies. As the frequency increased G'' increased faster than G' and eventually there was a cross-over which indicated that, at faster deformations, the liquid-like character of the sample was more pronounced. On the other extreme, particularly the samples of maize starch with a high concentration of stearic acid (Fig. 74), the G' and G'' become nearly frequency independent and the behaviour of the sample approached that of a strong gel. Moreover, the effect of the ratio of amylopectin to amylose is obvious for the two starches employed. Complexed maize starch with a relatively low amylose

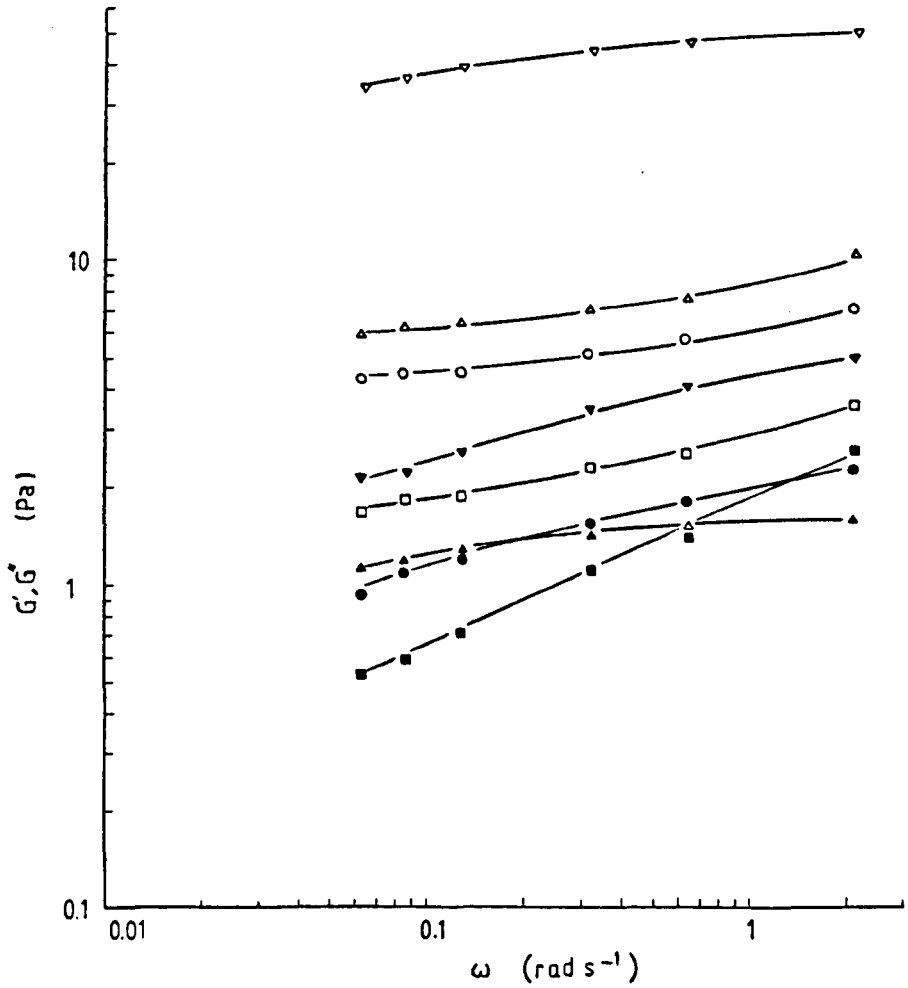

Fig. 70 Frequency dependence of G' (open symbols) and G'' (filled symbols) for maize starch (31 mg/ml) in 0·01M KOH with added fatty acid K salt: □, ■, control; ○, ●, myristic (0·043); △, ▲, palmitic (0·043); ▽, ▼, stearic (0·043). Molar ratio FA/AM in parentheses, is based on the amylose content of the starch.

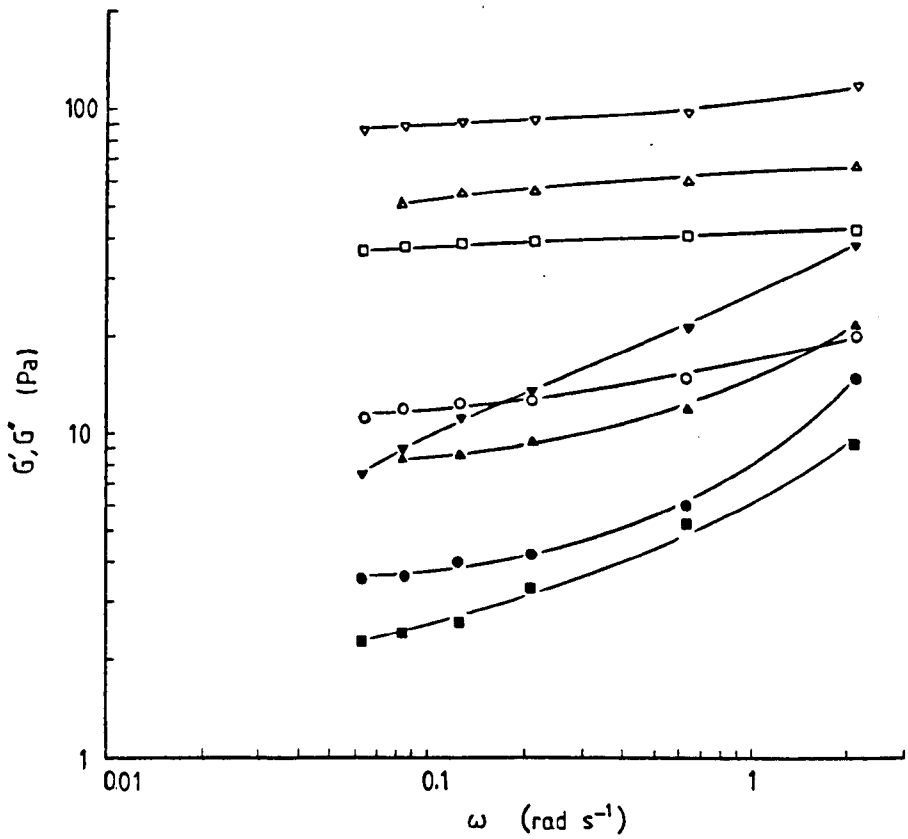

Fig. 71 Frequency dependence of G' (open symbols) and G'' (filled symbols) for faba bean starch (33 mg/ml) in 0·01M KOH with added fatty acid K salt: □ , ■ , control; ○ , ● , myristic (0·045); △ , ▲ , palmitic (0·045); ▽ , ▼ , stearic (0·045). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

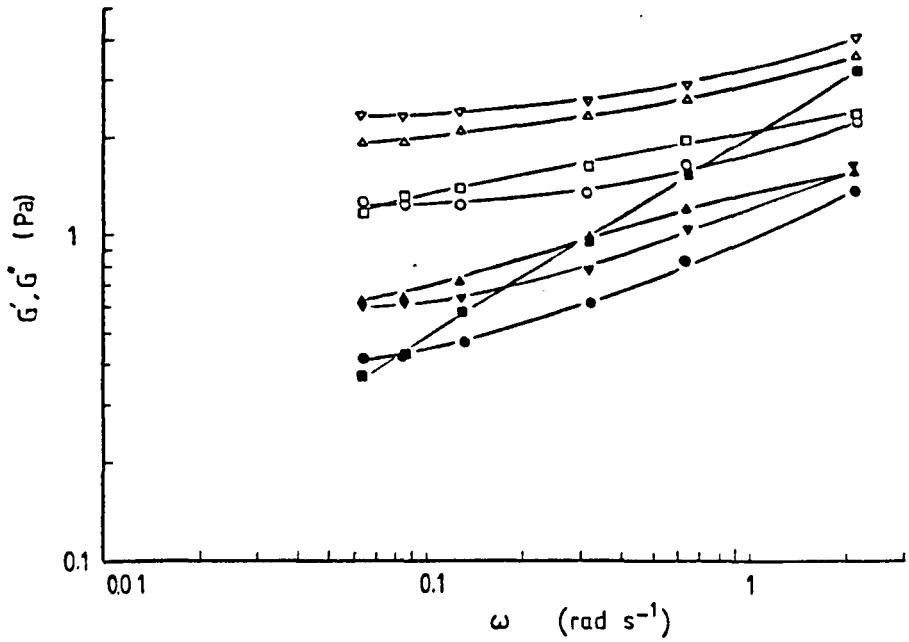

Fig. 72 Frequency dependence of G' (open symbols) and G'' (filled symbols) for maize starch (28·5 mg/ml) in 0·01M KOH with added myristic acid K salt: □ , ■ , control; ○ , ● , (0·021); △ , ▲ , (0·043); ▽ , ▼ , (0·065). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

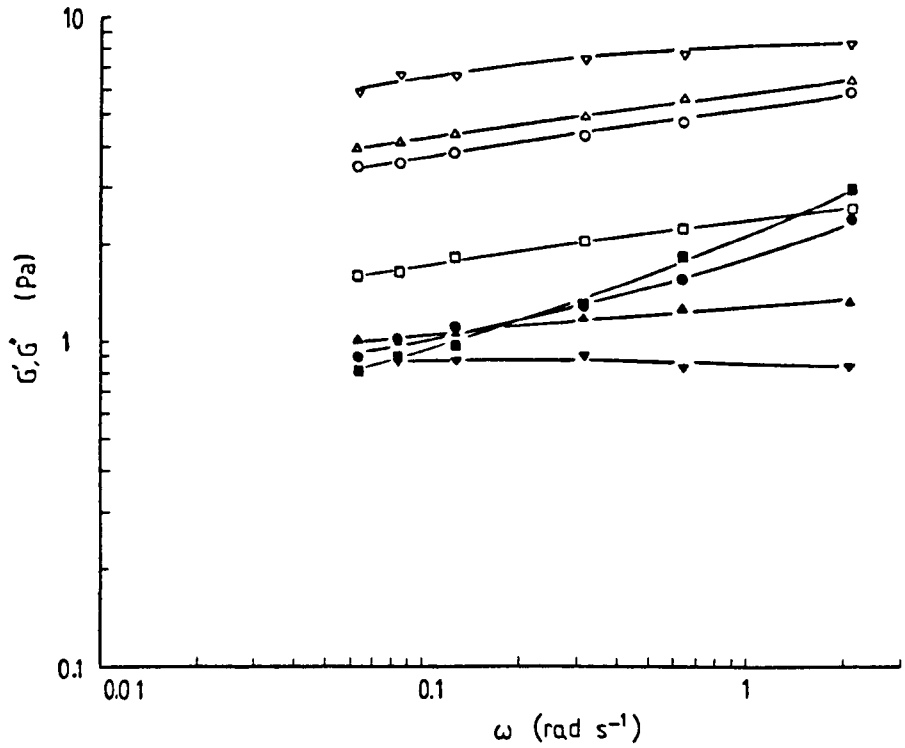

Fig. 73 Frequency dependence of G' (open symbols) and G'' (filled symbols) for maize starch (29·5 mg/ml) in 0·01M KOH with added palmitic acid K salt: □ , ■ , control; ○ , ● , (0·018); △ , ▲ , (0·037); ▽ , ▼ , (0·056). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

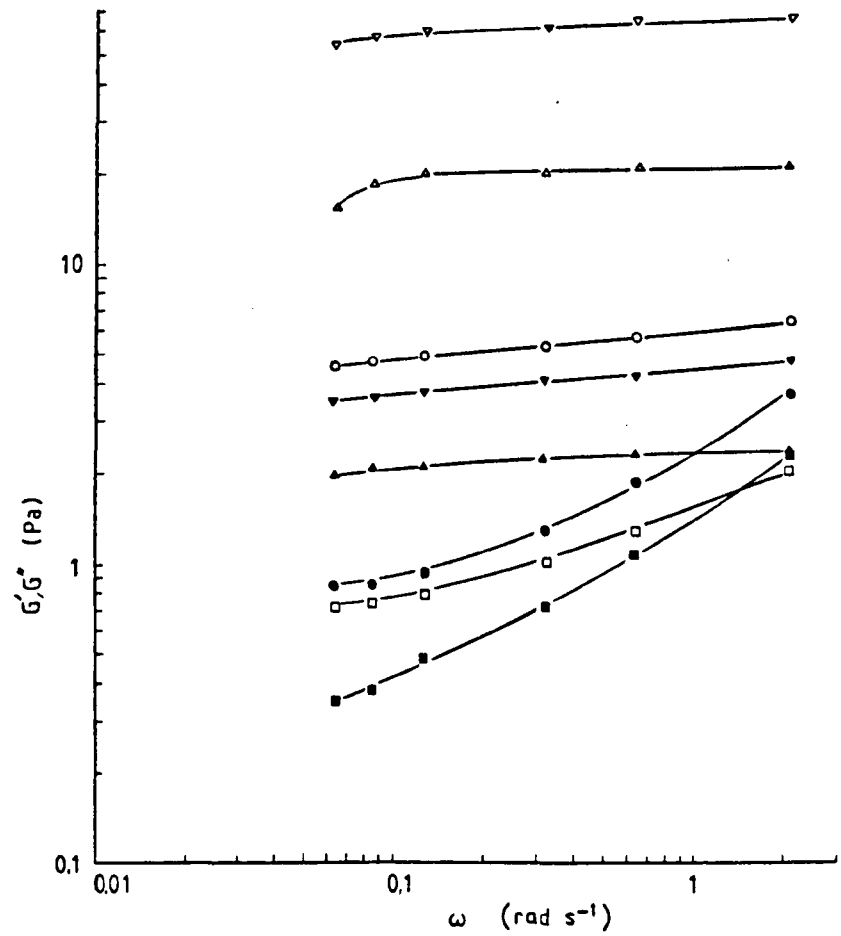

Fig. 74. Frequency dependence of G' (open symbols) and G'' (filled symbols) for maize starch (26·7 mg/ml) in 0·01M KOH with added stearic acid K salt: □ , ■ , control; ○ , ● , (0·018); △ , ▲ , (0·037); ▽ , ▼ , (0·056). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

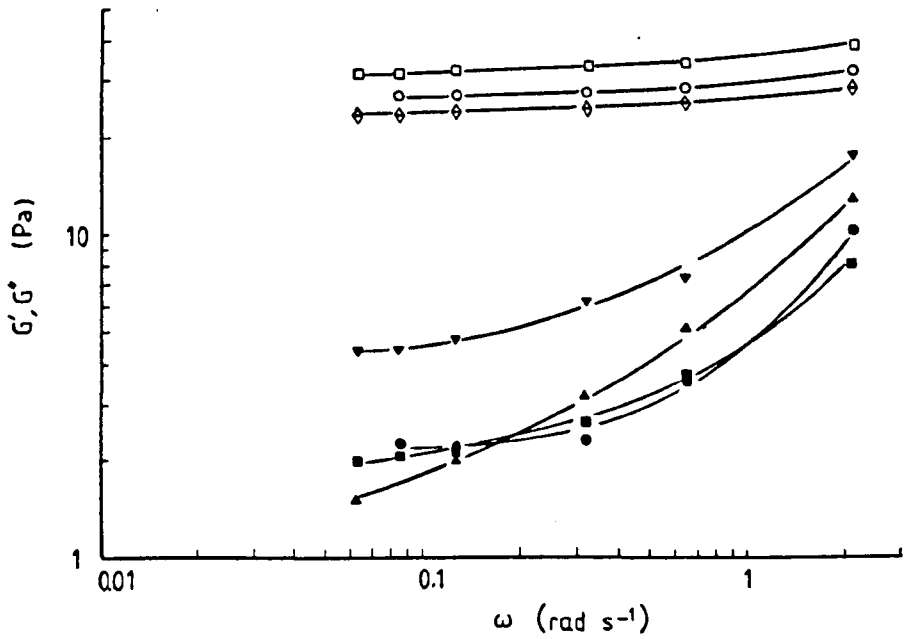

Fig. 75 Frequency dependence of G' (open symbols) and G'' (filled symbols) for faba bean starch (31 mg/ml) in 0·01M KOH with added myristic acid K salt: □ , ■ , control; ○ , ● , (0·022); △ , ▲ , (0·044); ▽ , ▼ , (0·066). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

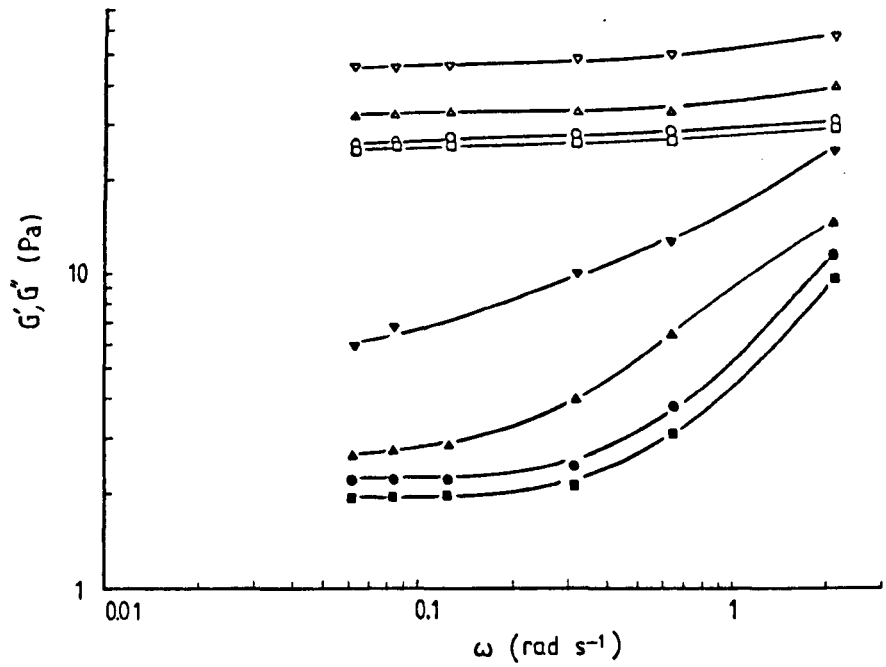

Fig. 76 Frequency dependence of G' (open symbols) and G'' (filled symbols) for faba bean starch (30 mg/ml) in 0·01M KOH with added palmitic acid K salt: □ , ■ , control; ○ , ● , (0·0225); △ , ▲ , (0·045); ▽ , ▼ , (0·0676). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

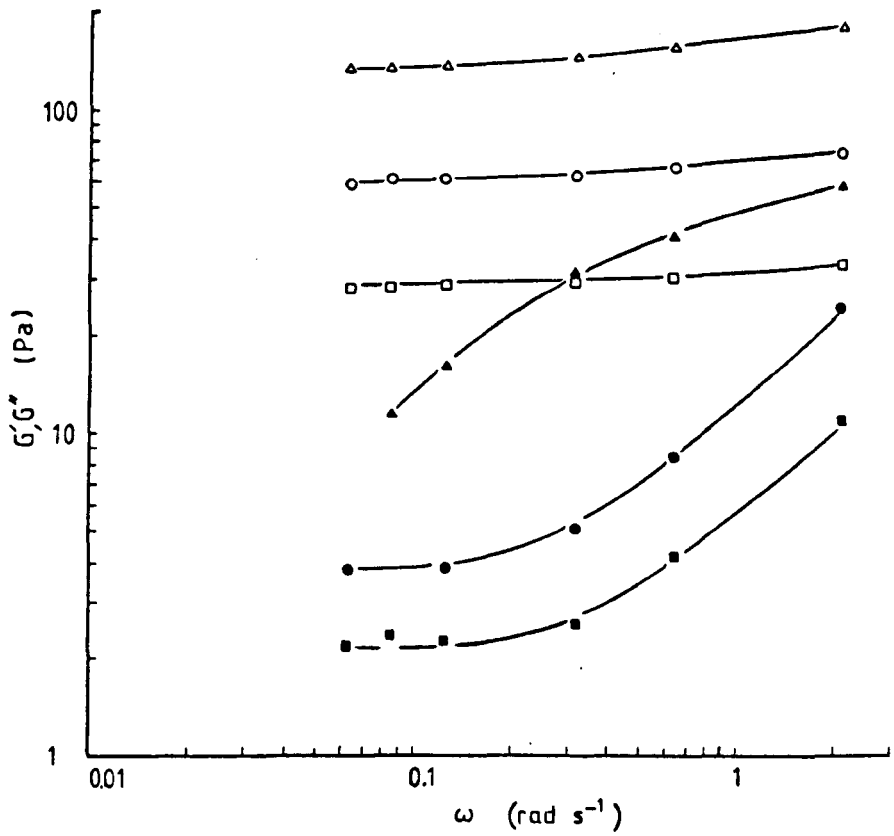

Fig. 77 Frequency dependence of G' (open symbols) and G'' (filled symbols) for faba bean starch (31 mg/ml) in 0·01M KOH with added stearic acid K salt: □ , ■ , control; ○ , ● , (0·021); △ , ▲ , (0·042). Molar ratio FA/AM, in parentheses, is based on the amylose content of the starch.

content, shows a more pronounced gel-like behaviour compared with complexed faba bean starch which contains a higher percentage of amylose.

3.4 Surface tension measurements of amylose-fatty acid complexes in solution

Surface tension is a property of air/liquid interface which can be affected by the presence of molecules with surface active properties such as fatty acids. In the present work, the effect of potassium salts of palmitic and stearic acids on the surface tension of an aqueous solution of 0·01M KOH was investigated both in the absence and in the presence of amylose. Surface tension was measured by the du Nouy ring method in which the maximum force required to pull a small platinum ring vertically from the surface of the liquid was determined. Complexes were prepared as described in section (2.3.14). The results obtained are shown in Figures 78 and 79.

In the absence of amylose, the fatty acids (as the potassium salts) caused a rapid decrease in the surface tension of the solution. This decrease continued as the concentration of the acids in solution was increased until the critical micelle concentration (CMC) was reached. CMC is denoted as the solute (amphiphilic molecules) concentration, in an aqueous solution, at which micelles are spontaneously formed by association of the hydrophobic parts of the molecules (Small, 1986). At this point no further change in surface tension occurs because of micelle formation. In the presence of amylose low levels of addition

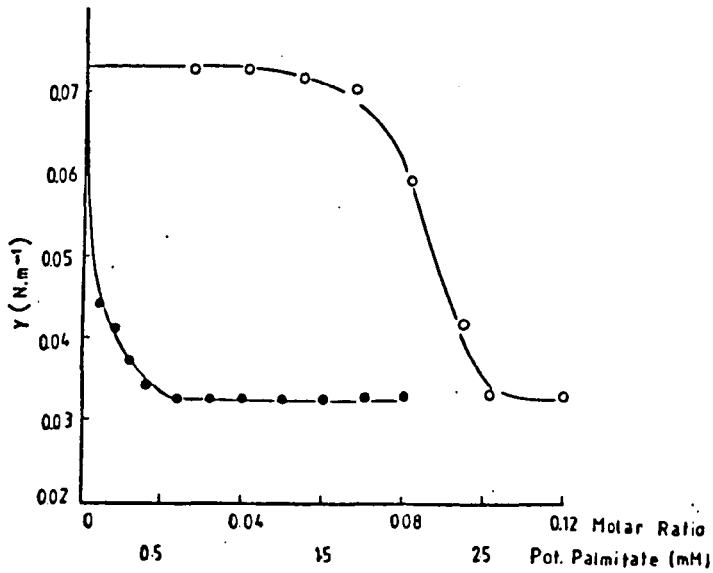

Fig. 78 Surface tension of potassium palmitate solution in 0·01M KOH in the absence (●) and presence (○) of 4 mg/ml amylose.

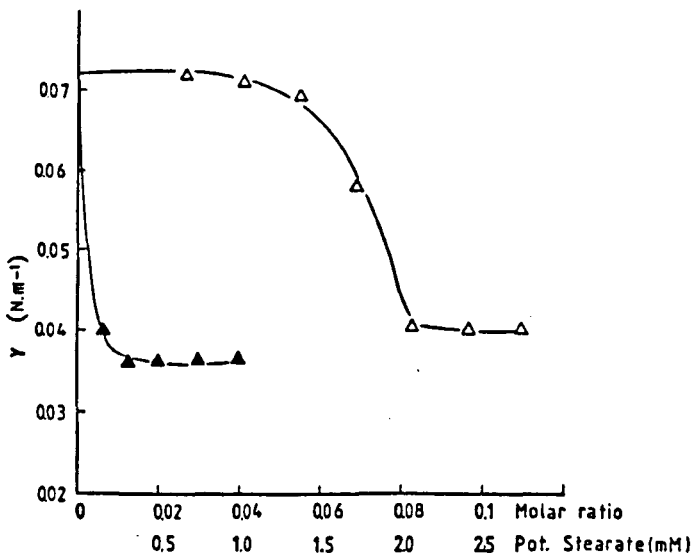

Fig. 79 Surface tension of potassium stearate solution in 0·01M KOH in the absence (▲) and presence (△) of 4 mg/ml amylose.

of fatty acids had practically no effect on the surface tension of the solvent. This could be explained by suggesting that the acid molecules inside the helical cavity were prevented from expressing their surface active properties. As soon as the amylose helix became saturated the surface tension of the solution was lowered due to the free fatty acid anions.

As seen from the graphs, the saturation molar ratios found by the surface tension method were in very good agreement with those found by other methods (section 3.2.4) as well as those calculated on the basis of the dimensions of the amylose helix (Table 6). As more acid was added the surface tension reached a limiting value similar to that observed for the fatty acid alone at the CMC. However, this limiting condition was reached at a higher fatty acid concentration than in the absence of amylose.

The results presented in Figures 78 and 79 provide clear evidence that complex formation of amylose with fatty acid anions is not hindered because of the aggregation of the anions in the form of micelles. It is known that the micellar aggregates of fatty acids are in equilibrium with the free molecules in solution although this equilibrium requires some time to be established (Small, 1986).

Similar results were reported by Bulpin *et al.* (1986) for the amylose-sodium myristate complex in water-DMSO solvent system.

3.5 Thermal properties of the complexes

DSC data were obtained for the insoluble amylose-fatty acid complexes, in suspension, containing not less than 85% water. Typical thermograms of complexes precipitated after neutralisation of alkaline amylose-fatty acid solutions are shown in Figures 80 and 81. Thermograms of complexes prepared with palmitic acid which was added over a range of molar ratios to a solution of amylose of a fixed concentration are shown in Fig. 82. When the saturation molar ratio for palmitic acid/amylose was exceeded a small endothermal peak was observed near the melting point of palmitic acid (62·7°C) and this suggests that the excess fatty acid was situated outside the helix of the complex, probably entrained in an adsorbed or occluded form between the folded chain of the complexed amylose. No free (uncomplexed) fatty acid was observed in any of the samples when the FA/AM saturation molar ratio was not exceeded during the preparation of complexes (Fig. 80). By contrast, when oleic and linoleic acids were used at ratios exceeding the saturation requirements of the helix, the free fatty acids could not be detected (Fig. 81) because their melting point was below the starting temperature of the instrument. Capric acid (m.p. 31°C) used above the saturation requirement of the helix was not detectable by DSC because it remained in solution due to its relatively high solubility in water (15 mg/100 g at 20°C). For lauric, myristic and palmitic acids it was possible to calculate the amount of free fatty acid entrained by the precipitated complex, as a percentage of added fatty acid, from the observed enthalpy due to melting and from published data for the specific enthalpy of melting. Dissociation

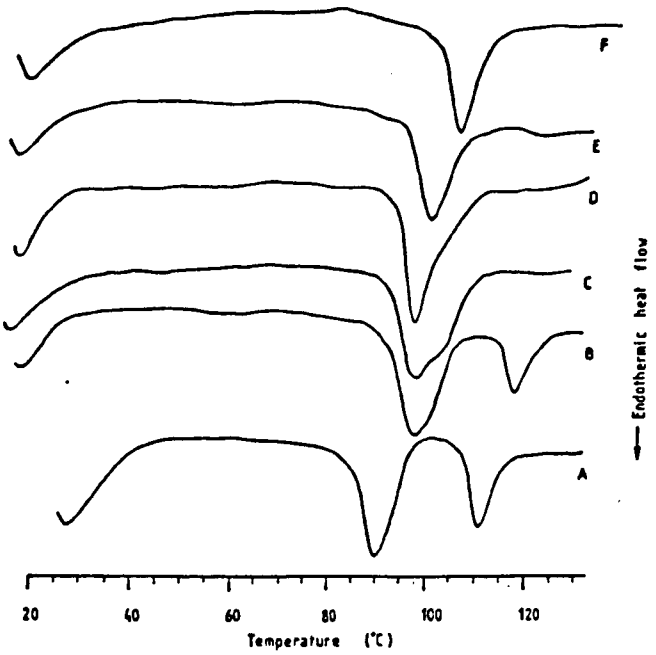

Fig. 80 DSC thermograms of amylose complexes with saturated fatty acids: A, capric; B, lauric; C, myristic; D, palmitic; E, stearic; F, arachidic.

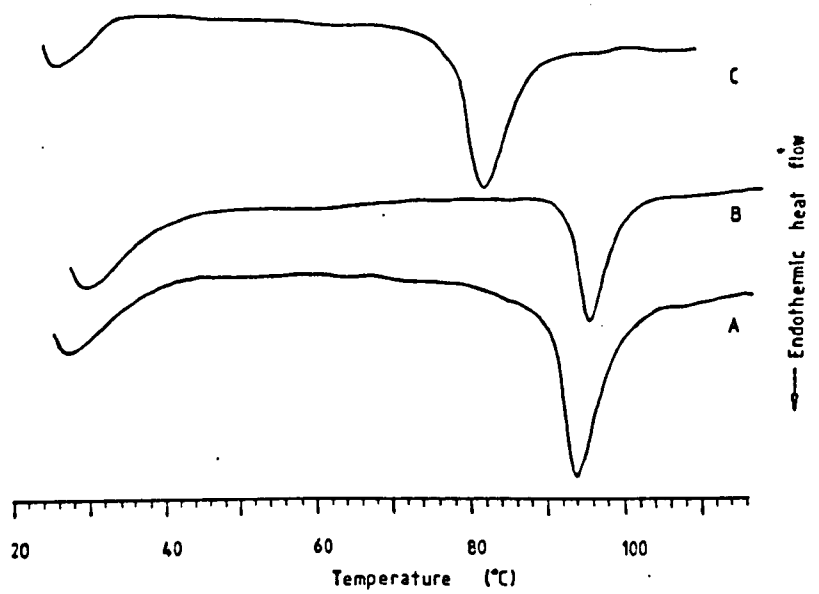

Fig. 81 DSC thermograms of amylose complexes with unsaturated fatty acids: A, oleic; B, elaidic; C, linoleic.

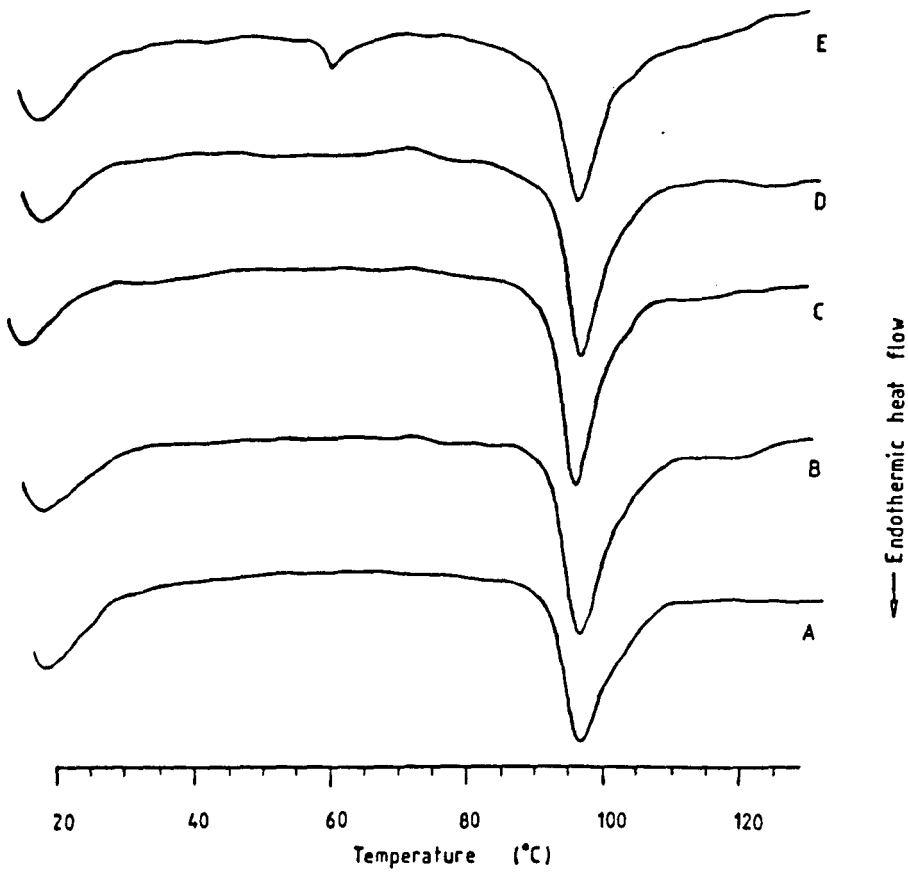

Fig. 82 DSC thermograms of amylose complexes, with palmitic acid at various molar ratios. MR = FA/AM: A, MR = 0·0185; B, MR = 0·0246; C, MR = 0·0370; D, MR = 0·0494; E, MR = 0·0616.

temperatures and enthalpies of complexes of saturated and unsaturated fatty acids are given in Tables 12 and 13 respectively together with the molar ratios at which the fatty acids were added to amylose. The molar ratios at which free lauric, myristic and palmitic acids could be detected in the DSC thermograms (Table 12) were in a very good agreement with calculated values for the saturation molar ratios and with experimental data obtained by different techniques (Table 6). It is also noteworthy that, within the experimental error, the enthalpy of dissociation of the complexes remained essentially the same ($29{\cdot}6 \pm 2{\cdot}1$ J/g) regardless of the chain length of the fatty acids. In the case of arachidic acid the dissociation enthalpy was somewhat higher ($33{\cdot}3 \pm 0{\cdot}9$ J3g) while for oleic and linoleic acids it was lower ($24{\cdot}6 \pm 0{\cdot}8$ J/g). This is probably due to the melting characteristics of the pure acids which may affect the dissociation of the respective complexes. Moreover, for each fatty acid, the dissociation enthalpy of the precipitated complexes was relatively constant and almost independent of the level of addition of fatty acid, suggesting that saturated complexes were preferentially removed from solution rather than partially filled amylose helices. Kugimiya and Donovan (1981) obtained very low dissociation enthalpies at low levels of addition of lipids to amylose. The discrepancy in their results is probably due to their method of preparing the complexes. They prepared amylose-lysolecithin complexes inside the DSC pans by mixing amylose and lysolecithin (both in the solid form) with excess of water followed by heating to 127°C. This temperature should be considered rather low, since it has been reported (Stute and Konieczny-Janda, 1983; Biliaderis et al., 1985), as it was also found

Table 12 Dissociation temperatures and enthalpies of fatty acid-amylose complexes at various molar ratios of fatty acid/amylose[a]

Fatty acid	Molar ratio	T_1[b] (°C)	ΔH_1[b] (J/g)	T_2[c] (°C)	ΔH_2[c] (J/g)	Free acid[d] (%)
Capric	0·079	87·7±0·6	18·5±1·0	108·0±0·3	10·6±1·0	0
	0·106	87·8±0·7	19·4±0·8	108·8±1·0	9·2±0·6	0
	0·132	87·2±0·5	19·2±0·2	107·9±0·8	9·5±0·8	0
	0·159	87·3±0·6	21·0±1·8	108·2±0·6	9·2±0·8	0
Lauric	0·040	94·1±0·2	22·4±1·8	114·0±0·8	6·0±0·5	0
	0·053	94·6±0·8	23·2±0·9	114·5±1·0	5·9±0·8	0
	0·079	93·6±0·8	23·0±1·2	114·0±0·9	5·5±0·6	4
	0·106	96·0±0·7	23·1±1·5	114·6±1·2	5·6±0·7	15
Myristic	0·036	93·7±1·0	24·6±0·2			0
	0·048	94·2±0·7	29·1±0·8			0
	0·072	93·3±0·9	29·0±2·0			10
	0·096	93·0±1·0	28·2±0·2			32
Palmitic	0·019	94·1±0·7	30·0±1·0			0
	0·025	94·0±0·5	26·0±0·7			0
	0·037	94·4±0·8	29·7±0·4			0
	0·049	94·5±1·0	30·2±0·8			0
	0·062	94·4±0·7	29·0±1·6			15
Stearic	0·012	97·3±0·9	26·5±0·6			0
	0·018	98·0±0·7	29·3±2·0			0
	0·024	98·1±0·1	26·6±0·9			0
	0·030	98·3±0·6	29·7±1·8			0
Arachidic	0·020	101·2±0·2	32·4±1·2			0
	0·026	101·6±0·8	34·6±1·5			0
	0·032	102·0±0·6	33·3±2·2			0
	0·039	101·0±0·4	33·1±1·2			0

[a] Means of four replicates ± standard deviation

[b] First dissociation temperature and enthalpy respectively

[c] Second dissociation temperature and enthalpy respectively

[d] As per cent of added fatty acid

Table 13 Dissociation temperatures and enthalpies of unsaturated fatty acid-amylose complexes at various molar ratios of fatty acid/amylose[a]

Fatty acid	Molar ratio	T (°C)	ΔH (J/g)
Elaidic	0·028	94·4±0·9	28·4±0·5
	0·037	94·2±0·3	30·2±2·0
	0·046	94·2±0·4	28·7±0·9
	0·056[b]	93·8±0·4	28·5±1·5
Oleic	0·028	91·2±0·5	24·1±0·9
	0·037	91·6±0·7	25·0±0·8
	0·046	91·2±0·6	25·1±1·0
	0·056	90·9±0·7	23·7±0·8
Linoleic	0·031	81·5±0·1	25·0±1·0
	0·039	80·4±0·8	25·6±1·2
	0·047	81·5±0·8	23·0±1·0
	0·055[c]	81·6±0·3	25·0±0·8

[a] Means of four replicates ± standard deviation

[b] Free fatty acid (11%) detected in the complex at a molar ratio of 0·056.

[c] Free fatty acid detected in the supernatant.

in the present work, that the melting point of crystalline amylose is 150-155°C. It is, therefore, not surprising that only part of the amylose participated in complexing.

The dissociation temperature of complexes of unsaturated fatty acids decreased with increasing number of double bonds and was highest for the trans-isomer in accord with the findings of Stute and Konieczny-Janda (1983) and Morrison (1985). Complexes made by mixing amylose and fatty acids in DMSO, followed by dilution with water to 10% DMSO (v/v) gave invariably two peaks; the first peak corresponded to the melting of the uncomplexed fatty acid, presumably entrained or occluded in the complex, and the second to the dissociation of the complex. For palmitic acid (Fig. 83), for example, the dissociation temperature remained constant, while the dissociation enthalpy showed a slight increase with increasing addition of fatty acid (Table 14).

Table 14 Dissociation temperatures and enthalpies of palmitic acid-amylose complexes, prepared in the presence of 10% DMSO, at various molar ratios of fatty acid/amylose[a]

Molar ratio	T (°C)	ΔH (J/g)	Free acid[b] (%)
0•027	97•2	19•2	53
0•039	96•6	20•7	
0•050	96•5	21•3	30
0•066	97•0	22•0	
0•078	96•5	22•5	38

a Data in duplicate
b As per cent of added fatty acid

The dissociation enthalpy was lower than that of the complexes

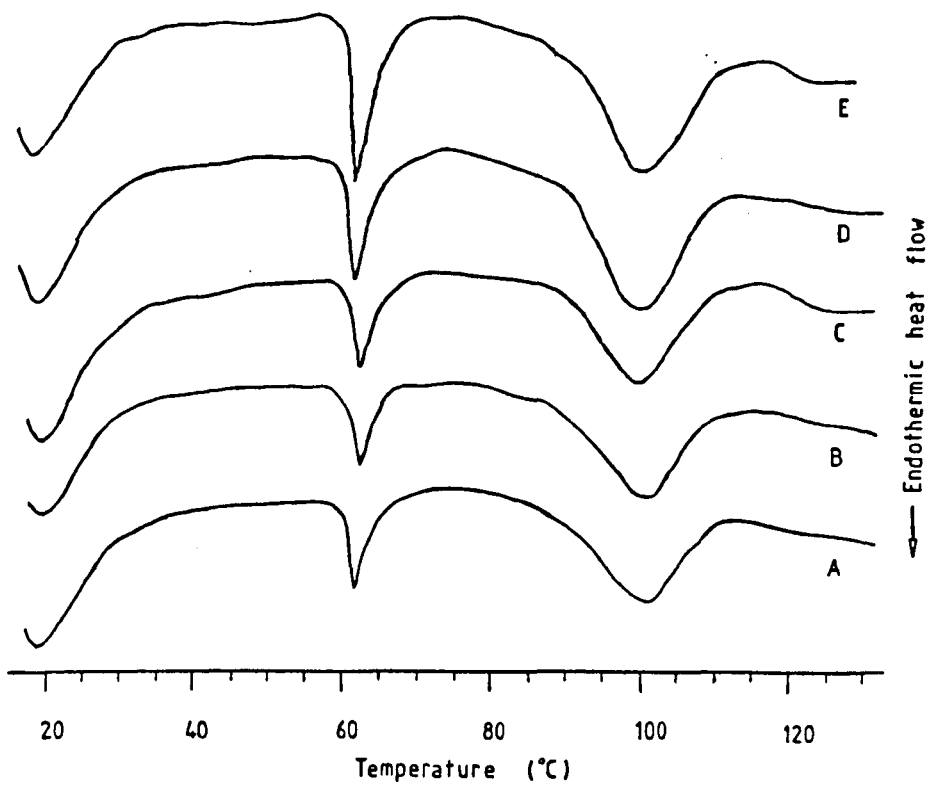

Fig. 83 DSC thermograms of amylose complexes with palmitic acid, from original DMSO solutions, precipitated with citrate buffer (pH 4·6) and heated at 85°C for 15 min: A, MR = 0·027; B, MR = 0·039; C, MR = 0·050; D, MR = 0·066; E, MR = 0·078. MR is the molar ratio FA/AM.

originating from alkaline solution suggesting imperfect structure of the helix.

After reaching maximum temperature in the pan (135°C), followed by rapid cooling, reheating of the complexes at a rate of 10°C/min gave rise to a single peak corresponding to the fatty acid released from the helix and no endotherm due to the complex could be detected (Fig. 84). However, capric and lauric acids, both of which showed double peaks for the complex, did not give any signs of dissociation, and the peaks merged into a single peak on reheating as shown in Fig. 85. This phenomenon is further discussed below.

3.5.1 Thermal behaviour of heat treated complexes

Stute and Konieczny-Janda (1983) were the first to demonstrate that amylose-lauric acid complexes heated in water to 80 - 100°C and cooled rapidly showed a shift of the dissociation temperature from 98° to 115°C. The complex with the higher dissociation temperature was birefringent and gave a crystalline X-ray diffraction pattern. The same authors found also that amylose-palmitic acid complexes (in aqueous suspension containing 20% dry complex) could be dissociated by heating to 135°C; after rapid cooling two peaks were obtained on DSC analysis. The first peak corresponded to the melting of the free fatty acid, while the second at about 150°C was due to the dissociation of crystalline (retrograded) amylose. The results of similar experiments performed during the present work are shown in Figures 86 - 88, for lauric, palmitic and arachidic acid complexes.

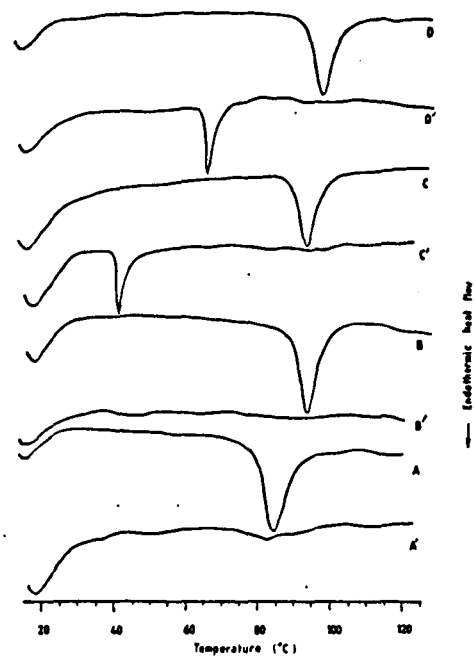

Fig. 84 DSC thermograms of amylose complexes with fatty acids: A, A', linoleic; B, B', oleic; C, C', elaidic; D, D', stearic. In A, B, C and D the complexes were heated to 135°C at 10°C/min and cooled rapidly. In A', B', C' and D' the same samples were reheated to 135°C, at 10°C/min.

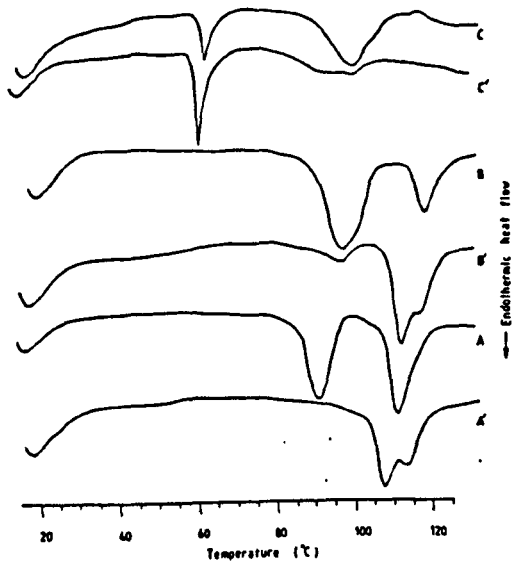

Fig. 85 DSC thermograms of amylose complexes with fatty acids: A, A', capric; B, B', lauric; C, C', palmitic (complex precipitated from original DMSO/water solutions and heated at 85°C for 15 min). In A, B and C the complexes were heated to 135°C at 10°C/min and cooled rapidly. In A', B' and C' the same samples were reheated to 135°C at 10°C/min.

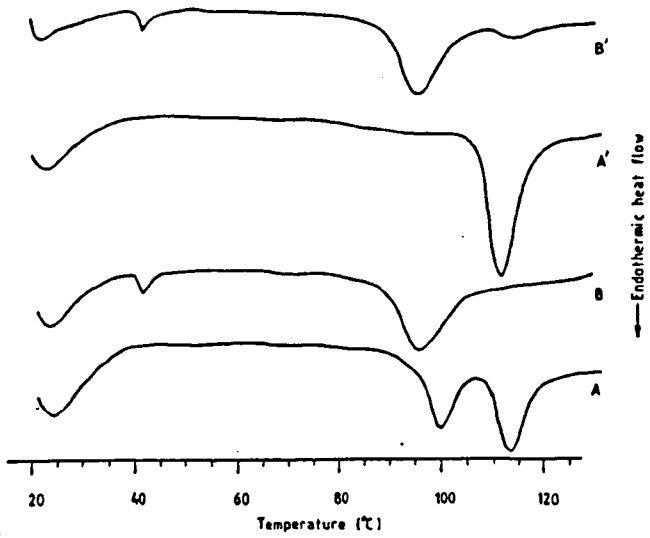

Fig. 86 DSC thermograms of amylose complexes with lauric acid formed: A, from original alkaline solution (0·01M KOH), neutralised, precipitated and heated at 90°C for 16 h; B, from original DMSO solution, precipitated after dilution with H_2O and buffer (pH 4·6) and heated at 90°C for 16 h; A', prepared as A but heated at 135°C for 30 min and then at 90°C for 16 h; B', prepared as B but heated at 135°C for 30 min and then at 90°C for 16 h.

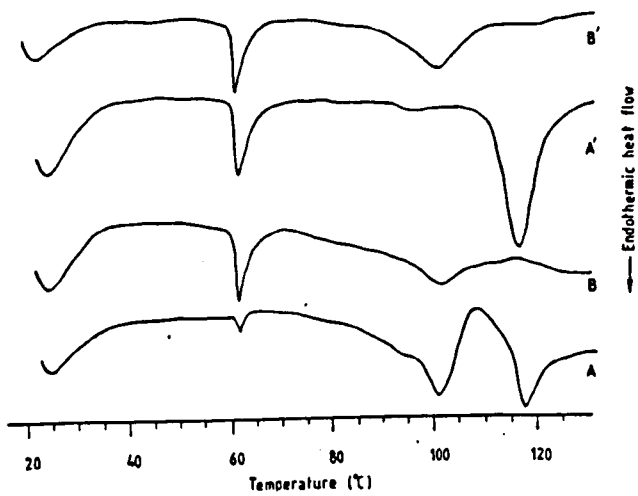

Fig. 87 DSC thermograms of amylose complexes with palmitic acid formed: A, from original alkaline solution (0·01M KOH), neutralised, precipitated and heated at 90°C for 16 h; B, from original DMSO solution, precipitated after dilution with H_2O and buffer (pH 4·6) and heated at 90°C for 16 h; A', prepared as A but heated at 135°C for 30 min and then at 90°C for 16 h; B', prepared as B but heated at 135°C for 30 min and then at 90°C for 16 h.

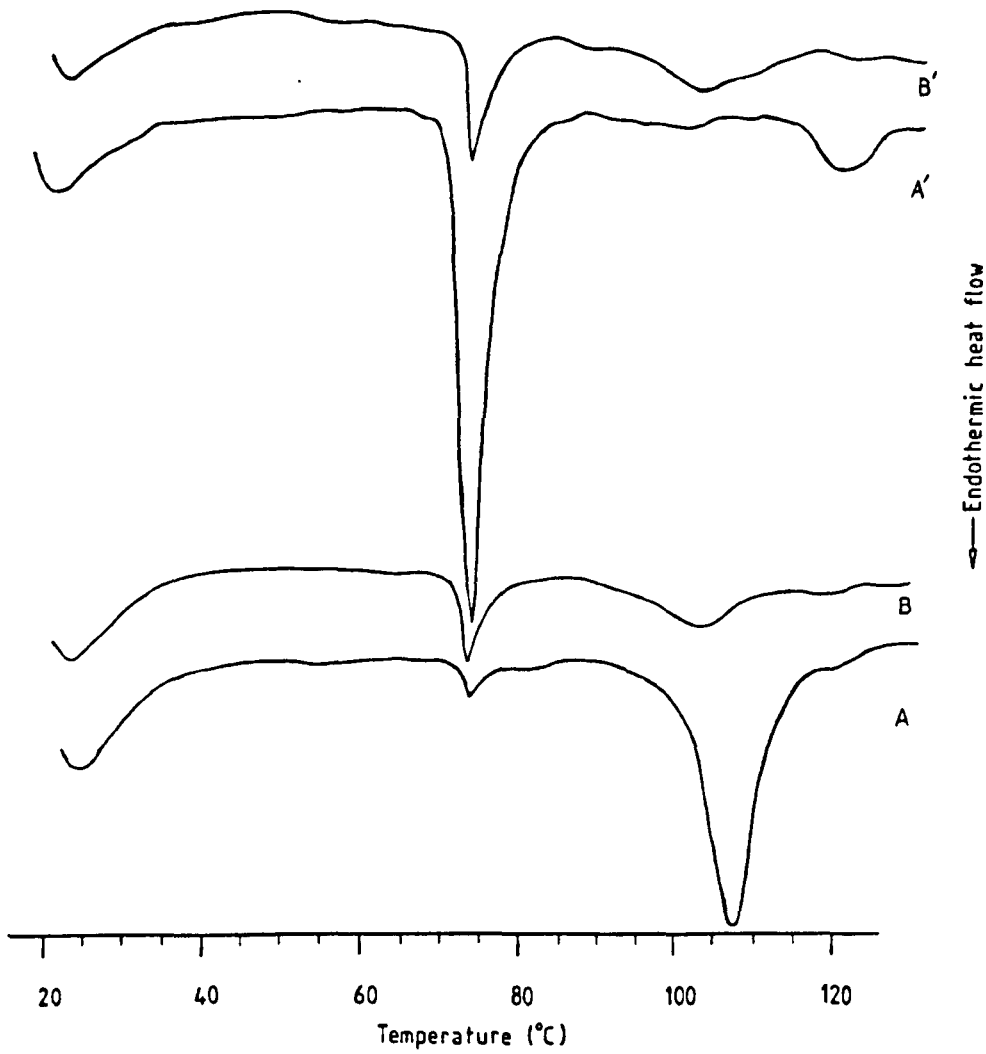

Fig. 88 DSC thermograms of amylose complexes with arachidic acid formed: A, from original alkaline solution (0·01M KOH), neutralised, precipitated and heated at 90°C for 16 h; B, from original DMSO solution, precipitated after dilution with H_2O and buffer (pH 4·6) and heated at 90°C for 16 h; A', prepared as A but heated at 135°C for 30 min and then at 90°C for 16 h; B', prepared as B but heated at 135°C for 30 min and then at 90°C for 16 h.

The complexes of lauric and palmitic acids formed from KOH solution, heated at 90°C only and cooled rapidly, gave two peaks while a single peak was obtained on heating first to 135°C followed by heating at 90°C and rapid cooling. Arachidic acid gave a single peak at 90°C, but dissociation of the complex occurred at 135°C giving rise to a large peak due to the free fatty acid and a very small peak due to the complex. Complexes prepared by dilution of the DMSO solutions dissociated at a lower temperature, and contained entrained free fatty acid as shown in the same Figures. These complexes appeared to be more stable during heat treatment than the respective complexes prepared by the KOH method. No shift in their endothermic peak of dissociation towards higher temperatures was observed when they were heated at 135°C. Dissociation enthalpies are given in Table 15. The free acids, measured in the supernatants, increased in the order lauric, palmitic, arachidic in the ratio 1:10:100. The amount of entrained acids, precipitated with the complex, also increased in the same direction. In all probability this could be explained on the basis of the increase in the melting point of the acids from lauric, through palmitic to arachidic. Due to the rapid cooling rate, after maintaining the complexes at 90°C, the fatty acids crystallised faster than they could complex with amylose with the result that only a small fraction of the acid with a high melting point was available to form a complex. Thermograms of partly dissociated complexes with a peak at 150°C due to crystalline amylose are shown in Fig. 89.

Fig. 90 shows the thermograms of amylose-lauric acid complexes obtained at 50°, 60° and 70°C by slow cooling. The increase in the

Table 15 Dissociation temperatures and enthalpies for fatty acid-amylose complexes prepared as described in the text and heated as shown[a]

Fatty acid		T (°C)		ΔH (J/g)	Free acid[b] (%)	Adsorbed acid[c] (%)	Molar ratio
Lauric[d]							
KOH 90°C	(T1)	98·5±1·0	(ΔH1)	8·1±0·3	0·1	0	0·048
	(T2)	111·5±0·2	(ΔH2)	11·5±0·7			
DMSO 90°C		94·4±0·5		22·6±1·1	0·00	e	0·039
KOH 135°C		110·2±0·5		30·5±1·2	0·1	0	0·048
DMSO 135°C		92·5±0·5		19·5±0·6	0·00	e	0·039
Palmitic[d]							
KOH 90°C	(T1)	99·5±1·0	(ΔH1)	17·4±0·8	0·8	14±1	0·048
	(T2)	115·1±0·7	(ΔH2)	7·9±0·6			
DMSO 90°C		98·6±0·5		13·8±1·0	0·2	e	0·041
KOH 135°C		114·1±0·3		28·3±0·2	0·8	46±2	0·048
DMSO 135°C		97·2±1·2		12·5±0·6	0·9	e	0·041
Arachidic[d]							
KOH 90°C		105·2±0·1		35·0±0·7	0·6	11±1	0·030
DMSO 90°C		100·5±0·5		18·6±1·0	0·5	e	0·025
KOH 135°C		121·3±0·2		21·0±2·0	7·2	f	0·030
DMSO 135°C		100·0±0·5		16·0±1·2	0·8	e	0·025

[a] Means of four determinations ± standard deviation

[b] As per cent of added acid extracted from the supernatant with diethyl ether and determined by GLC

[c] As per cent of added acid calculated from the thermogram showing an endothermic peak for free acid

[d] Method of preparation of complexes, KOH or DMSO, and heating temperature; for details see text.

[e] Not calculated because it was impossible to distinguish between free and adsorbed value

[f] Very large value beyond the range of the instrument

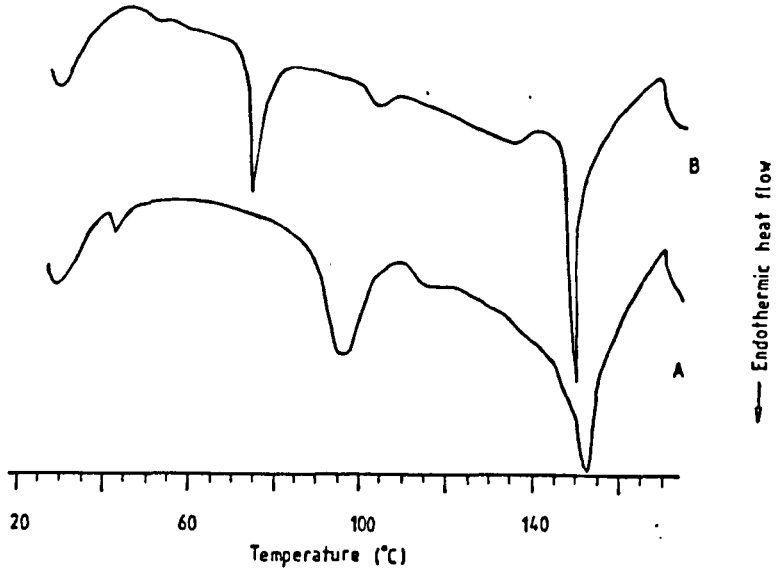

Fig.89 DSC thermograms of amylose complexes with: A, lauric acid from original DMSO solution, precipitated after dilution with water and buffer (pH 4·6), heated at 135°C for 30 min and then at 90°C for 16 h; B, arachidic acid prepared and heattreated as A.

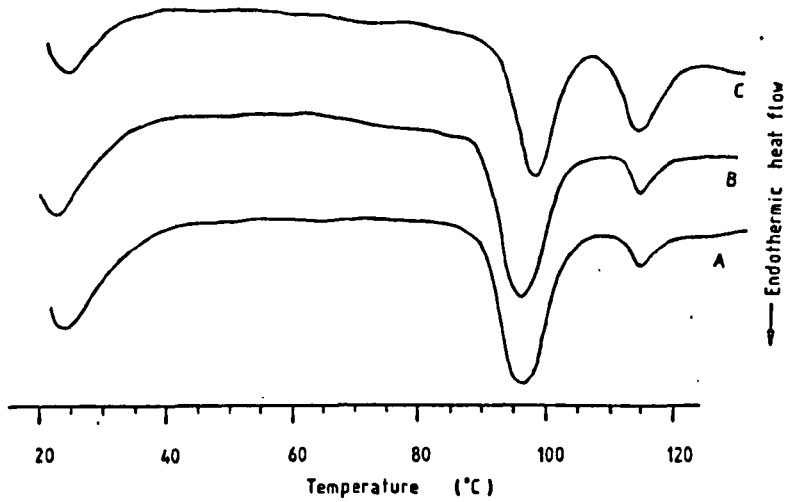

Fig. 90 DSC thermograms of amylose-lauric acid complexes formed from original alkaline solutions (0·01M KOH), neutralised (pH 4·6) and precipitated at temperatures: A, 50°C; B, 60°C; C, 70°C.

size of the peak at 115°C is in accord with published data (Stute and Konieczny-Janda, 1983). The enthalpies of dissociation are given in Table 16.

Table 16 Dissociation temperature and enthalpy for amylose-lauric acid complexes prepared at various temperatures[a]

Temperature of preparation	T_1 (°C)	ΔH_1 (J/g)	T_2 (°C)	ΔH_2 (J/g)	Free acid (%)
50	93•3±1•0	27•7±0•6	111•5±0•5	0•6±0•2	0
60	93•3±0•8	22•4±0•7	112•6±0•7	2•0±0•2	0
70	94•5±0•5	15•8±0•6	111•0±0•2	7•6±0•2	0•1

[a] Molar ratio (FA/AM) = 0•046

In another experiment amylose-palmitic acid complexes were prepared by the alkaline method. Some of the precipitated complexes were freeze-dried and some were left wet. To the wet complexes toluene (1:8 by volume) was added and heated respectively to 80°, 90° and 100°C for 4 hours. The freeze dried precipitates showed no endothermic peaks because of complete dissociation of the complexes. Wet precipitates were also treated with methanol (1:8 by volume) at 50°, 60° and 70°C for 1 hour, respectively. GLC analysis of the supernatant did not show any free palmitic acid. DSC analysis of the freeze dried precipitates did not reveal any change in the endothermic peak of dissociation in relation to the unheated (control) sample. Dried complexes (appr. 15% moisture content), were treated with anhydrous methanol, at 20°C, from 1 to 4 hours. Thermograms of the freeze dried precipitates show two endothermic peaks, one at 94°C, identical but broader to that of the untreated sample and the other at

118°C; the sum of the enthalpies of the two peaks was equal to the enthalpy of the dissociation peak of the control (Fig. 91).

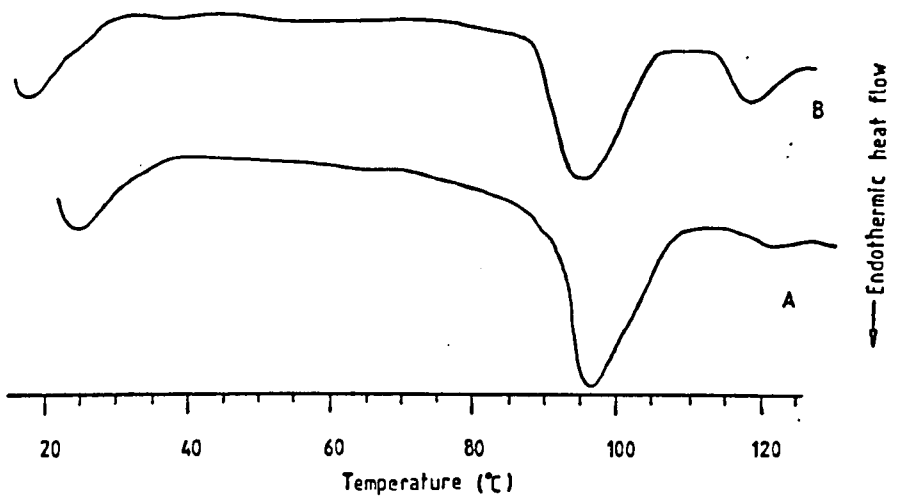

Fig. 91 DSC thermograms of amylose complexes with palmitic acid formed from original alkaline solutions (0·01M KOH), neutralised, precipitated and extracted with methanol: A as wet precipitate at 50°C for 1 h; B as freeze-dried precipitate at 20°C for 1 h.

This may be explained by considering the dehydrating effect of methanol. It is possible that anhydrous methanol attracted part of the water from the complex thus causing the formation of a more compact structure. Yamashita and Hirai (1966) found, by X-ray analysis, that wet amylose-propan-2-ol complexes had helices composed of 7 glucosyl residues, while crystals of the complexes treated with methanol gave helices with 6 glucosyl units.

3.5.2 Factors determining the nature of the complexes

The results in Table 17 indicate that there are considerable differences in the dissociation temperature of the complexes as reported by various investigators. These differences could be attributed mainly to the methods used in the preparation of the complexes. Morrison (personal communication) used high temperatures and high ionic strengths for the preparation of complexes; Whittam *et al.* used high temperatures, and Stute and Konieczny-Janda moderately high temperatures and high ionic strength.

Table 17 Dissociation temperatures (^{o}C) of amylose-fatty acid complexes

Reference	Capric	Lauric	Myristic	Palmitic	Stearic	Arachidic
This work	88/108	95/114	94	94	98	102
Morrison (1985)	66	84	94	109	115	119
Whittam *et al.* (1986)	82	86	89	95	103	-
Stute and Konieczny-Janda (1983)	93	98	-	103	100/120	-
Eliasson and Krog[a] (1985)	-	85	90	99	104	-

[a] Corresponding monoacyl glycerides

There is no doubt that complexes prepared from media containing DMSO invariably show a peak for the free lipid as in Fig. 83. The DSC data by Eliasson and Krog (1985) and by Biliaderis *et al.* (1985) also show the presence of non-complexed lipids. It is, therefore, suggested that DMSO and lipids compete for occupancy of the helix.

The enthalpies of dissociation found in the course of this work are in excellent agreement with those of Eliasson and Krog (1985) while the values reported by Morrison (1985) and by Biliaderis et al. (1985) are considerably lower. The direct determination of amylose in the DSC pan is particularly useful for accurate results. However, if part of the determined amylose remains uncomplexed, the dissociation enthalpy will appear low.

The appearance of two isotherms (double dissociation peaks) for capric and lauric acids, and for palmitic acid after heating to 90°C (annealing), may be due to the formation of a more compact helix, i.e. a transition from 7 to 6 glucosyl residues per helix. The process of partial melting followed by recrystallisation proposed by Biliaderis et al. (1985) is most unlikely because such a phenomenon has never been observed with other crystalline substances. It is also interesting that these authors observed this phenomenon at intermediate and low water contents only, while the present work and the work of Stute and Konieczny-Janda (1983) shows that two transition endotherms are possible at high moisture contents as well.

It can, therefore, be concluded that the nature of the amylose-lipid complexes is primarily a function of the method of preparation, particularly the solvent used as well as the temperature and the rate of cooling. Rearrangement of the helix on heating at 90°C, or dissociation at 135°C, may also be of technological significance in bread making and in the preparation of extruded products containing starch and emulsifying agents.

3.6 X-ray diffraction studies of the amylose-fatty acid complexes

X-ray diffraction patterns for three fatty acid complexes are shown in Fig. 92. All samples have been freeze-dried. The patterns are typical of V-amylose complexes as reported by many other workers. Samples a, c and e of the three complexes prepared at room temperature by the KOH method, showed identical patterns and their intensity was the same thus contradicting the argument of Takeo *et al.* (1973) that the longer the chain length of the fatty acid complexed, the lower the crystallinity of the complex. All samples had low crystallinity as reported by other workers such as Ghiasi *et al.* (1982). Diffractograms a and b are due to the capric acid complex. Sample b, which was neutralised and precipitated at 50°C had a higher crystallinity than sample a. Increased crystallinity has been reported by Stute and Konieczny-Janda (1983), for the amylose-lauric acid complex heated to 135°C and kept for 16 h at 90°C, in comparison with a non-heated sample. Diffractogram d, due to the amylose-palmitic acid complex prepared by the DMSO method, had a lower crystallinity compared with its counterpart c which was prepared by the KOH method.

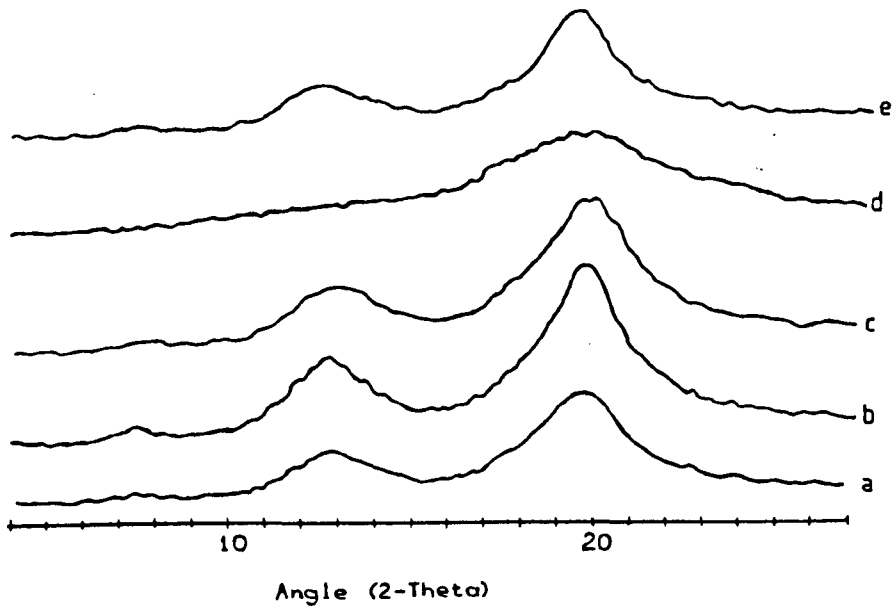

Fig. 92 X-ray diffraction diagrams of freeze-dried amylose-fatty acid complexes: a, capric acid-amylose complex (0·150); b, capric acid-amylose complex precipitated at 50°C (0·200); c, palmitic acid-amylose complex (0·045); d, palmitic acid-amylose complex precipitated from DMSO (10%)-water solution; e, arachidic acid-amylose complex (0·045). Molar ratio FA/AM in parentheses.

CONCLUSIONS

1. Under favourable experimental conditions the formation of helical inclusion complexes of amylose with fatty acids (12:0 to 22:0) occurs in a strictly stoichiometric fashion. The quantity of fatty acid (or lipid in general) required for saturation of the amylose helix can be predicted from the calculated chain length of the guest molecule and the dimensions of the amylose helix. This relationship is valid for complexed amylose in alkaline solution (pH 12) and for insoluble complexes precipitated at pH 4·6. The amylose helix may contain 6 or 7 anhydroglucose residues per turn with an accepted spacing of 0·8 nm between adjacent helices.

2. Unsaturated fatty acids with 1 to 4 cis-double bonds form complexes almost as effectively as their saturated analogues.

3. Fatty acids with short chain lengths (below 10:0) do not complex at pH 12 although they give insoluble complexes at pH 4·6 with the exception of butyric acid. Complexing at pH 12 may be prevented because of electrostatic repulsions between the linearly disposed fatty acids anions, the absence of adequate hydrophobicity and the expansion of the amylose helix because of partial ionisation.

4. Precipitated amylose-lipid complexes can be completely hydrolysed by amyloglucosidase; when the enzyme concentration is sufficiently high (1 mg/ml ≡ 6 units/ml) in relation to that of the complex (1 mg/ml) hydrolysis is complete within 30 min. at 60°C.

5. Fatty acids in alkaline solution (pH 12) even above their CMC interact with amylose to give inclusion complexes. Evidence is provided by the high and constant value of the surface tension of

solutions containing amylose and fatty acids as potassium salts. Surface tension is rapidly diminished when saturation of the available amylose has taken place.

6. The saturation of amylose helices with linearly arranged fatty acid molecules can be demonstrated by DSC experiments whereby the dissociation enthalpy of precipitated insoluble complexes remains virtually the same regardless of the molar ratio and the chain length of the fatty acid used.

7. DSC data demonstrate that precipitated insoluble complexes heated in the presence of water at temperatures near or above their dissociation temperature, and cooled rapidly, are subject to structural changes. It is conceivable that a conformational transition takes place from 7 glucosyl residues per helix to 6 residues per helix. Supportive evidence is provided by the increased crystallinity of the complexes.

8. Rheological tests performed with three types of rheometer, provide evidence that amylopectin interacts with lipids in alkaline solution, although the nature of this interaction is not clear. The longer the fatty acid, the stronger the interaction. Precipitation of complexed amylopectin is never observed under any circumstances.

9. The elastic modulus of gels formed on complexing gelatinised starch suspensions with fatty acids at pH 12, depends on the concentration and chain length of fatty acids involved, as well as the relative concentration of amylose and amylopectin present.

10. Concentrated amylose and starch solutions (35 mg/ml) at pH 12 readily form gels with long chain fatty acids, *viz.* stearic, elaidic and oleic. The longer the fatty acid the faster the gelation and the

greater the rigidity of the gel. Granular starch dispersions at pH 12 show similar behaviour.

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APPENDICES

1 Determination of the friction torque of the air-bearing of the Viscoelastic-analyser

The friction torque was determined by applying increased torque values on the measuring unit (cone) of the Viscoelastic analyser, which was allowed to rotate freely in the air, and by recording the respective velocities developed. The recorded angular velocities as a function of the applied torque values are shown in Fig. 93. The friction torque was determined from the equation:

$$\text{friction torque} = \text{intercept} + \text{slope} = 0{\cdot}0174\,\Omega$$

The intercept denotes the degree of balance of the instrument and the slope denotes the friction of the air bearing.

It can be seen that the friction torque becomes significant only at high shear rates and it is probably due to air turbulence developed inside the motor air bearing or due to some mechanical friction.

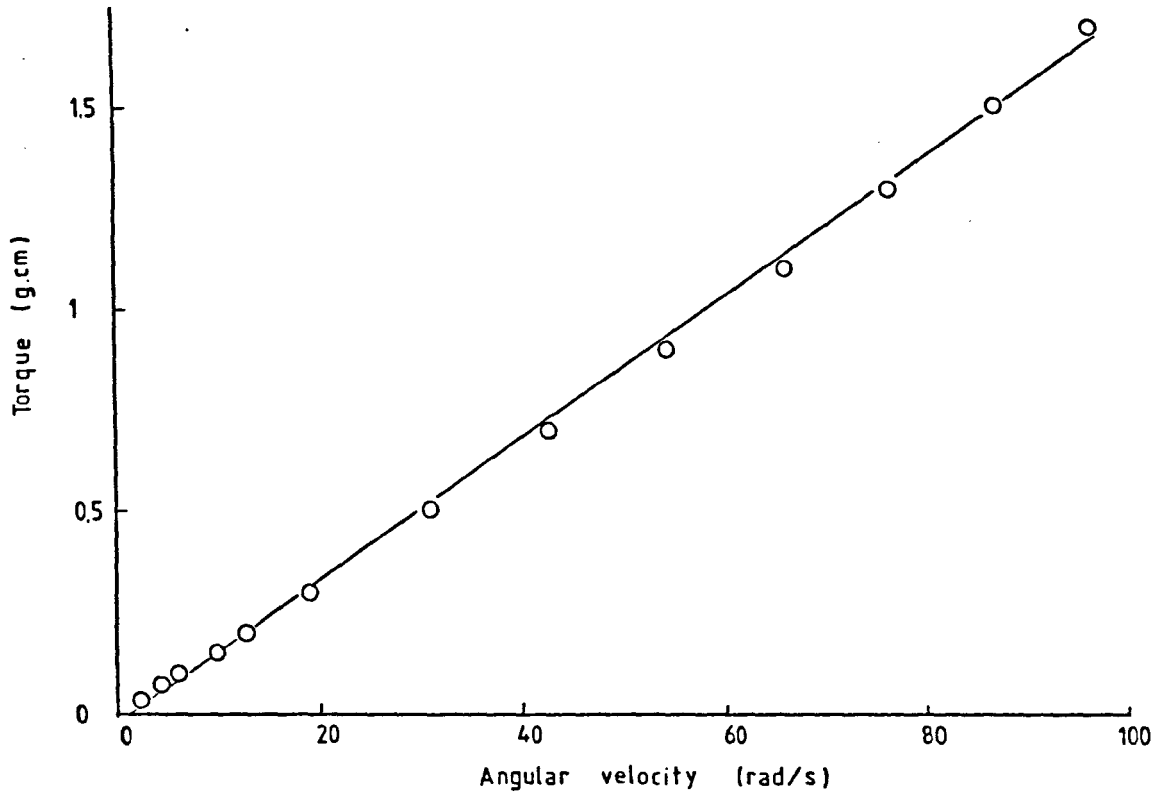

Fig. 93 Measured angular velocity as a function of the applied torque for the air bearing friction of the Viscoelastic analyser (Sangamo). Regression equation: Friction torque: $y = -0{\cdot}0205 + 0{\cdot}0174\Omega$; $r = 0{\cdot}9995$.

2. Determination of the moment of inertia of the measuring system of the Viscoelastic analyser

The moving part of the measuring system consisted of the motor air bearing and the cone. The moment of inertia of the system I is given as the sum of the moment of inertia I_m of the motor and the moment of inertia of the cone I_c.

I_c was determined using equation $I_c = \sum_{i=1}^{N} \frac{\rho \pi l_i r_i^4}{2}$

where, ρ is the density of the cone (stainless steel) 7·9 g/cm^3

l_i is the length of the individual parts of the cone unit

r_i is the radius of the individual parts of the cone unit

For the determination of I_m a torsion wire was used which was firmly attached to the air bearing and to the static part of the measuring system, (plate) in order to make use of the equation $I = \frac{K}{\omega_o^2}$, where K is the restoring constant of the torsion wire and ω_o is the natural frequency of the wire, determined using the free vibration test, i.e. a torque was applied to the measuring system connected with the wire and it was allowed to vibrate freely. K was calculated from equation $K = \frac{Gr^4}{321}$ where, G for stainless steel wire is 8×10^{10} pa, r is the diameter of the wire and l its length (Adams, 1975).

SUGGESTIONS FOR FURTHER WORK

1. Application of spectroscopic e.g. NMR and optical methods in order to elucidate the mechanism of gel formation in amylose-fatty acid complexes.

2. The effect of temperature on the development of rigidity modulus on amylose-fatty acid gels.

3. The effect of pH and ionic strength on the gelling properties of the complexes.

4. Aging of amylose and starch-fatty acid gels.

5. Kinetics, using microcalorimetry, to determine the enthalpy changes of amylose-fatty acid interactions in solution with time.

6. Determination of the DP of amylose fractions which interact with a quantity of fatty acid. Proposed method: Identical samples of amylose solutions in 0·01M KOH are mixed with fatty acid solutions containing progressively increased acid concentrations. After neutralisation and precipitation, the supernatant amylose could be analysed by gel filtration.

7. The effect of temperature and water content during thermal phase transition studies of the complexes.

8. The effect of heat on the structure of the complexes to be investigated in depth in an effort to elucidate the mode of action of lipids in bread, in extruded products and in starch based foods. The investigation is suggested to be carried out by DSC and X-ray analysis.