THE VISCOSITY AND MOLECULAR CONFORMATION OF GALACTOMANNANS IN SOLUTION

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To Sharyn.

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Abstract

Two galactomannans, from the seeds of Guar (Cyanopsis tetragonoloba) and Carob (Ceretonia siliqua) also known as Locust Bean, have found widespread use as industrial hydrocolloids. Many other galactomannans have been isolated from legume seeds, but so far none have been widely employed in industry.

Measurements of the viscosity of the solutions of some of these latter galactomannans (from Red Clover, Lucerne, Lotus pedunculatus, Sophora japonica, and Soybean) were made along with solutions of Guar and Carob galactomannans. A wide range of viscosities was found for the various samples, with the viscosity of Guar and Carob galactomannan solutions being the highest.

The effects of shear rate, temperature, and galactomannan concentration on the solution viscosities were examined. The measured viscosity changes correlated well with Schutz's (1970) empirical equations explaining the viscous solution behaviour of pseudoplastic carbohydrates (including Guar galactomannan). The effects on viscosity of two common industrial additives, sucrose and sodium chloride, were also examined, and interpreted in terms of a dehydration mechanism.

In order to explain the viscosity results, the galactomannan molecular weights were determined by measuring their sedimentation and diffusion coefficients in an Analytical Ultracentrifuge. Their molecular weight distributions were obtained both from their Schlieren sedimentation diagrams using the method of Gralen and Langermalm (1952), and from the ratio of their apparent diffusion coefficients to their weight-average diffusion coefficients (Henley, 1962).

The dependence of galactomannan intrinsic viscosities, sedimentation coefficients, and diffusion coefficients on molecular weight (weight-weight average and sedimentation-diffusion average) was

examined, and a good linear correlation obtained in each case, with two exceptions. Lucerne galactomannan had a very wide distribution of molecular weights. The galactomannan from Lotus pedunculatus II was thought to differ slightly in structure from the other galactomannans due to its high substitution ratio of one galactose sidechain for every mannose in the molecular backbone which changed the conformation of the mannose backbone.

The dependence of the galactomannan intrinsic viscosities, sedimentation coefficients and weight-average diffusion coefficients on the weight-weight average degree of polymerisation and/or the weight-weight average molecular weight was examined in the light of recent hydrodynamic theories. By use of the theories of Debye, Beuche and Brinkman; Kirkwood and Riseman; Kuhn and Kuhn; Peterlin; and Flory, Fox and Mandelkern it was deduced that the galactomannan molecule approximates to a random coil in solution. The molecule is highly extended, and the presence of the galactose sidechains alters its draining characteristics, making its solution behaviour deviate from that expected from the theories.

Comparison of galactomannan solution behaviour with that of two soluble cellulose ethers, ethylhydroxyethyl cellulose (Manley, 1956) and hydroxyethyl cellulose (Brown, 1962) shows many similarities.

It was concluded that galactomannans in solution can be treated as a linear polymer series provided that the galactose : mannose ratio is not less than 1 : 5 or more than 1 : 1.1, and also that their molecular weight distributions are very similar. This implies that other galactomannans could be used industrially instead of those of Guar and Carob, provided some problems of yields and solubility could be surmounted.

It was also concluded that galactomannans in aqueous solution have conformations very similar to water-soluble cellulose derivatives. Similarities between mannans and cellulose have already been proved in the solid state.

Preface

This work was originally intended to be a comparison of the viscosity of various galactomannan solutions with that of some of their derivatives (e.g. sulphates).

The purpose of this was to determine whether the derivatives had more desirable properties from an industrial viewpoint than the original galactomannans, especially with regard to viscosity. The idea for this came from the large number of water-soluble cellulose derivates that are used industrially.

For the above work, as simple a picture as possible of viscosity was required, and this led to the neglecting of shear rate in the measurement of the variation of galactomannan viscosity with temperature and concentration.

After these first measurements had been carried out, it was realised that galactomannan solution viscosities were much more complex than had previously been imagined. Accordingly, the original aim of this project was altered, and more complete measurements of viscosity (including shear rate effects) and galactomannan molecular parameters in solution were made, to provide a basis for any future work on galactomannan derivatives.

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SECTION I

INTRODUCTION

Gums* have been isolated from many natural sources such as plants, animal hides and bones, fermentation slimes, and seaweeds. Other gums unknown in nature have been synthesised in the laboratory. Many of these gums are polysaccharides and have been grouped according to plant origin or solution properties. However a gum may show adhesive (i.e. gumlike) or slimy (i.e. mucilaginous) properties under different conditions (e.g. pH changes), thus although a separate classification for mucilages has been attempted by some authors, such a classification is not necessary.

More recently it has been suggested (Glicksman 1969) that there are three main categories of gums:- (a) Natural gums, (b) Modified natural gums - produced by chemical modification of natural gums or gumlike materials, (c) Synthetic gums, prepared by total chemical synthesis.

The natural gums of plant origin, into which category galactomannans fall, are extracted from three main sources:-

(i) Exudates from the fruit, branches or trunks of trees, arising spontaneously or through a protective mechanism when the plant has been injured.

(ii) Seed endosperm, where the polysaccharide is a food store for the embryonic plant, and may also have a structural function.

(iii) Sea plants, from which a large proportion of the dry weight can be extracted as polysaccharide gums. These polysaccharides fulfill the same purpose as celluloses and hemicelluloses do in land plants, in that they bind the cells together. (Glicksman, 1969).

Galactomannans are found in the second class above, they normally occur in the endosperm of legume seeds, although other seed sources have been reported, and galactomannans have also been isolated from yeasts,

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* Definition, see Appendix VII p197.

molds, and fungi (see Appendix II). Gums today have a very widespread useage (especially in the food and paper and pulp industries), based on their viscosity at low concentration in solution, their gelling properties and their general hydrocolloid and solution properties. In order to understand these uses and properties, it is necessary to understand something of the structure of gums in general. Since the great majority of gums are polysaccharides of various types, the discussion will be based on these. [This discussion is based on Whistler (1959 and 1960)]

For a polysaccharide to produce a solution of high viscosity at low concentration, or even a gel, it must be extremely hydrophilic. The forces causing this hydrophilicity are mainly those of hydrogen bonds, but intermolecular secondary forces such as those of dipoles, ion-dipoles, and induced dipoles also act. In water polysaccharides exhibit vastly different degrees of hydration. Some polysaccharides are partially hydrated, with the remaining secondary forces satisfied by intermolecular hydrogen bonding, giving rise to a gel structure. At the other extreme the molecule may be completly surrounded with associated water molecules, and thus completely dissolved.

The degrees of hydration, and of intermolecular bonding, are not the only factors which determine the solution properties of polysaccharides. Molecular weight, shape, charge effects, salt effects, and interaction with any other solution components are also important.

Polysaccharides containing carboxyl groups, and the polysaccharide half-esters of sulphuric acid have special properties. Both the carboxylate group and sulphuric acid group are highly hydrated, and therefore increase both the amount of water bound to the polysaccharide and its solution stability. Furthermore, the anionic groups keep the molecule in extended form by coulombic repulsion, and prevent molecules coming into close proximity. If neutral polysaccharides are considered, then charge effects will become important only at high or very low pH. Under normal conditions (i.e. near neutrality) the shape and degree of hydration of these molecules will undergo little change with pH.

Linear neutral polysaccharides will be more viscous than branched ones of the same molecular weight, since the linear molecules, although not fully extended, will sweep out a greater volume in solution. However linear polysaccharides tend to precipitate rapidly out of solution as their shape allows intermolecular association over large areas of the molecule. Consequently particles of associated molecules will form quickly, grow beyond colloidal size, and then precipitate.

Although highly branched neutral polysaccharides produce much less viscosity, they are more readily soluble and do not tend to precipitate out of solution. Intermolecular bonds are possible only over very short contact areas of the molecule, and molecular shape precludes a high degree of association between any two molecules. One highly branched molecule is able to form intermolecular bonds to many other molecules, resulting in a gel network.

In both linear and branched molecules it can be easily seen that the molecular weight (or degree of polymerisation) will affect the polysaccharide solution viscosity. However natural polysaccharides are not normally isolated as a homogeneous species consisting of molecules of one molecular weight, but rather as a continuous series or spectrum of molecular weights. The molecular weight distribution will also affect the solution viscosity.

The structure of galactomannans isolated from seed endosperms (see Appendix I) has been shown to consist of a main chain of β-(1,4) linked D-mannopyranose units with single D-galactopyranose units linked α-(1,6) to the main chain units at intervals (Smith and Montgomery, 1959; Pigman and Horton, 1970; Aspinall, 1970). Evidence from the analysis of the products of enzymic hydrolysis (Courtois and Le Dizet; 1966, 1970) indicates that the galactose side-chain units tend to occur in blocks with few in isolated positions. A generalised galactomannan structure is shown in fig. (I-1). (Galactomannans isolated from yeasts, molds and fungi (see Appendix II) have somewhat different structures.)

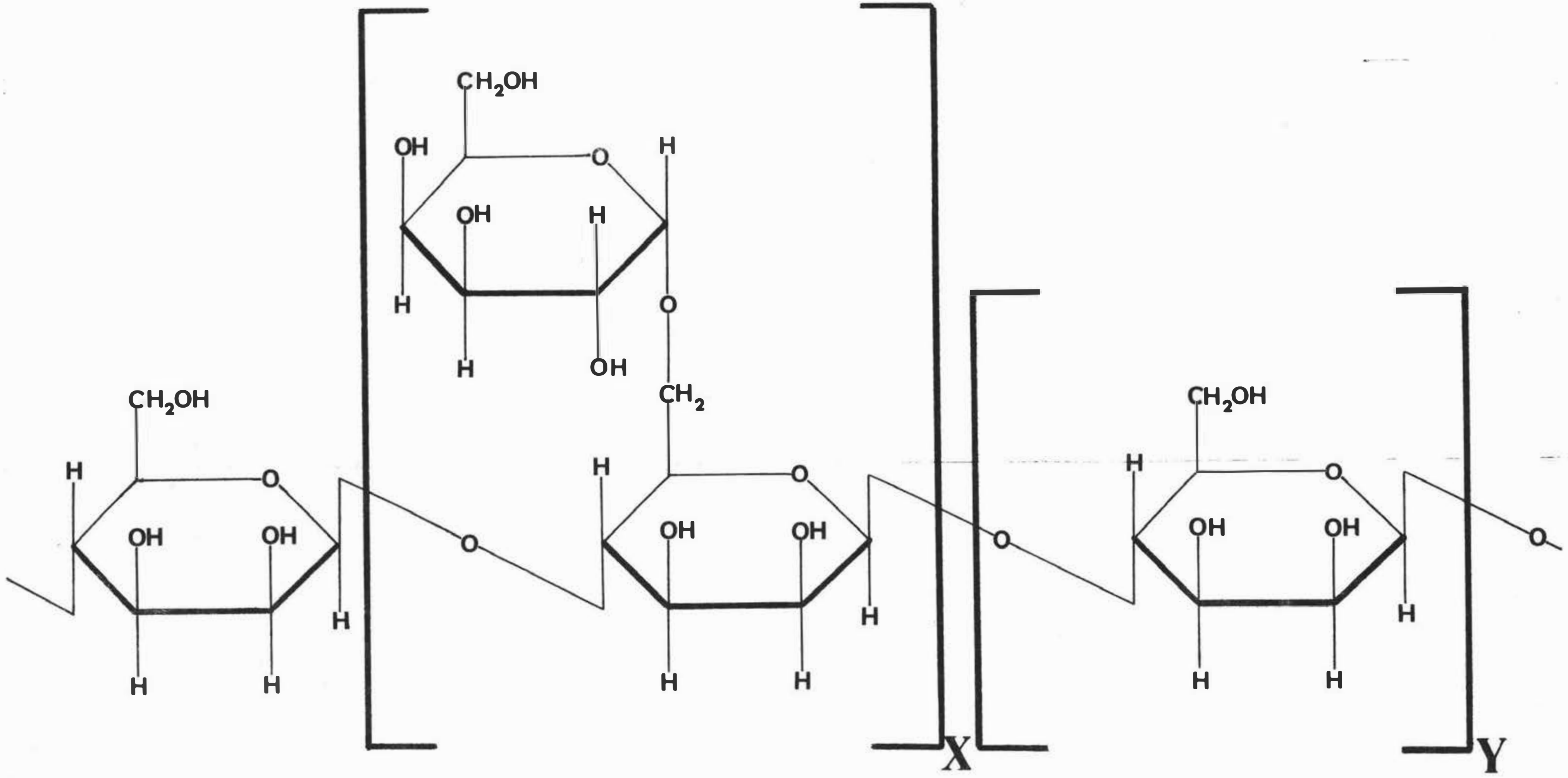
The endosperm galactomannans, with their unique structure, have many of the advantages of both branched and linear neutral polysaccharides. As long polysaccharides they are highly viscous, but the single unit galactose side chains prevent the associative lining up of molecules that would occur for a truely linear polysaccharide. This function of the galactose side units may be clearly demonstrated by a comparison between the solubilities of guar and carob galactomannans. Guar galactomannan, which has approximately twice as many galactose side chains per molecule as carob, is soluble in cold water, while carob galactomannan with its greater potential for molecular alignment, (in the solid state as well as in solution) is soluble only in hot water.

/‾It is of historical interest to note that both guar and carob galactomannans have been of importance since ancient times.

The locust tree, or carob tree (Ceratonia siliqua L.) is indigenous to the Near East and Mediterranean regions. In Biblical times the seed pods were used for animal feed, and the beans made into bread. The ancient Egyptians used carob bean paste to bind their mummies, while later the Arabs used the seeds to weigh precious metals and gems (the word "carat" is cognate with the botanical name, Ceretonia (Glicksman, 1969)).

The guar plant (Cyamopsis tetragonoloba L), which is a herbaceous annual, has been grown in Pakistan and India for thousands of years, where it is used as a food crop for humans and animals.

Figure (I–1):– General Structure of Galactomannans from Legume Seeds as Indicated by Experimental Evidence



Today both plants are grown commercially, and the endosperm of the guar and carob seeds is extracted and ground. This ground water-soluble material finds wide use in the food, paper and pulp, pharmaceutical and mining industries, due to the high viscosity of its dilute solutions and its flocculating properties.7

The main difference in galactomannans isolated from plant seeds of different species lies in their molecular weights (and possibly molecular weight distributions) and in the ratio of galactose to mannose in the molecule. It can be seen in Appendix I that although the galactose to mannose ratio of galactomannans varies somewhat within a species, this variation tends to reflect the different analytical methods rather than changes in the galactomannan composition. It may also be noted that the galactose:mannose ratio of galactomannans from the species within a Genus are very similar. This has been observed by a number of workers (Jones and Smith, 1949; Tookey et al 1962; Reid and Meier, 1970) and it has been suggested (Bailey 1971a) that this may be of taxonomic value.

Further evidence for the unique structure of seed galactomannans has come from studies comparing the monosaccharide content with the molar optical rotation. Tookey et al (1962) showed that a high degree of positive correlation existed between optical rotation and galactose to mannose ratio. Leschnizer and Cerezo (1969), and Kooiman (1972) obtained a quantitative relationship between the optical rotation and the molar ratio of galactose to mannose by using an equation based on the principle of optical superposition, and derived originally by Timell (1963) for native xylans.

The gelling of galactomannan solutions on the addition of borates, and their ability to form insoluble complexes with Fehling's solution (Smith and Montgomery, 1959) has been interpreted as being due to coordination of the adjacent cis hydroxyl groups at carbon atoms 2 and 3 on mannose. The change in optical rotation on the formation of these complexes has also been cited as evidence for the β-(1,4) linkage of the mannopyranose units (Smith and Montgomery 1959).

Extensive measurements of galactomannan solution viscosities to date have been confined to guar and carob galactomannans since they have appeared the most interesting from an industrial viewpoint. Individual measurements on some other galactomannans have been made, however (see Appendix III).

Whistler (1959) collated (mainly from manufacturer's brochures) work up to 1959 on the solution viscosities of guar and carob galactomannans. This showed that both guar and carob galactomannans followed pseudoplastic behaviour in solution (i.e. the viscosity of their solutions decreased with increased rate of shear). The effects of temperature and time on solution viscosity were also studied. The viscosity was found to decrease with temperature, and also with time of standing after hydration of the galactomannan. In the latter case, viscosity loss was shown to be caused by bacterial and enzymic degradation of the galactomannan in solution (the enzymes were present in the ground endosperm along with the galactomannan). Bollinger (1960) showed that the use of heat treatment and/or preservatives to deactivate enzymes and prevent bacterial degradation resulted in guar galactomannan solutions of stable viscosity.

The effects of added salts on galactomannan solution viscosity have also been tabulated (Whistler 1959). They showed that until salt concentrations greater than 10% were reacted, little effect on guar galactomannan solution viscosity is noticeable. In a similar investigation into the effects of added sodium chloride on carob galactomannan solution, however, the solution viscosity decreased markedly at quite low concentrations of additive. Guar and carob solution viscosities were found to be almost pH independent over the range pH 3 to pH 10.

Carlson et al (1962) carried out an extensive study of the viscosity of guar galactomannan in solution, investigating the effect of temperature and time on galactomannan hydration and solution stability. The effect on galactomannan hydration and consequent viscosity of added salts and sucrose was also examined, the latter more extensively by Carlson and Zeigenfuss.(1965).

It was found that guar galactomannan solutions developed their maximum viscosity more rapidly with increasing temperature, but that extended heating led to a viscosity decrease. Hydration of the galactomannan in solutions containing added salts had varied effects, while hydration in sucrose solution was found to delay viscosity development, as the galactomannan hydration was delayed.

A more intensive study made (Kassem and Mattha, 1969a) of the rheological properties of guaran (guar galactomannan) confirmed that it showed pseudoplastic behaviour in solution. It was also found (Kassem and Mattha, 1969a) that the flow curves of guaran (i.e. shear rate and shear stress dependence of viscosity) could be represented by an emperical formula derived by Steiger-Trippi and Ory (1960) for pseudoplastic fluids at medium to high rates of shear in rotational viscometers.

The effect of added salts, and of added alcohols and glycols on guaran solution viscosity was studied by Kassem and Mattha (1969b). The effect of the salts was variable, while alcohols and glycols generally increased the viscosity of the solutions.

Schutz (1970) carried out a comprehensive study of the rheological properties of polysaccharide gums, including guaran. He obtained a series of emperical equations, each of which described a part of the total flow curve (see fig.III-3). The concentration and temperature dependence of the viscosity in terms of each equation was also determined.

An interpretation of the physical factors necessitating the use of the different equations was suggested (see page 27). Molecular weight studies have also largely been confined to guar and carob galactomannans, although the molecular weights of galactomannans from a few other species have been measured (see Appendix III). The main methods used were chemical determination of the number of reducing end groups giving the number average molecular weight ($\bar{M}_n$), and ultracentrifugation or light scattering measurements which normally (in the former case, and always in the latter) give weight average molecular weights ($\bar{M}_w$).

For polydisperse samples (i.e. those having a continuous spectrum of molecular weights), the ratio of $\frac{\bar{M}_w}{\bar{M}_n}$ is taken as an index of the degree of polydispersity.

$\bar{M}_n$ values of galactomannans determined to date have been low ($<$30,000) apart from the values obtained by Hui and Neukom (1964) for guar and carob. $\bar{M}_w$ values have all been in excess of 100,000. As the $\bar{M}_n$ and $\bar{M}_w$ values have seldom been determined on the same galactomannan however, no conclusions can be drawn regarding their molecular weight distributions. Methods other than the use of $\frac{\bar{M}_w}{\bar{M}_n}$ ratios have been suggested for measuring polydisperity. Kubal and Gralen (1948) studied the sedimentation of carob galactomannan in an ultracentrifuge, and estimated its' polydispersity by measuring the spreading of the sedimentation boundary with time. They concluded that carob galactomannan had a small degree of polydispersity.

The shape of galactomannan molecules in solution has not been extensively investigated. Deb and Mukherjee (1963) assumed a spherical shape for guar galactomannan in solution, and from their light-scattering measurements deduced the radius of the sphere to be 2000 Å. They were also able to determine that the galactomannan was highly hydrated in solution.

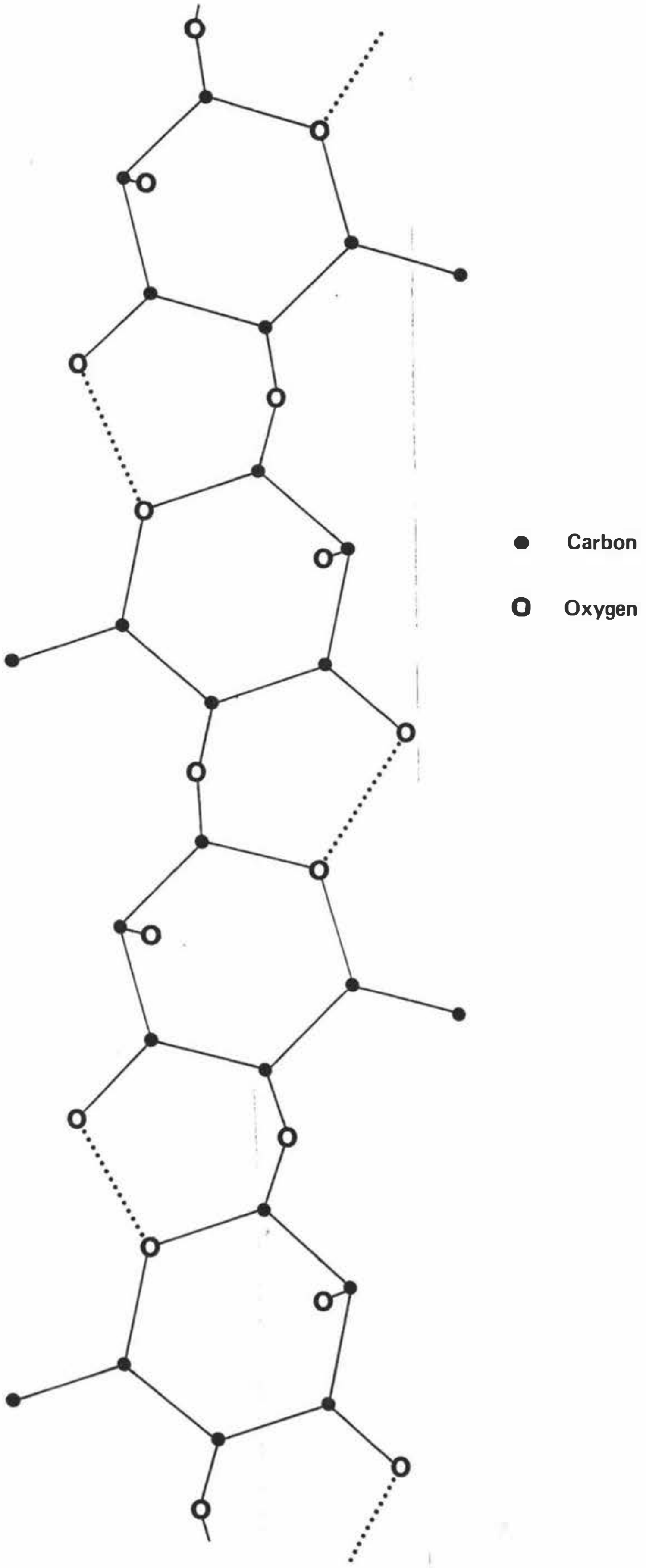
On the basis of physical evidence (e.g. X-ray data, solubility, nitrated mannan properties) it has been suggested (Meyer and Mark, 1930) that a β-1,4' linked mannan (which constitutes the backbone of galactomannans) should have a structure similar to cellulose (β-1,4' linked glucan). This conclusion has recently been supported (Frei and Preston, 1968) on the basis of x-ray measurements on mannan powder.

Sundararajan and Rao (1970) calculated the theoretical conformation of a β-1,4 linked mannan chain from a consideration of non-bonded interaction energies. They concluded that the conformation is extremely similar to that of cellulose with slightly more rotation about the β-1,4 bond possible. An $O_5 - O_3'$ type hydrogen bond (as is found in cellulose) was also found to be feasible. Their proposed extended helical ribbonlike conformation with all the linking oxygen atoms lying in the same plane is shown in fig. (I-2).

It is noteworthy that in their calculation, Sundarajan and Rao ignored both the hydroxyl hydrogens and the atoms bonded to carbon C_6, as their positions will vary with the environment. On this basis it is possible to postulate that galactomannans should also show conformations in solution similar to cellulose provided the galactose side chains do not interfere with the conformation of the mannan chain.

Evidence for a strong similarity between galactomannan and cellulose conformation in solution is provided by a study of the solution properties of guaran (guar galactomannan) triacetate, carried out by Kurath and Koleske (1964). They fractionated a sample of acetylated guaran to obtain a series of samples with a weight average degree of polymerisation of 171 to 17,400. These fractions were characterized by light scattering and viscometry. It was found that a plot of the intrinsic viscosity $[n]$ of the fractions as a function of $\bar{M}_w$ consisted of two distinct regions. At low molecular weights, $(< 10^6)$, the molecule could be

Figure (I–2):-Proposed Helical Structure of a β1–4′ linked Mannan Chain (after Sundararajan and Rao, 1970)
(the Hydrogen-bonded Oxygen Atoms lie in the Same Plane)



represented by a rigid rod model, whereas at higher molecular weights the shape in solution was closer to that of a random coil. The dependence of the radius of gyration (of an equivalent sphere representing the molecule in solution) on chain length of guaran triacetate was found to be identical to that observed for hydroxyethyl cellulose (as calculated by Brown, 1962) and cellulose tricaproate (Krigbaum and Sperling (1960). Kurath and Koleske interpreted this as an indication that the β-1,4 linked D-glucose chain of cellulose derivatives and the β-1,4 chain of guaran triacetate have similar conformations in solution, in spite of chemical and structural differences between the anhydro sugar units in the chain. They further suggested that the α-1,6 linked D-galactose side-chains of guaran triacetate did not affect the main (mannose) chain conformations to any great extent.

Swenson et al (1965) included the data of Koleske and Kurath (1964) in a comparison of the configuration of mainly substituted β-1,4 linked hexosans and wood xylan pentosans by means of the Eizner-Ptitsyn viscosity equations. They found that cellulose, glucomannan (from orchid salep tuber) and the galactomannan from guar had a common conformation which was independent of the length of derivative side chains and of the single α-1,6 linked galactose groups in the case of guar galactomannan.

On the basis of the theoretical calculations of Sundarajan and Rao (1970), and the experimental observations of Koleske and Kurath (1964) and Swenson et al (1965) it would appear that the conformation of galactomannans in solution should be independent of the galactose to mannose ratio (i.e. the degree of substitution of the molecule) and dependant only on the molecular weight. The conformation should also be very similar to that found for cellulose in solution.

Cellulose is not water soluble, so for comparisons to be made between it and galactomannans it has been necessary to make derivatives of both which are soluble in a sommon solvent (e.g. acetates; Kurath and Koleske, 1964; Swenson et al 1965) or solvents of similar properties.

Industrially, galactomannans are important only for their solution properties in water, so that comparisons of the behaviour of galactomannan derivatives with those of cellulose in solvents other than water are of limited value in explaining their hydrodynamic properties, as these properties must be somewhat solvent dependent.

A comparison of the hydrodynamic behaviour of a water-soluble cellulose derivative with that of galactomannans in solution would be much more informative, bearing in mind Swenson et al's (1965) prediction that the conformation of cellulose should be independent of its substituent side chains.

An extensive study of the properties of water soluble hydroxyethyl cellulose has been carried out by Brown (1962). He used four hydroxyethyl-cellulose fractions of differing molecular weight, and for each fraction studied viscosity (including the effects of temperature, shear rate and concentration dependence), as well as determining sedimentation and diffusion coefficients, weight-weight average molecular weight, number average molecular weight, and weight average molecular weight. The polydispersity of each fraction was also estimated, and the viscosity and sedimentation-diffusion data considered in the light of recent hydrodynamic theories developed for macromolecules.

It therefore seems feasible that a study on a series of galactomannans carried out on similar lines to that of Brown (1962) should yield considerable information on the solution properties of galactomannans provided that the assumptions made earlier apply; i.e. that the conformations of galactomannans and cellulose in solution are similar, and that the behaviour of both polysaccharides is independent of the nature and length of the sidechains.

It can be seen from the structures of hydroxyethyl cellulose (fig. (I-3a)) and of ethylhydroxyethyl cellulose (fig. (I-3b)), that they are similar to galactomannans (fig. (I-2)), with substitution at carbon atom 6 occurring mainly, with a lesser amount of substitution at carbon 2. Provided that the substituent at carbon 2 is not so large as to distort the cellulose chain (a possible danger with the poly(ethylene-oxide) sidechains of hydroxyethyl cellulose), a similarity in solution behaviour between galactomannans and these water-soluble cellulose ethers could be expected.

Figure (I-3a):- Structure of Ethylhydroxyethyl Cellulose (Manley,1956).

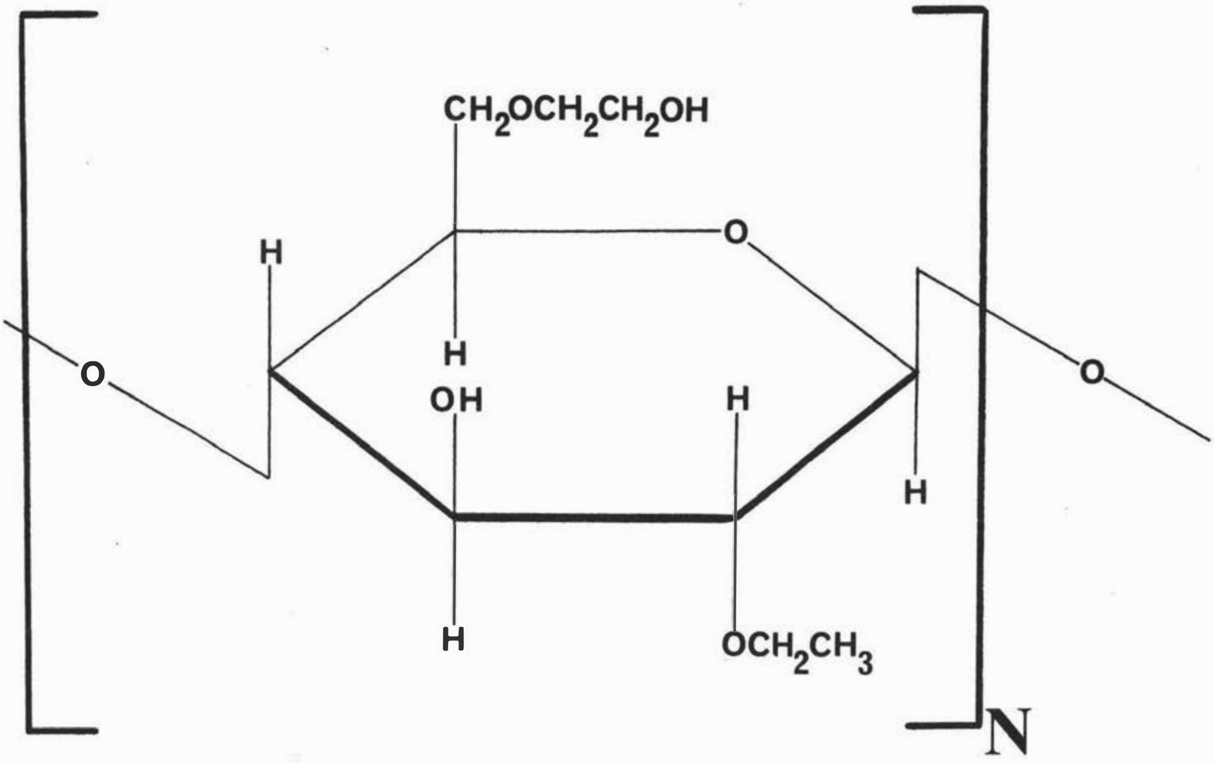
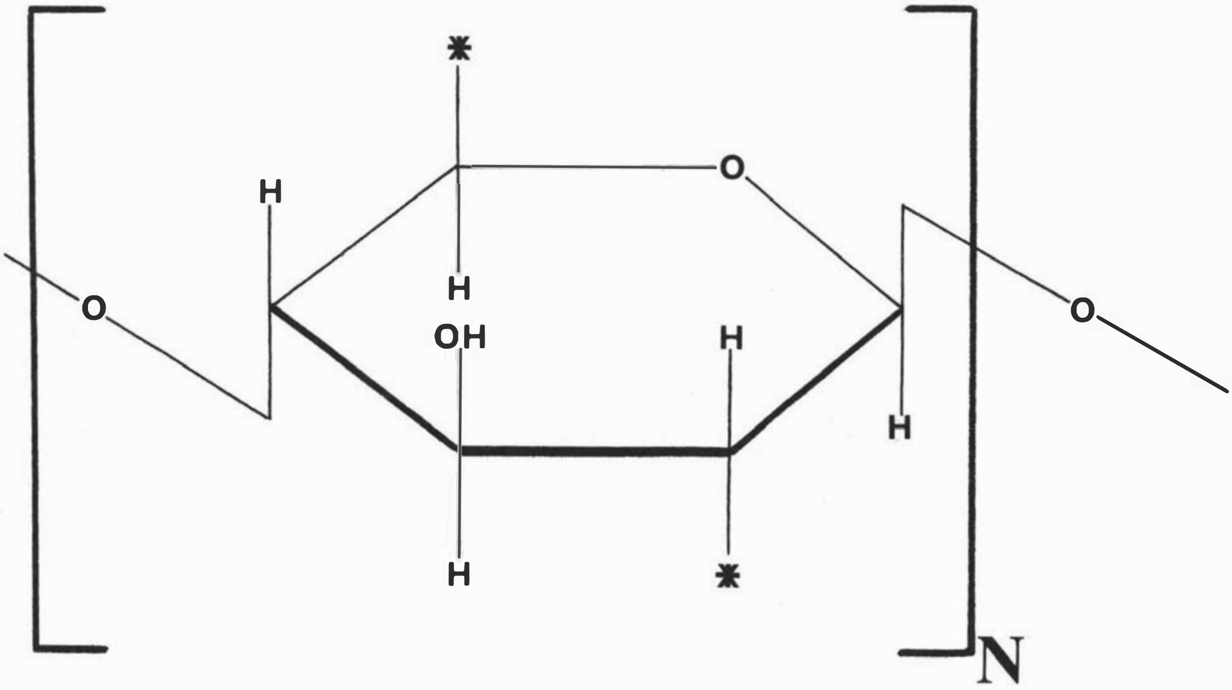


Figure (I-3b):- Structure of Hydroxyethyl Cellulose (Brown,1962)



$* = -OCH_2CH_2OH$ or $(-OCH_2CH_2)_nOH$

SECTION II

PREPARATION OF GALACTOMANNANS

A. Isolation and Purification

(a) Materials

(i) Carob: Milled carob seed endosperm (Supercol 912) was obtained from General Mills, U.S.A. For average analysis see Table 1.

(ii) Guar: Milled guar seed endosperm (Supercol U) was also obtained from General Mills. For average analysis see Table II-1

Table II-1

Average Analyses of Milled Guar and Carob Seed Endosperm

Galactomannan	Moisture (max)	Fibre (max)	Protein (max)	Fat	Ash
Guar	12.0%	2.0%	4.0%	0.4%	0.8%
Carob	12.0%	1.0%	5.0%	0.8%	0.8%

(iii) Lotus pedunculatus I: Degraded galactomannan sample obtained from Richards et. al. (1968).

(iv) Lotus pedunculatus II: Seed obtained from Grasslands Division, D.S.I.R., Palmerston North.

(v) Lucerne: Seed obtained commercially.

(vi) Red Clover: Seed obtained commercially.

(vii) Sophora Japonica: Seed obtained from a Sophora japonica tree in the Central City Square, Palmerston North.

(viii) Soybeans: Soybeans obtained commercially.

(b) Extraction and Purification

Galactomannans were extracted from materials (i-vii) by a modification of the method of Andrews, Hough and Jones (1952) as follows:-

Isolation and Purification of Galactomannan

Mill Seed
↓
Extract with Water
↓ (filter)
Centrifuge
↓
Precipitate Copper Complex
↓ (filter - (sintered glass))
Decompose Copper Complex (cold dil. HCl)
↓ (filter - (sintered glass))
Precipitate galactomannan by pouring into 2 vols. of ethanol
↓ (filter - (sintered glass))
Redissolve precipitate
↓ (filter - (sintered glass))
Precipitate galactomannan by pouring into 2 vols. of ethanol
↓ wash until acid and Cu^{++} free
Redissolve precipitate
↓ (filter - (sintered glass))
Dialyze 24 hours
↓
Freeze Dry

The milled seed was extracted by stirring with ten times its weight of water, filtered, and then centrifuged. An excess of Fehling's Solution was added to the clear supernatant and the resultant insoluble copper complex filtered off (sintered glass funnels were used in all but the first filtration). The copper complex was decomposed by suspending it with stirring in cold 0.1M hydrochloric acid (1-2 litres) until no further solution took place. The solution was filtered and the filtrate poured into ethanol (2 vols.).

The precipitated galactomannan was collected, redissolved in distilled water, filtered and again poured into ethanol (2 vols.). The reprecipitated polysaccharide was washed with ethanol (95%) until acid (pH meter) and copper (test filtrate with potassium iodide crystals) free, and then redissolved in distilled deionised water. After filtration, the galactomannan solution was dialyzed (24 hours) against deionised distilled water and then freeze-dried. An outline of this method is given in Scheme I.

Individual variations from the above method are outlined below. Yields are shown in Table II-2.

(i) Carob: The ground endosperm (20g) was extracted with water (2 litres) at 70°C for two hours.

(ii) Guar: The ground endosperm (20g) was extracted with water (3 litres) at room temperature for three hours (It was found that a solution containing 20g/2l became too viscous to stir).

(iii) Lotus pedunculatus I: This sample, which appeared to be degraded, was isolated by Richards et. al. (1968).

(iv) Lotus pedunculatus II: The milled seed (400g) was extracted with water (2 L) for four hours at room temperature. The procedure was repeated and the extracts combined for further treatment.

(v) Lucerne: The milled seed (400g) was extracted with water (4 litres) at 70°C for two hours.

(vi) Red Clover: The milled seed (400g) was extracted with water (4 litres) at 70°C for two hours.

(vii) Sophora japonica: The black seeds (240g) were carefully crushed with a hammer and then exhaustively extracted in a Soxhlet apparatus with benzene / ethanol (2:1). The dark, water-soluble pigment in the seeds was largely removed by exhaustive extraction with methanol/water (2:3). (Although this galactomannan has been isolated by Anderson (1949) and Kooiman (1971), no specific pretreatment to remove this pigment has been given. If the pigment is not removed as far as possible, it tends to colour the final product). The defatted brown seeds (200g) were extracted in water (4 litres) at room temperature for four hours. This extraction was repeated, the extracts were combined and reduced to 3 litres on a rotary evaporator (40°C) for further treatment.

(viii) Soybean (method after Aspinall and Whyte (1964)). Commercial soybeans (10Kg) were fragmented by coarse grinding and the hull fragments separated by air flotation. The hulls (590g) were exhaustively Soxhlet extracted with acetone, n-hexane and ethanol/water (4:1), then air dried and ground. Twelve hourly extractions of the ground hulls with water (6 x 3.3 litres) were carried out at room temperature. A layer of toluene on top of the water helped to prevent decomposition during extraction.

The combined, centrifuged extracts were concentrated on a rotary evaporator (40°C) to 9.9 litres, adjusted to pH 4.5 and filtered to remove precipitated protein. The filtrate was further concentrated to two litres and poured into ethanol (4 litres), the precipitate filtered out and redissolved in water (2 litres). After filtration, an excess of 7% cupric acetate was added. The resulting insoluble copper complex (A) was removed

TABLE 2

Galactomannan Yields

Galactomannan	Yield	Reported Yield	Source
Guar	63.1% (12.7gm)	69%	Hui & Neukom (1964)
Carob	58.5% (11.7gm)	52%	Hui & Neukom (1964)
Lotus pedunculatus I	—	—	—
Lotus pedunculatus II	2.1% (8.4gm)	2.2%	Richards et. al. (1968)
Lucerne	4.2% (16.8gm)	5.5%	Andrews et. al. (1952)
Red Clover	2.9% (11.6gm)	3.5%	Andrews et. al. (1952)
Sophora japonica	3.9% (7.8gm)	4.4%	Kooiman (1971)
Soybean	1.5% (6.0gm)	1.4%	Aspinall & Whyte (1964)

by centrifugation and discarded. The centrifugate was poured into ethanol (2 vols.) and the precipitated copper complex (B) centrifuged out. This complex was decomposed by suspension with stirring in ethanol containing 1% hydrogen chloride, the resulting galactomannan was filtered off and dissolved in water (2 litres). After filtering the polysaccharide was reprecipitated by pouring into ethanol (4 litres), isolated by filtration and washed until acid and copper free. The purified galactomannan was redissolved in deionised distilled water, filtered and dialyzed for 24 hours before being freeze-dried.

Further extraction of the hulls with water at 60°C (6 x 3.3L x 12 hours) gave a similar polysaccharide mixture which was separated via the copper complexes. The first complex was discarded and the second was treated exactly as above. But after three attempts an uncontaminated galactomannan could not be obtained without resorting to column chromatography, and since Guar galactomannan had a similar mannose:galactose ratio as the one being sought, this part of the isolation was abandoned.

B. Chemical Analysis

a) Hydrolysis of Galactomannans

Samples of galactomannan (25 - 30 mg) were hydrolysed in (0.5 mol/l) H_2SO_4 (4 ml) in sealed tubes at 100°C for 5-8 hours (samples having low mannose:galactose ratios being hydrolysed for the longer time). All hydrolysates were neutralised with saturated aqueous $Ba(OH)_2$, and the centrifugates decationised by swirling with a small portion of Amberlite IR-120 (H^+ form) cation exchange resin. Solutions were concentrated on a rotary evaporator, and the volume adjusted to 2 mls prior to paper chromatographic identification and analysis.

b) Paper Chromatography

(i) Qualitative

Galactomannan hydrolysates were chromatographed on Whatman No. 1 paper by descending paper chromatography using as solvent n-butyl acetate: pyridine:ethanol:water (8:2:2:1) (Bailey et. al. 1971). The chromatography paper was serrated along the lower edge to allow this fast solvent to drip off evenly.

Chromatograms were run for 30-36 hours, and good separation of mannose, galactose and glucose was obtained (mannose had R_G (= relative to glucose) = 1.09, galactose R_G = 0.78 Bailey et. al., Mannose R_G = 1.12, galactose R_G = 0.80). Pentoses ran much faster than these three

Chromatograms were developed by spraying with ammoniacal silver nitrate and heating at 110°C until the colour developed. All the galactomannans isolated were found to contain only mannose and galactose (apart from the second soybean galactomannan, which was discarded).

(ii) Quantitative

Galactomannan hydrolysates were chromatographed in the same manner as above, on Whatman No. 1 paper. The paper was pretreated by washing with 1% Oxalic Acid for 36 hours followed by washing with distilled water for

48 hours. Standard galactose/mannose solution was applied to the paper in amounts corresponding to 25,50,75 and 100 µgm of each. Two standard sets were applied per paper, with one hydrolysate spot (concentration 30 - 70 ugm approx.) being applied with each set. External markers were run on the outer edges of the paper with another down the centre.

After the chromatograms had been run and dried, the marker strips were cut out, sprayed with ammoniacal silver nitrate, and the position of the standard and hydrolysate sugars estimated. A square of paper containing each sugar was cut out, each square being the same size and as small as possible. These squares plus a blank were eluated with distilled deionised water for 10 - 14 hours giving a volume of 0.6 - 0.8 mls of solution. Each solution was made up to 1.00 ± 0.02 gms in a 25 ml pyrex test tube fitted with a ground glass stopper, and analyzed.

e) Quantitative Estimation of Sugars

Eluate samples were analyzed by an anthrone method suitable for hexoses (Bailey 1958) except that the reagent was modified after Richards (1959). Anthrone (0.75 g) was dissolved in 70% (V/V) A.R. sulphuric acid (500 mls) and aged at 0°C overnight. 10 ml of anthrone reagent was added to 1 ml of sugar solution in a 25 ml pyrex test-tube standing in an icebath. The contents were thoroughly mixed in the icebath, the tube heated in a boiling water-bath for six minutes and then cooled in ice for 30 minutes. The optical density of this solution was measured at 625 mµ in a Hitachi 101 spectrophotometer using a cell of 1 cm pathlength, and compared to a water/anthrone reagent blank. Standard curves were plotted for both galactose and mannose standards, and eluted standards (see fig. II-1), and the correlation coefficients (r) for each compared. As $r > 0.99$ for all the above cases, the concentration / optical density relationship was considered to obey Beer's Law for sugar concentrations up to 100 µgm/ml.

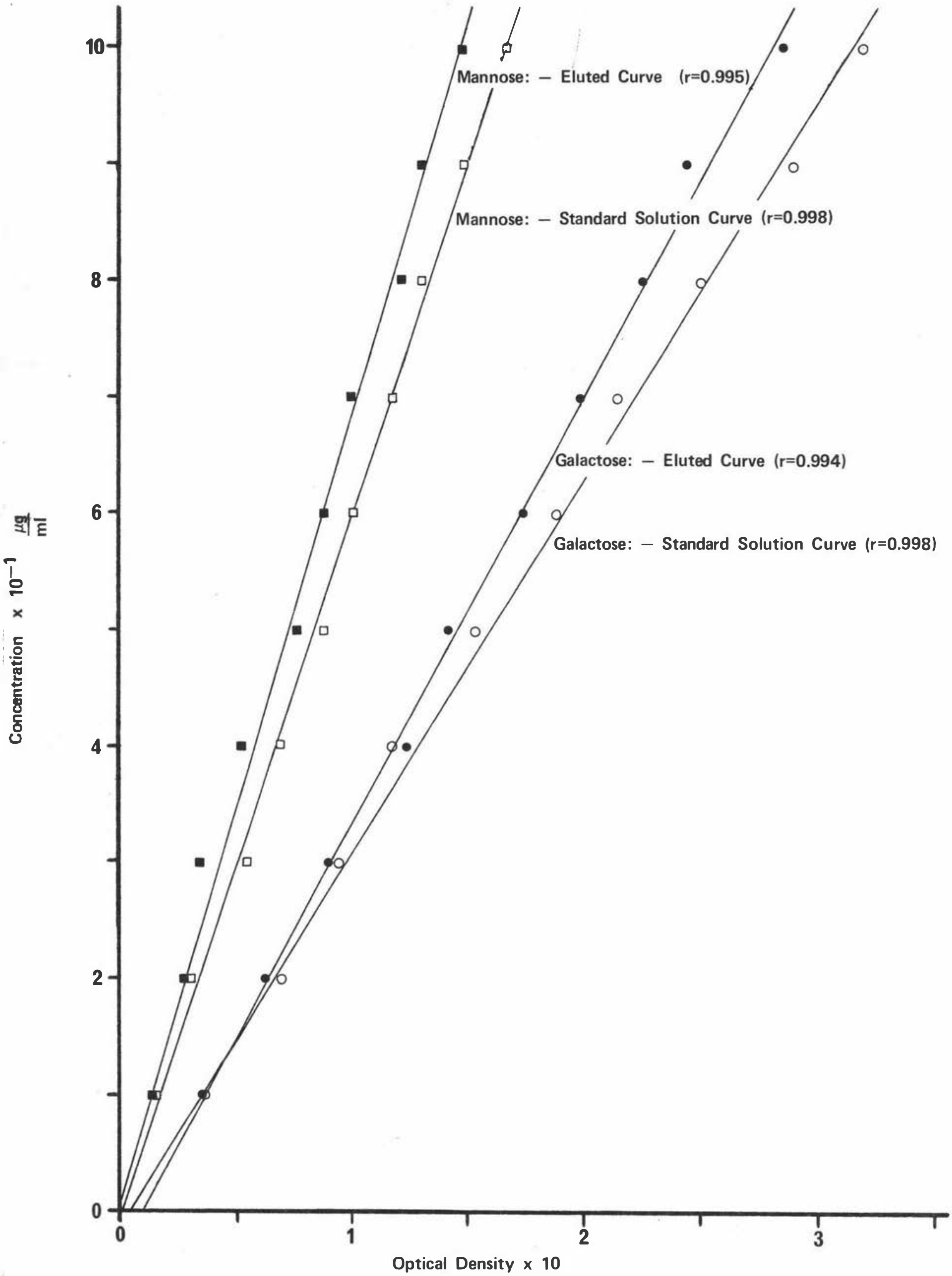


Figure (II–1):– Determination of Mannose and Galactose by the Anthrone Method– Standard Curves

It is obvious from fig. II-1 that not all the hexose is eluted from the paper but binding appears to occur linearly at least up to 100 μgm/ml. Analyses were carried out on at least three separate hydrolysates of each galactomannan. Results are shown in Table (II-3).

d) Ash and Protein Analyses

Microanalyses for both ash and protein (as N) were carried out in the University of Otago's microanalytical laboratory, under the supervision of Professor A. Campbell. Results appear in Table (II-3).

Table II-3

Galactomannan, Protein and Ash Analyses

Galactomannan	% Ash	% Protein (as N)
Guar	0.4	0
Carob	1.3	0
Lotus pedunculatus I	0.7	0
Lotus pedunculatus II	0.2	0
Lucerne	3.0	0
Red Clover	1.0	0
Sophora japonica	6.6	0.6
Soybean	1.6	0

Table II-4

$\frac{\text{Mannose}}{\text{Galactose}}$ Analyses of Galactomannans

Galactomannan	$\frac{\text{Mannose}}{\text{Galactose}}$	Average	Reported Values	Source
Guar	1.75,1.72, 1.78	1.75	2.00 2.00 1.84 1.78 1.70 1.62	Hui & Neukom (1964) Rafique & Smith (1950) Heyne & Whistler (1948) Ahmed & Whistler (1950) Tookey et. al. (1962) Swanson (1949)
Carob	3.79,3.86, 3.90	3.85	3.00 3.50 4.00 4.00 4.00	Hui & Neukom (1964) Hui & Neukom (1964) Hui & Neukom (1964) Smith (1948) Anderson (1949)
Lotus pedunculatus I	1.10,1.11, 1.13	1.11	1.04	Richards et. al. (1968)
Lotus pedunculatus II	1.00,1.02, 1.04	1.02	1.04	Richards et. al. (1968)
Lucerne	1.11,1.12, 1.15	1.13	1.25 1.10 1.00 1.00 1.00	Andrews et. al. (1952) Courtois et. al. (1958) Courtois et. al. (1958) Bourquelot & Herissey (1900) Hui & Neukom (1964)
Red Clover	1.14,1.13, 1.18	1.15	1.30	Andrews et. al. (1952)
Sophora japonica	5.08,5.11, 5.16	5.12	5.00 5.26	Anderson (1949) Kooiman (1971)
Soybean	1.40,1.38, 1.42	1.40	1.35 1.50	Aspinall & Whyte (1964) Whistler & Saarnio (1957)

SECTION II :- VISCOMETRY

A. Introduction

[Viscosity Measurements

(It should be noted that throughout this section viscosities are expressed in terms of centistokes (cS) and centipoise (cP). These units are related to the S.I. unit, the Pascal-second (Pa s) by:

$$1 \text{ cP} = \frac{1 \text{ cS}}{\rho} \approx 1 \times 10^{-3} \text{ Pa s}$$

(where ρ = density of the fluid)]

1) Definition of Viscosity (after Tanford (1961), p.321)

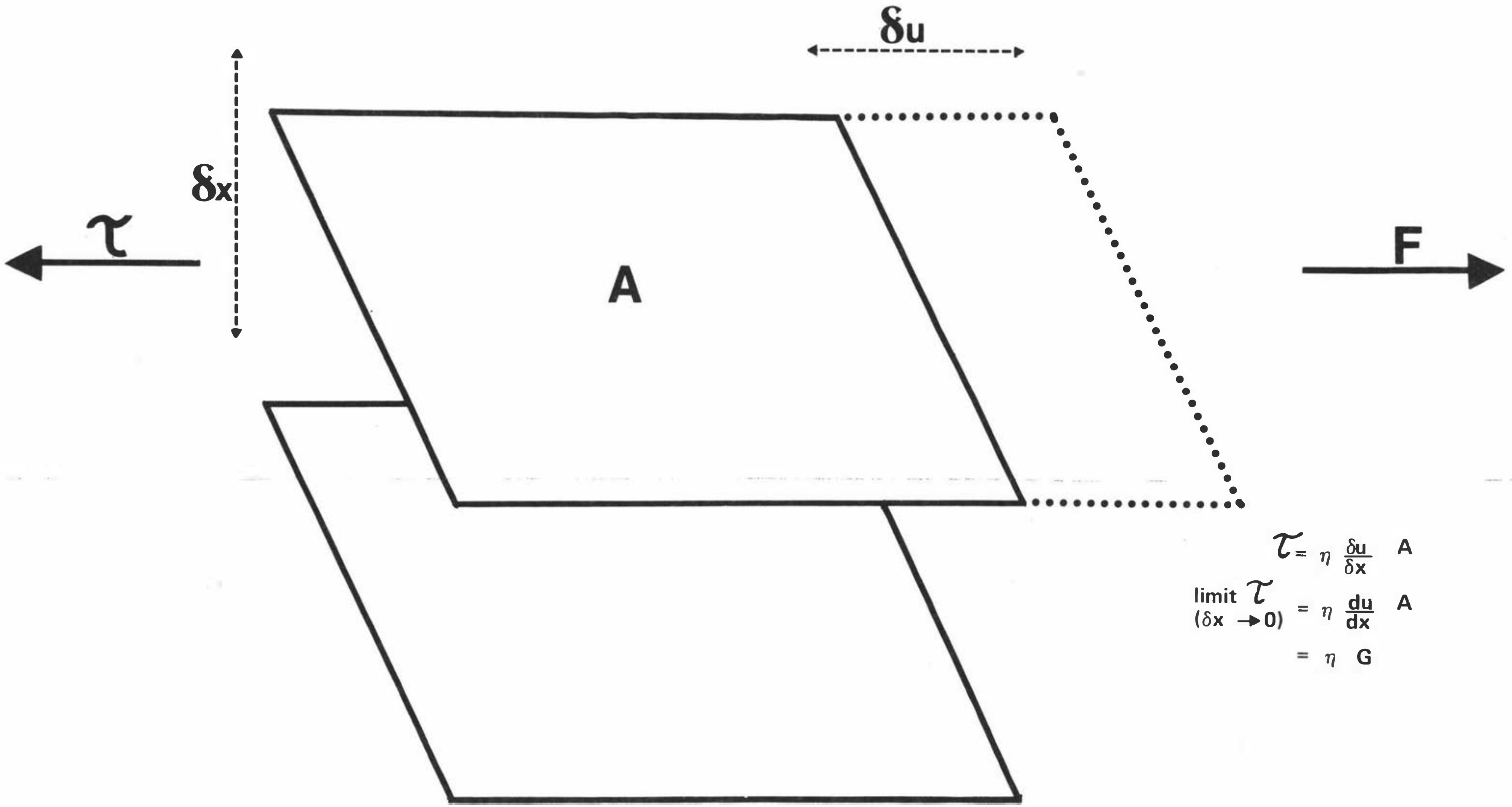
A viscous fluid is one in which there are attractive forces between neighbouring portions of the fluid; any motion of one part of the fluid relative to another is opposed by these attractive forces.

For a theoretical treatment, a liquid is considered to be a continuous, structureless fluid. If two parallel planes of infinite extent in such a fluid are at rest with respect to one another, then the net effect of the forces between them must be zero. However, if one of the planes is in motion relative to the other under the influence of an external force F, then the local forces will oppose such motion in an effort to restore the planes to their equilibrium positions. These opposing forces are acting as frictional forces.

To define viscosity in quantitative terms suppose that one of the parallel planes of fig. (III-1) is moving with velocity δu relative to the second. Then the frictional force (τ) will be proportional to both δu and the contact area between the planes A, and inversely proportional to the distance (δx) between the planes.

The proportionality constant relating the force to these variables is known as the coefficient of viscosity, or more commonly as the viscosity, η.

Figure (III—1) :— Definition of Viscosity



$$\tau = \eta \frac{\delta u}{\delta x} A$$

$$\lim_{(\delta x \to 0)} \tau = \eta \frac{du}{dx} A$$

$$= \eta G$$

$$\tau = \eta \left(\frac{\delta u}{\delta x} \right) A$$

$$\tau = \eta \left(\frac{du}{dx} \right) A$$

or in the limit ($\delta x \longrightarrow 0$)

For unit area, this is more usually written as $\tau = \eta G$ · (III-2)

where G = shear rate (S.I. unit s^{-1})

τ = shear stress (S.I. unit Nm^{-2})

η = viscosity (S.I. unit Pascal, second) (1 poise, $P = 10^{-1}$ Pa s)

Fluids for which η given by the ratio τ/G, has a constant value are known as Newtonian. That is, the viscosity of a Newtonian fluid is independent of both shear stress and shear rate.

The viscosity of other fluids may be dependent on shear rate and shear stress. In some cases viscosity increases, and in others decreases with increasing shear rate; also a discrete applied shearing stress may or may not be necessary before any motion occurs. A description of all these kinds of behaviour is given by Van Wazer et al (1963).

2) Preliminary Comments

In the early experiments when a simple comparison of the viscous behaviour of the galactomannan solutions at varying temperatures and concentrations was being studied, the type of viscometer required was one that could be easily thermostatted. The obvious choice was a capillary viscometer which could be immersed in a constant temperature waterbath, and accordingly a series of Ubbelohde viscometers was constructed.

For the final investigation of viscosities however, the measurement of shear rates in the viscometer at constant temperature was needed as well as the measurement of viscosity. Instruments often used for this work are "co-axial cylinder" or "cone and plate" viscometers, in which known viscometer geometry can be used to calculate shear rates (Van Wazer et al, 1963; Sherman, 1970).

Both a Ferranti coaxial cylinder viscometer and a Brookfield "Synchro-lectric" viscometer (which uses a rotating bob immersed in the fluid whose viscosity is to be measured) were available. However, tests using aqueous sucrose and glycerol solutions showed that these instruments, which are for industrial rather that research purposes, were unsatisfactory below 100 centipoise.

Mean shear rates can be calculated in capillary viscometers using Kroeplins (1929) equation:

$$\bar{G} = \frac{8V}{3\pi r^3 t} \qquad \text{(III-1)}$$

where v = volume of fluid (mls) passing through the viscometer in time t seconds.

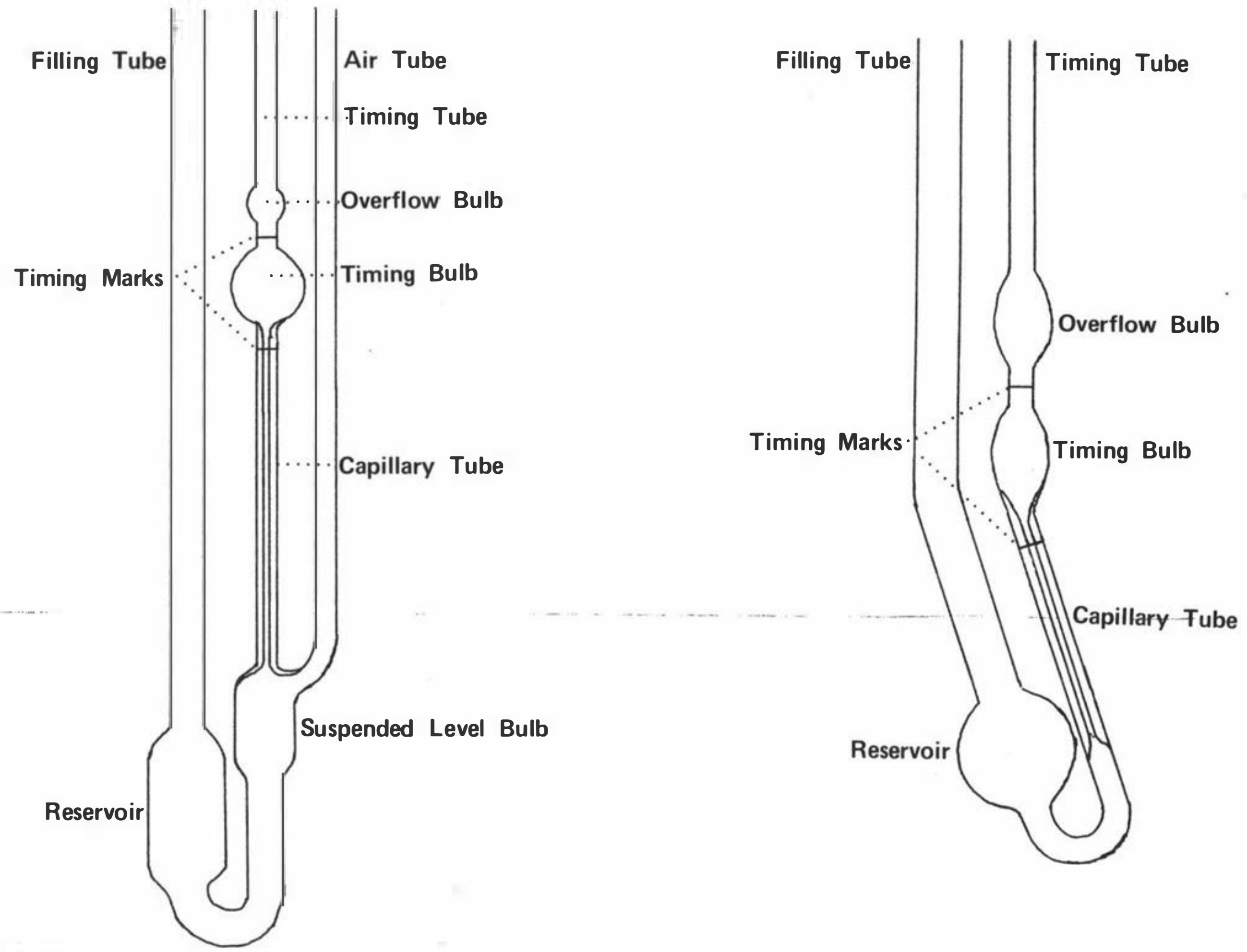
r = radius of capillary (cm)

$\bar{G}$ = mean shear rate

This method has been extensively used in measuring the dependence of viscosity on shear rate of cellulose in cupriethylenediamine solution (e.g. Browning and Sell, 1956). By application of external driving pressures to the viscometer a wide range of shear rates can be obtained.

This method was therefore used in the present investigation of viscosities. The construction of the Ubbelohde viscometers used in the first experiments involved hand drawing of the glass capillaries. Determination of the mean shear rate requires known uniformity of the capillary bore. Accordingly, for these determinations, a set of commercial Cannon-Fenske viscometers was used, which have machine drawn capillaries of more uniform bore than the hand-drawn ones. Commercial Cannon-Fenske viscometers were used rather than Ubbelohde solely on the basis of cost, as either would be suitable. Both types of viscometer are shown in figure III-2.

Figure (III–2) :– Viscometer Types



Ubbelohde (Suspended Level) Viscometer

Cannon–Fenske Viscometer

3) Viscosity of Galactomannan Solutions

(a) Observed Behaviour

Galactomannans in solution are known to show pseudoplastic behaviour [Schutz (1970), Kassem and Mattha (1969a)], which means that the viscosity of their solutions decreases with increasing shear rate. They have no yield value (i.e. no discrete applied shearing stress is necessary before motion occurs). Plots of log shear stress (log τ) against log shear rate (log G), and viscosity (η) against shear rate (G) have typically the shapes shown in fig. (III-3) a and b.

Figure (III–3) :— Typical Flow Curves of a Pseudoplastic Carbohydrate Solution (after Schutz, 1970)

Figure (III–3)a Log τ/Log G

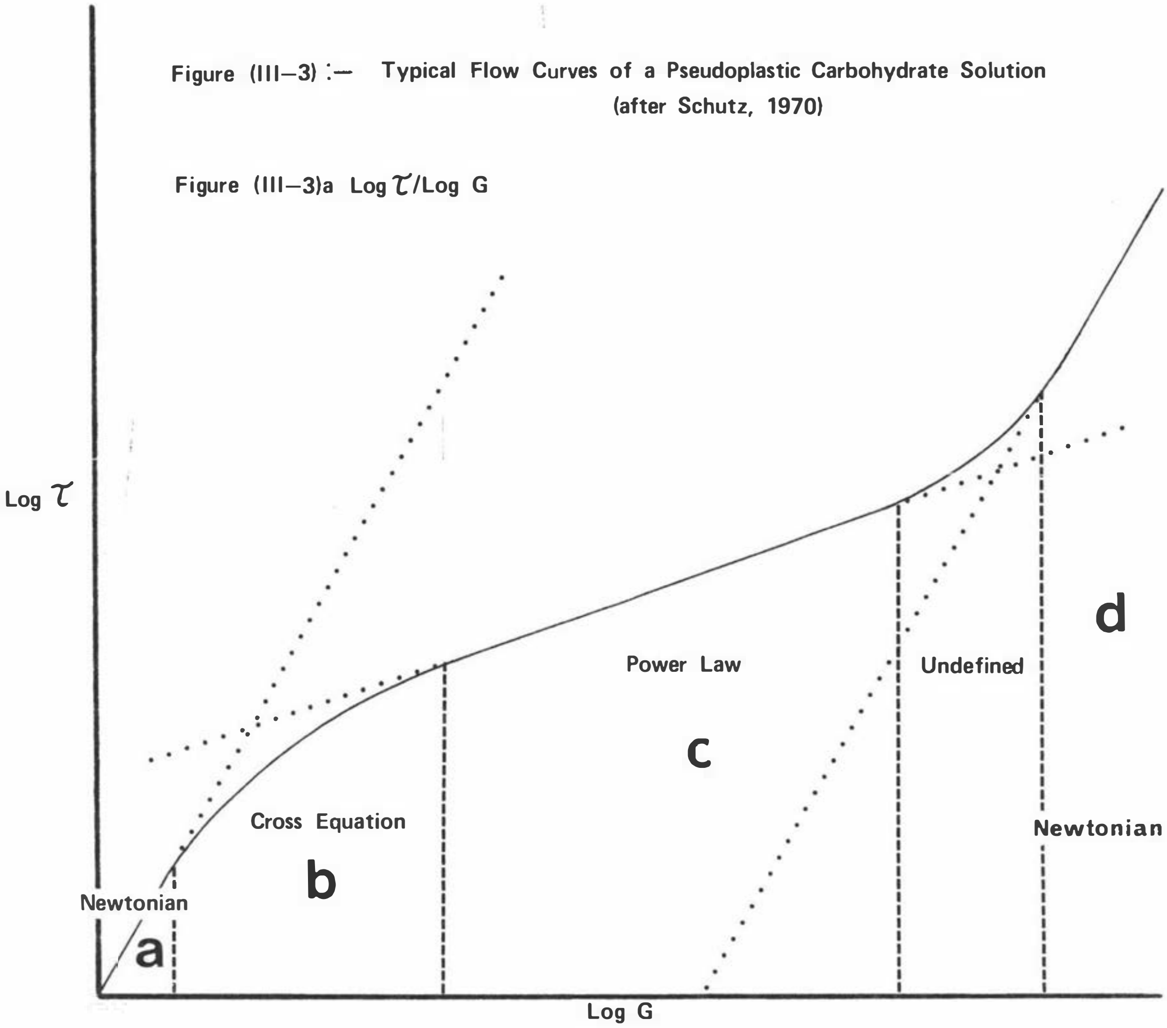
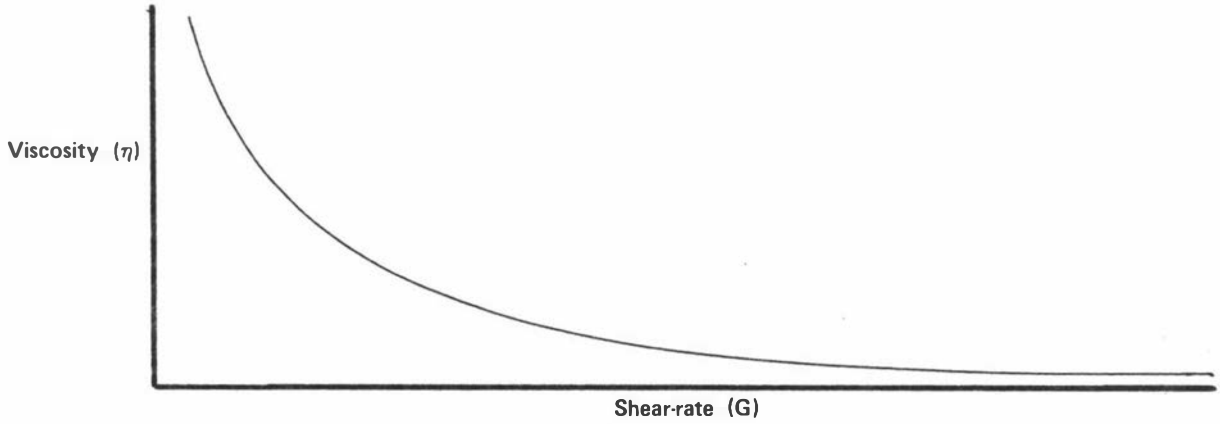


Figure (III–3)b η / G



(5) INTERPRETATION OF THE VISCOUS BEHAVIOUR OF CARBOHYDRATE SOLUTIONS (SCHUTZ, 1970).

(i) General Description

Schutz (1970) studied the viscosity behaviour of various types of carbohydrate solutions, including Guar galactomannan. He derived equations emperically to describe the behaviour of these solutions, and suggested that their behaviour could be summarized in the following way:-

The curve (often called rheogram) of fig. (III-3a) was divided into four parts, labelled (a) to (d) on the diagram. These four parts can be interpreted in terms of a solution containing:-

(a) large agglomerates resulting from preparation of the system at G = 0. The solution follows Newtonian behaviour.

(b) large agglomerates breaking up progressively with increasing G. The solution behaviour follows a Cross (1965) relationship (as discussed below).

(c) agglomerates breaking up with further increase in G; solution behaviour follows a Power Law (as discussed below).

(d) free particles of solute formed at still higher values of G. These particles may be single molecules or groups of molecules smaller than the agglomerates. These particles do not break up with further increase in G so that Newtonian behaviour is followed.

Section (a) of Schutz's curve will probably never appear, as some stirring (= shearing) motion is necessary to dissolve the carbohydrate solute.

(ii) Definition of the Cross Equation

The Cross (1965) equation is given by:-

$$\eta = \eta_{\infty} + \left(\frac{\eta_0 - \eta_{\infty}}{1 + \alpha G^{2/3}} \right) \qquad \text{(III-3)}$$

where η is the viscosity of the system under shear rate G

η_o and η_{∞} are limiting values of viscosity at zero and at infinite rate of shear, respectively.

α is a constant.

For disperse systems this equation was derived on the assumption that particles link together in the form of random chains, and that non-Newtonian flow can be attributed to the effect of shear rate on mean chain length. It has been extended to include polymer melts (Cross, 1966), and can be generally applied where non-Newtonian behaviour can be interpreted as arising from structural break-down of particles under increasing rate of shear.

Rearranging (III-3) gives:-

$$\frac{\eta - \eta_{\infty}}{\eta_o - \eta_{\infty}} = \frac{1}{1 + \alpha G^{\frac{2}{3}}} \qquad \text{(III-4)}$$

It has been pointed out by Schutz (1970) that very often $n_{\infty} << n_o$, $n_{\infty} << n$ and $\alpha < 1$, in which case equation (III-4) reduces to the modified form,

$$\eta = \frac{\eta_o}{1 + \alpha G^{\frac{2}{3}}} \qquad \text{(III-5)}$$

This will be called the Schutz equation.

(iii) Definition of the Power Law Equation

The Power Law relationship is emperical, and is given by

$$\tau = KG^a \qquad \text{(III-6)}$$

(Van Wazer et al, 1963)

When this equation is obeyed, the viscosity η is given by:-

$$\eta = \frac{\tau}{G} \quad \text{from (III-2)}$$

$$= KG^{a-1} \quad \text{from ((III-6)} \qquad \text{(III-7)}$$

According to this equation a plot of log η against log G will be linear with slope equal to (a-1).

It is also possible to eliminate G rather than τ between equations (III-2) and (III-6), which leads to the equation

$$\eta = K^{1/a} \; \tau^{(a-1)/a} \qquad \text{(III-8)}$$

(iv) Concentration Dependence of Carbohydrate Solution Viscosity

Schutz (1970) considered the change in viscosity with concentration for the portions of the shear stress / shear rate curve over which the Schutz Equation and the Power Law held.

For the Schutz equation, the most readily accessible parameter of the three (η_∞, η_0, a) is η_0, the viscosity at zero shear rate.

Emperically, Schutz found that

$$\eta_0 = A\,e^{Bc} \quad (\text{or } \eta_0 = \eta_0' e^{Bc}) \qquad \text{(III-9)}$$

where A and B are constants, and c is the concentration (g / 100 ml). In the second form of the equation the constant A is identified as η_0', the zero shear rate viscosity of some reference state.

In the Power Law case, Schutz measured the change in parameters 'a' and K (in equation III-6) with concentration. He determined emperically that for a limited concentration range, 'a' could be taken as constant. Over larger ranges 'a' decreased slowly as the carbohydrate solution concentration increased.

Schutz found an emperical relationship between K and the concentration. A reference state constant, K_c was defined so that when 'a' can be assumed constant:-

$$K = K_c e^{bc} \qquad \text{(III-10)}$$

(where b is a constant, and c is again the concentration of carbohydrate in solution.)

(v) Temperature Dependence of Carbohydrate Solution Viscosity

Schutz (1970) considered the temperature dependence of the Schutz equation and Power Law parameters. He did not find a simple relationship between η_0 and temperature for the section of the curve governed by the Schutz Equation.

In the Power Law case, 'a' varied little with temperature. The constant K, however, was inversely proportional to the absolute temperature. By defining a reference state with constant K_T analgous to K_c above, Schutz obtained emperically

$$K = K_T \, e^{D/T} \qquad \text{(III-11)}$$

This form is similar to the widely known proportionality between viscosity and absolute temperature, viz. $\eta = A \, e^{E/RT}$ (III-12) (E is the activation energy necessary for flow to occur). Equation (III-12) can be derived from Chemical reaction rate theories (Barrow (1966), p. 544; Van Wazer et al 1963) and is applied to Newtonian fluids.

c. ADAPTATION OF SCHUTZ'S EMPERICAL EQUATIONS FOR USE WITH UBBELOHDE VISCOMETERS.

(i) Estimation of the Effect of Changing Shear Rate

For the comparison of the viscosity behaviour of various galactomannan solutions, it was desirable to cover as many concentrations and temperatures as possible. To this end it would be useful to avoid having to measure the effects of shear rate on the solution behaviour. It would otherwise be necessary to make each measurement at several rates of shear, in order to ensure that the set of measurements for any one galactomannan included a common rate of shear.

Poiseuilles Law for flow in a capillary tube is given by (British Standards 188:1957).

$$\eta = \frac{\pi r^4 pght}{8lV} \qquad \text{(III-13)}$$

where r = capillary radius

h = mean head of the viscometer

p = density of the liquid in the viscometer

g = acceleration due to gravity

t = time of flow of liquid of volume V

l = length of capillary

(η will be a mean viscosity, since it is dependent on the mean head of the viscometer).

Kroeplin's (1929) equation for the calculation of mean shear rate $\bar{G}$ in a capillary was given as (III-1).

$$\bar{G} = \frac{8V}{3\pi r^3 t} \qquad \text{(III-1)}$$

Eliminating volume, V, between (III-13) and (III-1) gives:-

$$\eta = \frac{rhpg}{3lG} \qquad \text{(III-14)}$$

and from equations (III-14) and (III-2)

i.e. $$\eta\bar{G} = \tau = \frac{rhpg}{3l} \qquad \text{(III-15)}$$

l, r, and g are constants, p may regarded as constant since for all the galactomannan solutions used it varied between 0.998 and 1.002 g / ml at 20°C, and decreased only to 0.978 g / ml at 70°C. Since h is the mean head of the viscometer, it will be constant for any one viscometer.

Thus equation (III-15) can be rewritten as :-

$$\eta \bar{G} = \text{constant} \qquad \text{(III-16)}$$

If acceptable times of flow in the viscometer are taken as 200 seconds minimum and 600 seconds maximum, then by equation (III-1),

$$3\ \bar{G}\ \text{(for maximum flow time)} = \bar{G}\ \text{(for minimum flow time)} \qquad \text{(III-17)}$$

If the carbohydrate concentration in the solution is high, the viscosity η will be high, and $\bar{G}$ will be small. This follows from equation (1),

$$\bar{G} = \frac{8V}{3\pi r^3 t}$$

since $\bar{G} \sim \frac{1}{r}$, $t \sim \eta$, and for a highly viscous solution the radius of the capillary will have to be relatively large to obtain a reasonable ($<$ 600 seconds) flow time.

If $\bar{G}$ is small, then the range of mean shear rate, $\bar{G}$ (minimum flow time) - $\bar{G}$ (maximum flow time) = $2\bar{G}$ (maximum flow time) (III-18)

(from equation (III-17))

will also be small. Although non-Newtonian character (and therefore the shear rate dependance of viscosity) is high for high carbohydrate concentrations, because of the small shear rate range the solution viscosity should not change greatly with changing flow times.

Conversely, if the carbohydrate concentration is low, η is small hence r will be small and G will be large. The mean shear rate range will also be large. The shear rate dependence of viscosity is low for this case, however and again the solution viscosity should not change greatly.

Thus the assumption is made that within the maximum (600 seconds) and minimum (200 seconds) flow times suggested the viscosity of the galactomannan solutions is virtually a constant, independent of the rate of shear (i.e. the solutions show Newtonian behaviour).

(ii) Adaptation of the Equations for the Power Law Case to Eliminate Shear Rate Effects.

Concentration Effect

Schutz's emperical equation for the Power Law case of change in viscosity with concentration of carbohydrate in solution was:-

$$K = K_c e^{bc} \qquad \text{(III-10)}$$

Substitution back into the form of the Power Law equation (III-8)

$$\text{i.e. } \eta = K^{1/a} \tau^{(\frac{a-1}{a})} \qquad \text{(III-8)}$$

$$\text{gives } \eta = (K_c e^{bc})^{1/a} \tau^{(\frac{a-1}{a})} \qquad \text{(III-19)}$$

From equation (III-15), τ is constant for any one viscometer, so that equation (III-19) becomes:-

$$\begin{aligned} \eta &= (K_c e^{bc})^{1/a} \tau^{(\frac{a-1}{a})} \\ &= k(K_c e^{bc})^{1/a} \\ &= K' e^{bc/a} \qquad \text{(III-20)} \end{aligned}$$

where K_c is a constant, $k = \tau^{(\frac{a-1}{a})}$ and $K' = kK_c^{1/a}$

From equation (III-20)

$$\log_e \eta = \log_e K' + (\frac{b}{a})c \qquad \text{(III-21)}$$

Thus for any one viscometer, a plot of $\log_e \eta$ against concentration should be linear with slope $(\frac{b}{a})$ and intercept $\log_e K'$.

Temperature Effect

Schutz's emperical equation for the temperature dependence of the Power Law constant K was $K = K_T e^{D/T}$ (III-11)

Substitution of this into the form of the Power Law equation (III-8)

$$\eta = K^{1/a} \tau^{(\frac{a-1}{a})} \qquad \text{(III-8)}$$

$$\text{gives } \eta = (K_T e^{D/T})^{1/a} \tau^{(\frac{a-1}{a})}$$

$$\begin{aligned} \text{i.e. } \eta &= (K_T e^{D/T})^{1/a} k \\ &= K'' e^{D/aT} \qquad \text{(III-22)} \end{aligned}$$

where $k = \dot{\gamma}^{(a-1)/a}$ and $K'' = k\,K_T^{1/a}$.

From equation (III-22)

$$\log_e \eta = \log_e K'' + \frac{D}{aT} \qquad \text{(III-23)}$$

For any one viscometer a plot of $\log_e \eta$ against the reciprocal of the absolute temperature should be linear with slope $\frac{D}{a}$ and intercept $\log_e K''$.

Adaptation of the Schutz Equation to Eliminate Shear Rate Effects.

This adaptation was not attempted, as there was no method available for determining η_o, the viscosity at zero rate of shear (see equation (III-9)).

(d) Use of Schutz's (1970) Equations for Data Obtained with Cannon-Fenske Viscometers.

These viscometers were purchased following the decision to change the object of this thesis from a simple comparison of chemically modified galactomannans to a more comprehensive comparison of the solution properties of unmodified galactomannans.

To achieve this latter end it was necessary to eliminate the effect of shear rate on the solution viscosities. This was achieved by using Schutz's equations (III-9), (III-10) and (III-11) in their original form, and extrapolating experimental viscosity / shear rate plots to zero rate of shear.

B. Experimental Methods

(1) Ubbelohde Viscometers

(a) Construction of Ubbelohde Viscometers

Seven Ubbelohde viscometers (see fig. (III-2)) were constructed according to British Standard 188:1957. These were used together with one commercial model, giving a total range of measurement of 0-2000 centistokes.

(b) Construction of Waterbath

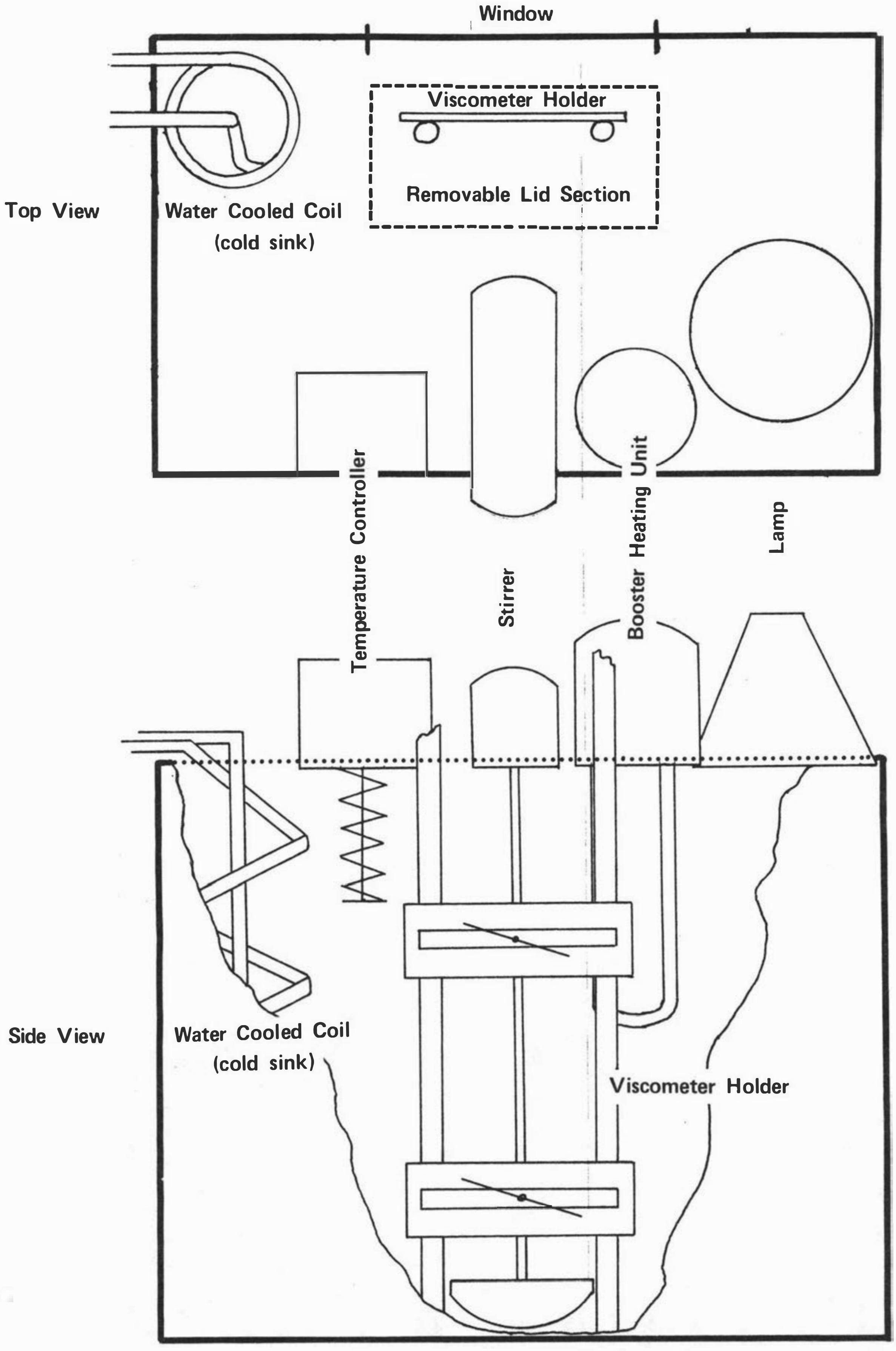
A glass tank (60 cm x 30 cm x 35 cm deep) was insulated with 5 cm thick polystyrene, and a 2 cm lid of the same material placed over the top. A small removable section of the lid slid up and down on a pair of vertical aluminium rods. To these rods were fitted two perspex clamps designed to hold a viscometer in a vertical position with its upper etched mark 6 cm below the water surface (see fig. (III-4).)

Temperature control was provided by a Technicon "Tempunit" adjusted to control to ± 0.05°C at 20.0°C, with this precision decreasing to ± 0.1°C at 70°C. A two kilowatt proportionally controlled heater was used to raise the temperature between sets of viscosity determinations. A cold sink, in the form of a water-cooled glass coil was used in conjunction with the "Tempunit" in maintaining the bath at 20°C. To ensure even heating, the bath was stirred by means of a large paddle attached to an overhead stirrer. Temperatures were measured on a mercury in glass thermometer calibrated to 0.1°C.

(c) Calibration of Viscometers

The viscometers were calibrated using the "step-up" procedure; i.e. the viscosity of a liquid was determined in one viscometer, and then this liquid was used to calibrate the next viscometer in the series. The primary calibrating liquid was water which has a known viscosity of

Figure (III–4):– Constant Temperature Water Bath



1.0038 cS at 20.0°C. This water was prepared by double distillation through an all-glass apparatus which had previously been well cleaned in chromic acid and rinsed in deionised water. The first three batches of distillate were discarded. Solutions of A.R. sucrose and A.R. glycerol in doubly distilled water (for use as secondary calibrants) were filtered, and then stored in tightly stoppered containers in the refrigerator when not in use as they tend to either absorb or lose water, depending on their concentration.

Viscometers were cleaned before use by soaking overnight in chromic acid. The acid was removed, and the viscometer rinsed thoroughly with doubly distilled water and then with acetone. After drying in a stream of dust-free air the viscometer was charged through the wide arm by carefully adding sufficient liquid to approximately one-third fill the lower reservoir. The viscometer was then placed in it's holder in the waterbath and allowed to equilibrate. Loosely fitting stoppers were used to exclude dust without restricting any air flow.

Timing was carried out using a Junghans stopwatch calibrated to 0.1 sec. which was frequently checked against two other stopwatches of the same make. This stopwatch was always used in the same face-up position. A flow time of 200 seconds or greater was used to reduce timing error to 0.1%, as the human error in starting and stopping the stopwatch was 0.2 seconds.

Viscosities were calculated from Poiseuille's Law (equation (III-13)) in the form:

$$\nu = ct \qquad \text{(III-24)}$$

where ν is the viscosity in centistokes.

c is the calibration constant.

t is the time of flow in seconds.

Although this equation can be corrected for the kinetic energy of efflux by putting:

$$\nu = ct - \frac{B}{t} \qquad \text{(III-25)}$$

where B is the coefficient of the kinetic energy of efflux, this was not done for the following reason:

A calculation of the possible magnitude of the kinetic energy corrections was made using a B value of 2.5 as suggested in the British Standard (1957). The calculated values (table (III-1)) appear small. That the kinetic energy correction was indeed small was verified by carrying out two separate calibrations of the viscometers, and noting the difference in c (the calibration constant). These results also appear in table (III-1). The difference between calibration constant pairs is less than 1% in all cases, and normal practice in viscometry measurements is to aim for an error of 1% or less (Jackson, 1972; Koleske and Kurath, 1964).

Except for the calibration of viscometer 1 flow times were kept above 200 seconds in viscometers 0 to 3, and above 180 seconds in viscometers 4 to 7. The deviation with viscometer 1 was unavoidable if water was to be used as a primary calibrant. Calibration of a viscometer was carried out by measuring the flow time of a liquid at 20.0 ± 0.05°C until three successive flow times agreed within 0.1%. Five such determinations were made, the average of each set of three readings taken, and these five averages averaged again. The resultant time was substituted into the Poiseuille's Law equation (III-24) to determine c.

Table (III-1)

Viscometer Constants and Calibrants for Ubbelohde Viscometers

Viscometer Number	Viscometer Range (cS)	Calibration Constant I	Calibration Constant II	Calibrant	Difference (%) between I & II	Approx. Max. K. E. Error (%)
0	0.4 - 1.0	0.00165	0.00164	water	0.6	3.5
1	1.0 - 4.0	0.00549	0.0050	water	0.18	1.0
2	4.0 - 12.0	0.0211	0.0211	34% sucrose	0.00	0.25
3	12.0 - 36.0	0.0624	0.0624	48% sucrose	0.00	0.1
4	36.0 - 100	0.180	0.180	56% sucrose	0.00	0.04
5	100 - 300	0.438	0.439	80% glycerol	0.22	negl.
6	150 - 600	0.696	0.697	87% glycerol	0.14	negl.
7	600 - 1800	3.44	3.45	97% glycerol	0.03	negl.

(d) Determination of Galactomannan Solution Viscosities at Varying Concentrations and Temperatures

(i) Preparation of Galactomannan Solutions:

Initial problems were encountered in attempting to dissolve Red Clover and Lucerne Galactomannans, and a minimum of 40% of any sample remained insoluble. Similar difficulties were reported by Andrews et al. (1952) who used material dried by solvent exchange. The use of freeze-drying in the preparation of the material used above, however, does not appear to have reduced the level of insoluble galactomannan.

The reason for the insolubility of 40% of the galactomannan is not known but may be due to the formation of extremely large aggregates in the legume seed, or on drying. The other galactomannans were more soluble, although heat was necessary in the case of Carob and Sophora japonica galactomannans. None apart from the Lotus pedunculatus I alactomannan appeared to dissolve completely. By taking Red Clover and Lucerne as a uide, it was found that maximum solubility could be achieved after approximately 90 minutes at 70°C. (although autoclaving or heating to higher temperatures would have reduced the time involved, and possibly the amount of insoluble material, galactomannans tend to depolymerise at higher temperatures in solution (Carlson et al., 1962)). Accordingly, all galactomannan solutions were prepared by suspending the finely-divided freezedried material (1g, more for Red Clover and Lucerne) in doubly distilled water (100 ml) and stirring it in a waterbath at 70°C for one and a half hours.

The resulting solution was cooled and filtered through a sintered-glass funnel. Samples of this approximately 1% solution were weighed out (2g, 5g, 10g, 15g) into flasks fitted with ground glass stoppers and adjusted to 20g with distilled water, giving additional galactomannan solutions of strengths of approximately 0.1%, 0.25%, 0.50%, 0.75%. Duplicate samples (5g) of the 1% solution were freezedried to determine the actual concentration of galactomannan in the solution, and the concentrations of the diluted solutions were calculated accordingly. No preservative was added to the solutions as measurements on the change in viscosity of a guar solution ((0.31%), see fig. (III-5)) with time showed that the solutions were stable for up to seven days if kept refrigerated when not in use, provided that they were aged for 24 hours in the refrigerator before use.

(ii) Viscosity Measurement on Galactomannan Solutions*

The time of flow of a galactomannan solution was measured until two successive values agreed to within 0.2 seconds. This measurement was repeated at 10°C intervals from 20°C to 70°C. As far as possible all measurements for one galactomannan solution were carried out in the same viscometer.

The variation of viscosity with concentration at constant temperature was plotted, and also the variation of viscosity with temperature at

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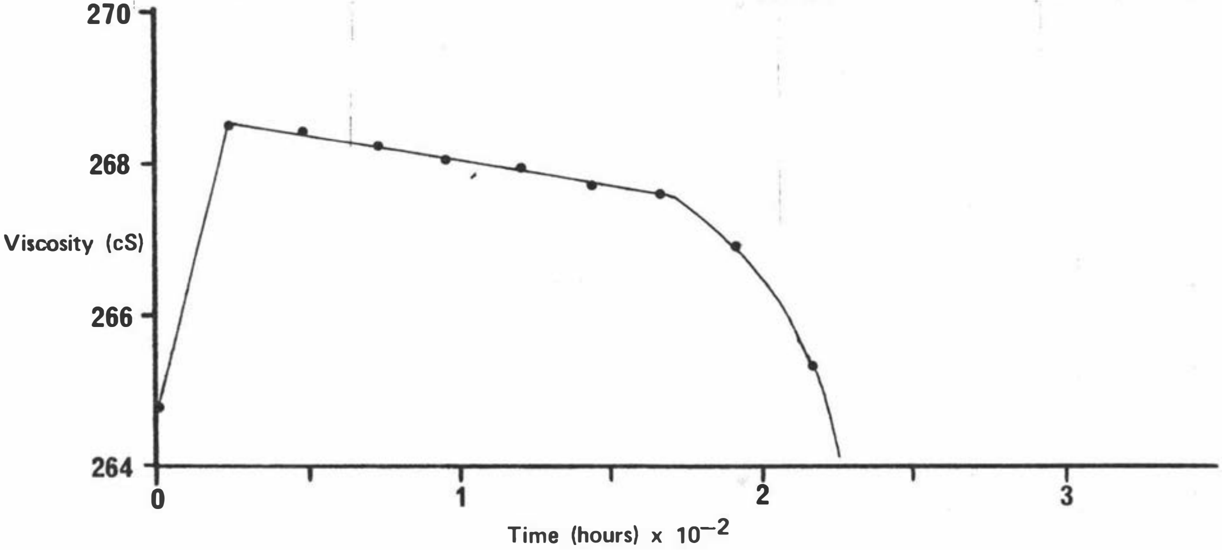
* For further information on technique of viscosity measurements, see Appendix VIII p197

constant concentration. Correlation coefficients were calculated to the modified form of Schutz's equations, and lines plotted by the method of least squares.

Figure (III–5):– Change in the Viscosity of Guar Galactomannan (0.31 $\frac{g}{100\ ml}$) with Time of Refrigerated Storage.



(2) Cannon-Fenske Viscometers

(a) Modification of the Constant-Temperature Waterbath

The waterbath used was the same as that in Section III, B (1) for the Ubbelohde Viscometers), except that the Technicon "Tempunit" was replaced by a mercury-toluene regulator connected through a percentage power controller to a one kilowatt heating element. By maintaining the percentage controller at a setting of 10% and adjusting the water flow through the cooling coil, it was possible to maintain the bath at 20.00 ± 0.02°C. Temperature was measured on a mercury in glass thermometer calibrated to 0.01°C.

(b) Calibration of Viscometers

(i) Flow Constants:

A series (10) of Cannon-Fenske viscometers (routine type) was purchased from the Cannon Instrument Co. (U.S.A.). Alternate viscometers in the series had been calibrated with two standard liquids of known viscosity by the company. By using the flow times of these standard oils and their known viscosity, it was possible to calculate their calibration constants (c) and kinetic energy correction constants (B) using the following formulae (British Standard 188:1957):

$$c = \frac{\nu_2 t_2 - \nu_1 t_1}{t_2^2 - t_1^2} \qquad \text{(III-26)}$$

$$B = \frac{t_1 t_2(\nu_2 t_1 - \nu_1 t_2)}{t_2^2 - t_1^2} \qquad \text{(III-27)}$$

where ν_1, ν_2 are the viscosities (cS) of the two calibrating liquids.

t_1, t_2 are their respective flow times (secs.)

$(t_1 < t_2)$

Table(III-2)

Calibration Constants for Cannon-Fenske Viscometers at 20.00 ± 0.02°C.

Viscometer No.	Calibrant	Calibrant Composition (%)	ν_1 (cS) ν_2 (cS)	t_1 (secs)	t_2 (secs)	c $\left(\frac{cS}{secs}\right)$	B (cS x sec)	$\frac{B/200}{200c} \times \frac{100}{1}$ %
25	Cannon Stds		0.7684	355.3		0.002163	0.4843	0.56
			0.9680		447.3			
75	Benzene / Decalin	30:70	1.846	205.2		0.009008	0.5140	0.14
	Hexadecane	100	4.477		497.1			
100	Cannon Stds		3.304	232.0		0.01425	0.7149	0.12
			6.202		435.2			
150	Generex / Whiterex	60:40	8.900	221.5		0.04025	4.078	0.25
	Generex / Whiterex	20:80	16.49		409.8			
200	Cannon Stds		24.39	240.3		0.1016	11.50	0.28
			35.48		349.2			
300	Whiterex / Stock 0032	30:70	55.57	246.8		0.2240	4.883	0.05
	Stock 0032/Stock 0050	80:20	105.2		467.1			
350	Cannon Stds		102.1	206.4		0.4946	6.684	0.03
			212.5		429.6			
400	Stock 0050	100	294.5	258.9		1.132	41.48	0.09
	Stock 0050/Stock 396/Stock 307	40:30:30	520.2		457.2			
450/1	Cannon Stds		635.9	249.5		2.549	118.5	0.12
			1209.5		474.3			
450/2	Stock 0050/Stock 396/Stock 307	40:30:30	520.2	319.3		1.629	58.20	0.09
	Stock 396/Stock 307	50:50	827.8		508.0			
500	Stock 0050/Stock 307	25:75	1422	196.4		7.345	144.0	0.05
	Stock 0050/Stock 307	10:90	1988		270.7			

* The Cannon calibrated 450 viscometer was broken, so another was constructed to the same dimensions using a length of standard bore 2 mm capillary.

By calibrating oils (ex-Mobil Oil Co.) in these standardised viscometers, at 20°C, it was possible to determine B and c for each of the remaining viscometers. Results are summarised in table (III-2). The percentage error arising from neglecting kinetic energy corrections for a flow time of 200 seconds has also been assessed, and because of the low values obtained (less than 0.3% apart from viscometer 25) kinetic energy corrections have been neglected in all further calculations. Flow times were accordingly kept above 200 secs. (Although the error arising from neglecting the kinetic energy correction with the 25 viscometer is 0.56% for a flow time of 200 seconds, this reduces to 0.35% for 250 seconds and 0.25% for 300 seconds. It seemed reasonable, since this viscometer was used only at high shear rate and low viscosity, with most flow times in excess of 250 seconds, to neglect the kinetic energy correction here also.)

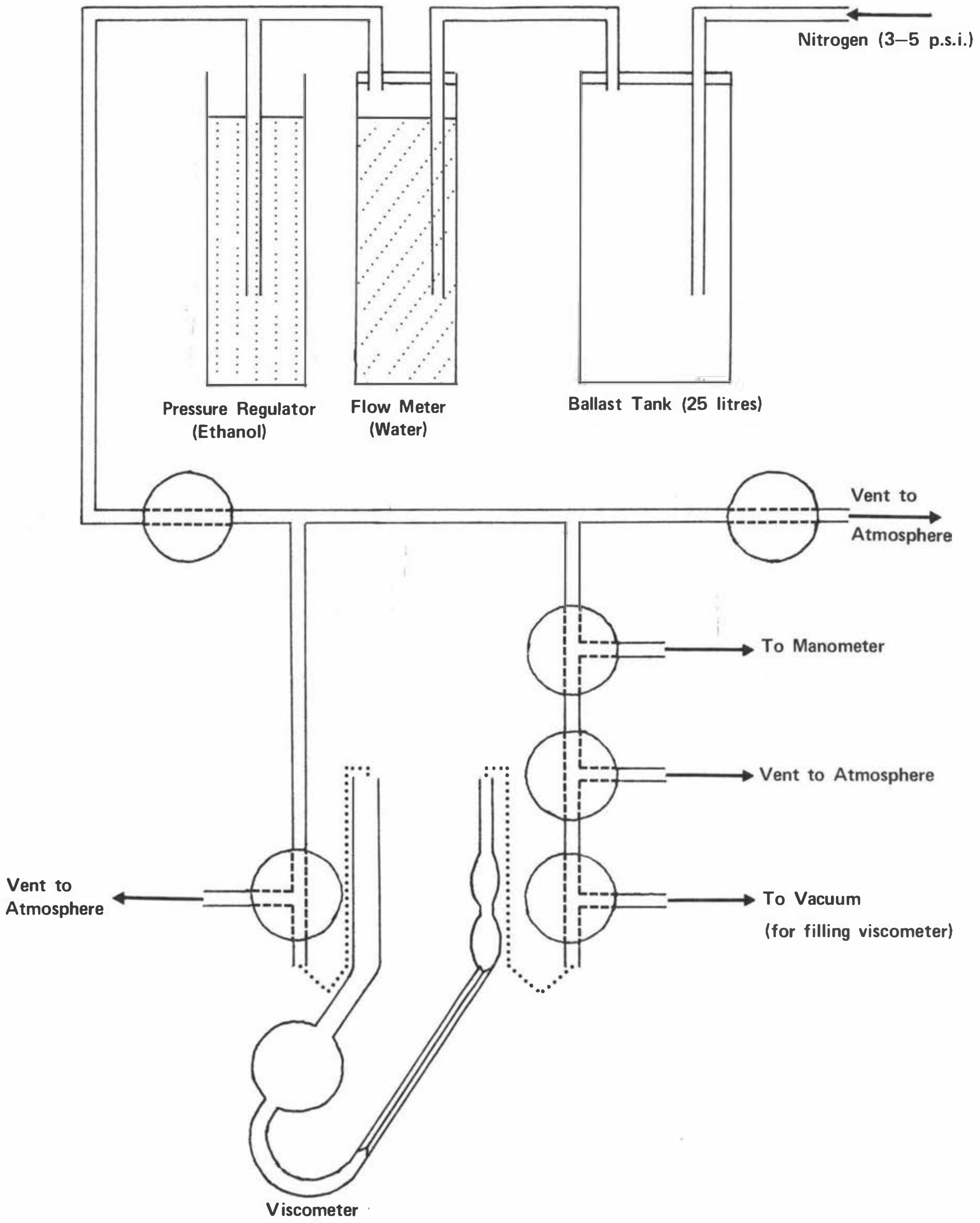
(ii) Mean Head:

A glass pressure line was constructed so that external pressures could be applied to either the driving arm or the filling arm of the viscometer (see fig.(III-6)). This line was pressurised with nitrogen, the actual pressure being controlled by the depth of immersion of a length of glass tubing in a cylinder of ethanol. Pressure was read on a manometer filled with dibutylphthallate by use of a travelling microscope, and corrected to 20.0°C.

The mean effective head (h_o) of each viscometer was determined from measurements of flow times of standard oils (at 20.0°C) with external pressures applied to the driving arm of the viscometer of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 cm of dibutylphthallate (=d.b.p.); h_o was calculated from the formula: (Browning and Sell, 1956):

$$h_o = \frac{t_2}{t_1 - t_2} \cdot \frac{(h_e d_e)}{d_o} \qquad \text{(III-28)}$$

Figure (III–6):– Pressure Line used for Driving Cannon–Fenske Viscometers



where h_o is the mean effective head,

h_e is the external pressure,

d_o is the density of the fluid in the viscometer,

d_e is the density of the manometer fluid,

t_1 is the time of flow without external pressure,

t_2 is the time of flow with external pressure.

Flow times without external pressure were calculated from Poiseuille's Law (equation III-24). Densities were measured at 20.0°C in a micropipette previously calibrated with doubly distilled water; measurements were repeated until two successive values agreed to within 0.1%. Results appear in table (III-3).

Table (III-3)

Determination of the Mean Heads of Cannon-Fenske Viscometers

Viscometer Number	Mean Head (cm of d.b.p.)		Standard Deviation	Standard Deviation (%)(= Coefficient of Variation.)
	Cannon Co. (approx.)	Determined		
25	9.6	9.404	0.013	0.14
75		9.731	0.018	0.18
100	9.1	9.080	0.011	0.12
150		9.974	0.013	0.13
200	9.5	9.259	0.011	0.12
300		9.304	0.016	0.17
350	9.9	9.446	0.018	0.19
400		8.954	0.021	0.23
450/2		9.229	0.032	0.35
500		8.584	0.057	0.66

The increase in Standard Deviation from the top to the bottom of the table reflects the increasing difficulty of accurately determining oil viscosities greater than 200-300cS.

(iii) Calculation of Mean Shear Rate Constants:

These were obtained from Kroeplin's equation (III-1),

$$\bar{G} = \frac{8V}{3\pi r^3 t} \tag{III-1}$$

using the following measurements:-

Volume

The volume between the etched marks on each viscometer was measured by inverting the viscometer, filling to the top mark with doubly distilled water, and running the water through a fine jet into a tared flask (10 ml) until it reached the bottom mark. The water was then weighed. This was repeated four times, and the volume calculated from the density of water at the calibration temperature. Results appear in table (III-4).

Table (III-4)

Calculated Volumes and Radii of Cannon-Fenske Viscometers

Viscometer No.	Volume (mls)	Standard Deviation (%)	Radius (cm)	Standard Deviation (%)
25	1.771	0.09	0.01668	0.02
75	3.084	0.05	0.02733	0.03
100	3.243	0.06	0.03148	0.03
150	3.102	0.04	0.04008	0.04
200	3.225	0.04	0.05152	0.02
300	3.267	0.06	0.06342	0.01
350	3.222	0.07	0.07655	0.01
400	3.317	0.05	0.09576	0.01
450/2	2.836	0.06	0.1005	0.01
500	3.219	0.04	0.1578	0.01

Table (III-5)

Constants (K) for Calculation of the Mean Shear Rate ($\bar{G}$), and Some Sample Shear Rates

Viscometer	Constant K $(\text{sec.})^{-1}$	Standard Deviation (%)	Flow Times (Seconds)				
			200	300	400	500	600
25	323900	0.15	1620	1080	810	648	540
75	128300	0.14	642	428	321	257	214
100	88190	0.15	441	294	221	176	147
150	40900	0.16	205	136	102	81.8	68.2
200	20000	0.10	100	66.7	50.0	40.0	33.3
300	10870	0.09	54.4	36.2	27.2	21.7	18.1
350	6097	0.10	30.5	20.3	15.2	12.2	10.2
400	3206	0.10	16.0	10.7	8.02	6.41	5.34
450/2	2372	0.10	11.8	7.91	5.93	4.74	3.95
500	695	0.10	3.48	2.32	1.74	1.39	1.16

Resulting Mean Shear Rate, $\bar{G}$

Capillary Radius

The viscometer was inverted and the capillary tube adjusted to the vertical by means of a plumb-bob. After filling the tube with mercury almost to the top, the mercury was run out through a fine jet into a tared flask (10 ml) until the meniscus was almost to the bottom of the capillary. The mercury was weighed, and the length run out measured by use of a travelling microscope. This was repeated four times. By using the density of mercury at the calibration temperature it was possible to calculate the radius of the capillary. Results appear in table (III-4).

Calculation of the Kroeplin "Constant".

From the calculated viscometer volumes and radii, a constant K for Kroeplin's equation (III-1) can be calculated, giving the equation the form:

$$\bar{G} = \frac{K}{t} \qquad \text{(III-29)}$$

Values of K and $\bar{G}$ for various times appear in table (III-5).

(c) Measurement of Galactomannan Solution Shear Rates and Viscosities

Galactomannan solutions were prepared as described earlier in Section B1(Ubbelohde Viscometers.). Samples of these solutions were diluted as before to give approximate concentrations of 0.8%, 0.6%, 0.4%, 0.2%, and 0.1% (all w/w.). The actual concentrations were determined as usual.

For each solution ten measurements of viscosity and associated shear rate (at 20.0°C) were made over a range of applied pressures from -6.0 cm d.b.p. (pressure applied to filling arm of viscometer) to + 60.0 cm (pressure applied to driving arm). These measurements were spaced at approximately equal intervals on a graphical plot of log shear rate versus log viscosity.

The viscosity of each solution was calculated from the equation:

$$\eta = A(h_o d_o + h_e d_e)t \qquad \text{(III-30)}$$

(Browning and Sell, 1956)

where η is the viscosity of the solution in centipoises,

A is the viscometer constant,

h_o is mean head of the viscometer,

h_e is the external head as indicated on the manometer,

d_o is the density of the liquid in the viscometer,

d_e is the density of the manometer fluid.

The viscometer constant A may be calculated in the following way:
Take Poiseuille's equation (III-24) in the form

$$\nu = ct \qquad \text{(III-24)}$$

Conversion of ν to centipoise gives

$$\eta = cd_o t \qquad \text{(III-31)}$$

If there is no external pressure, the combination of equations (III-30) and (III-31) gives $c d_o t = A h_o d_o t$

$$\text{i.e.} \quad A = \frac{c}{h_o} \qquad \text{(III-32)}$$

Values of the mean head and flow constants without external pressure have been previously determined, and were given in tables (III-3) and (III-2).

Correlation coefficients and straight-line equations for the Schutz Equation (III-5) and Power Law Equation (III-6) were calculated using the method of least squares.

Intrinsic viscosities at zero shear rate were calculated by extrapolation of viscosity/shear rate plots to zero shear rate for each concentration followed by extrapolation of plots of viscosity at zero shear rate divided by concentration versus concentration to zero concentration.

C. Results

(1) Ubbelohde Viscometers

(a) General Comments

Solutions of the galactomannans were clear at concentrations of 0.1%, but, with the exception of Soybean and Lotus pedunculatus I galactomannans, became increasingly opaque as concentration increased.

Soybean galactomannan solutions over a concentration of about 0.25% were difficult to work with as they formed very stable foams if agitated. The other galactomannans showed little sign of this property.

All the galactomannan solutions, even that of the very low viscosity Lotus pedunculatus I galactomannan had a slippery, mucilaginous texture.

Typical curves showing the dependence of the viscosity of several galactomannans on temperature and concentration are shown in fig. (III-7).

(b) Concentration Dependence of Viscosity

The concentration dependence of the viscosity of the galactomannan solutions was examined by fitting the measured values of viscosity and concentration to equation (III-21):

$$\log_e \eta = \log_e K' + (\frac{b}{a})c \qquad \text{(III-21)}$$

Some typical plots are shown in figs. (III-8) and (III-10). The parameters $\log_e K'$ and $\frac{b}{a}$ were determined by the method of least squares, and the correlation coefficient (r) of the line was also obtained. The results appear in table (III-6).

In all cases the results are well represented by an equation of the form of equation (III-21). The correlation coefficient was not less than 0.995 in all but two cases, and not less than 0.990 in all cases. Thus the concentration dependence of viscosity is essentially that found empirically by Schutz (1970) for carbohydrate solutions (including those of Guar galactomannan) in the region of their psuedoplastic behaviour

Figure (III–7a):– The Dependence of Galactomannan Viscosity on Concentration at 20°C

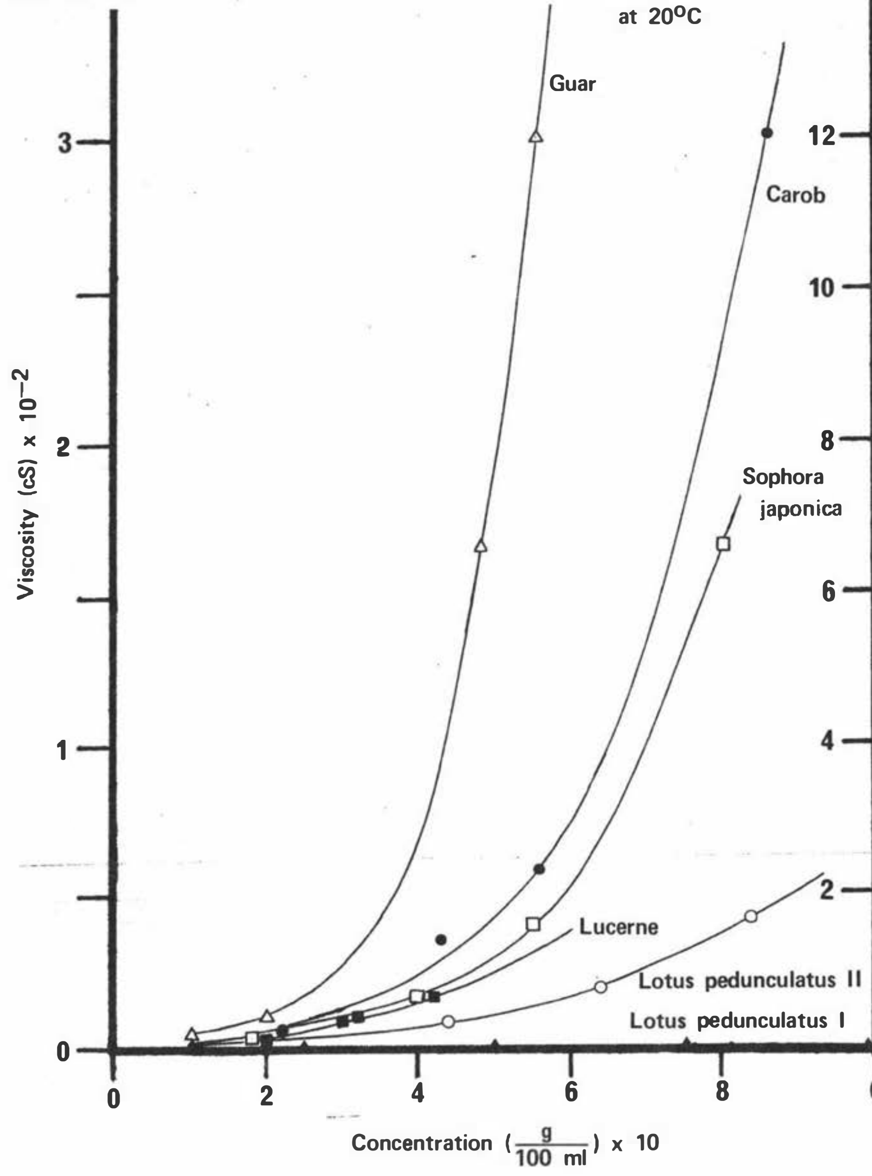
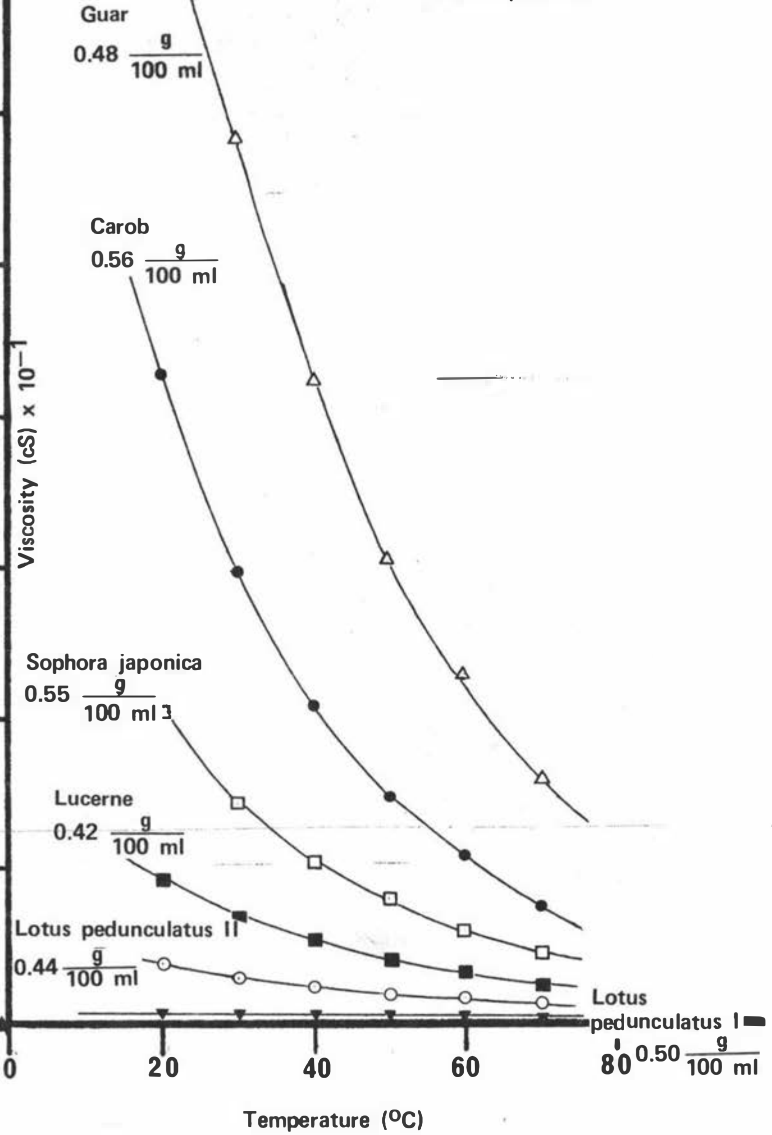


Figure (III–7b):–The Dependence of Galactomannan Viscosity on Temperature



Table(III-6)

Comparison of Experimental Results with an Equation of the Form $\log_e \eta = \log_e K' + (\frac{b}{a})c$ at a Series of Temperatures

Galactomannans	Temperature (^{o}C)	Coeff. of concentration $(\frac{b}{a})$	Constant $\log_e K'$	Correlation Coefficient (r)
Carob	20	0.650	0.440	0.990
	30	0.679	0.062	0.996
	40	0.662	-0.173	0.996
	50	0.641	-0.359	0.996
	60	0.624	-0.556	0.997
	70	0.608	-0.780	0.999
Guar	20	0.979	0.309	1.000
	30	0.959	0.062	1.000
	40	0.942	-0.167	1.000
	50	0.923	-0.374	1.000
	60	0.903	-0.571	1.000
	70	0.877	-0.777	1.000
Lotus pedunculatus (I)	20	0.0168	0.010	0.998
	30	0.0166	-0.211	0.995
	40	0.0172	-0.595	0.999
	50	0.0169	-0.420	0.999
	60	0.0162	-0.745	0.998
	70	0.0157	-0.881	1.000
Lotus pedunculatus (II)	20	0.429	0.173	0.999
	30	0.458	0.363	0.999
	40	0.382	-0.134	0.997

(to be cont)

Table (III-6)(cont)

Galactomannan	Temperature	Coeff. of concentration $(\frac{b}{a})$	Constant $\log_e K'$	Correlation Coefficient (r)
Lotus pedunculatus (II)	50	0.404	-0.458	0.999
	60	0.389	-0.612	0.999
	70	0.364	-0.730	0.999
Lucerne	20	0.689	0.449	0.996
	30	0.679	-0.124	0.997
	40	0.669	-0.344	0.997
	50	0.651	-0.519	0.997
	60	0.630	-0.677	0.998
	70	0.618	-0.856	0.998
Red Clover	20	0.605	0.002	0.997
	30	0.591	-0.222	0.997
	40	0.576	-0.426	0.997
	50	0.563	-0.616	0.997
	60	0.530	-0.993	0.996
	70	0.545	-0.787	0.998
Sophora japonica	20	0.644	0.082	0.998
	30	0.618	-0.128	0.998
	40	0.605	-0.344	0.998
	50	0.585	-0.529	0.998
	60	0.555	-0.664	0.998
	70	0.525	-0.779	0.998

(to be cont)

Table(III-6)(cont)

Galactomannan	Temperature	Coeff. of concentration $(\frac{b}{a})$	Constant $\log_e K'$	Correlation Coefficient (r)
Soybean	20	0.111	0.045	0.998
	30	0.109	-0.184	0.998
	40	0.105	-0.376	0.999
	50	0.105	-0.547	0.990
	60	0.097	-0.702	0.997
	70	0.093	-0.848	0.997

Figure (III–9):– Dependence of Guar Galactomannan Viscosity on Temperature at Varying Concentrations

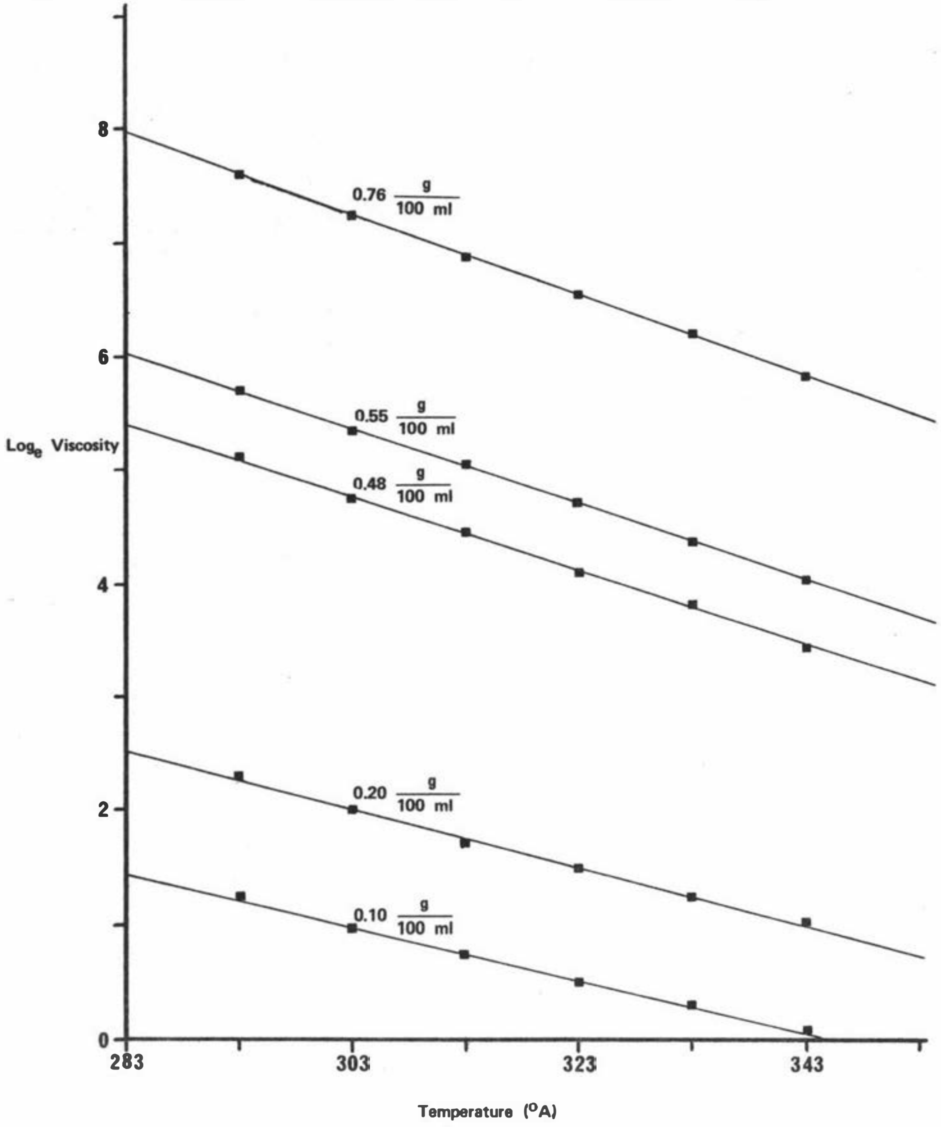


Figure (III–8):– Dependence of Guar Galactomannan Viscosity on Concentration at Various Temperatures

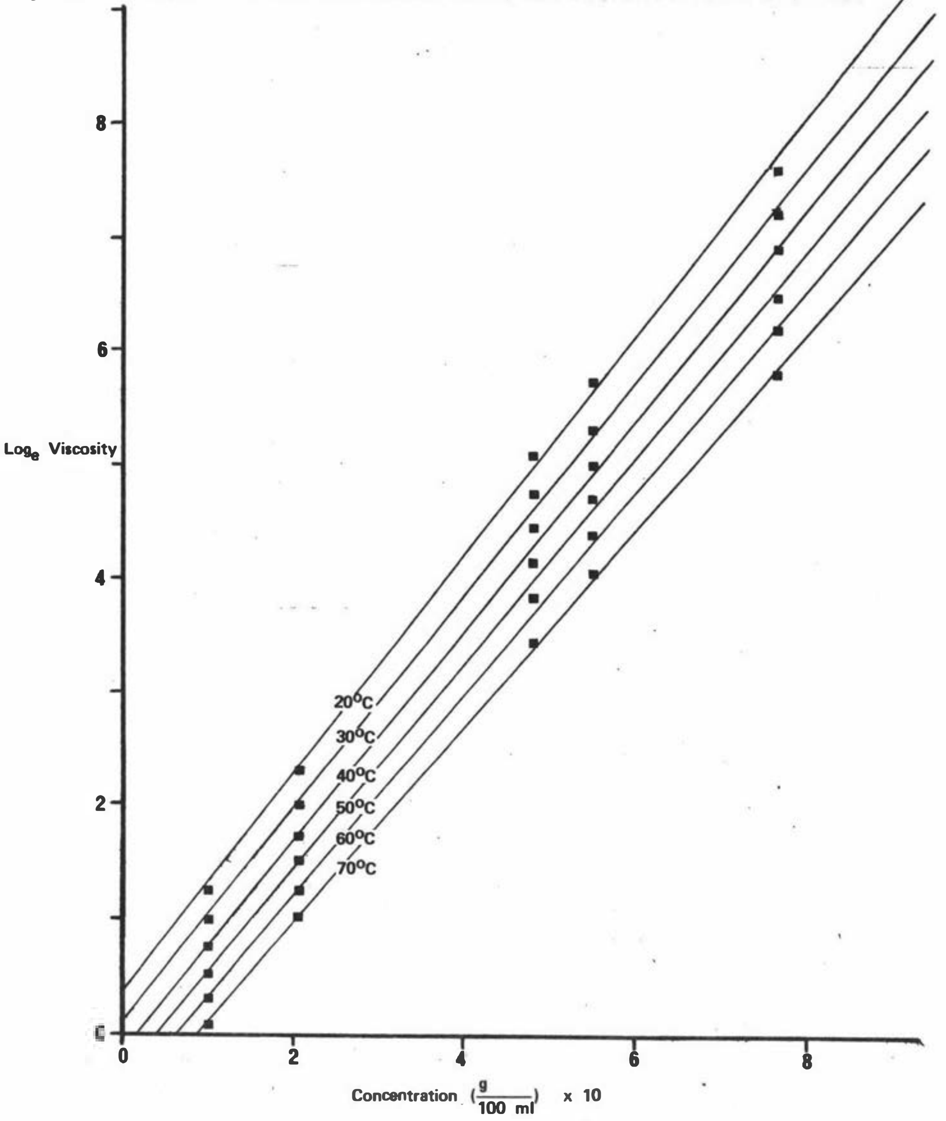


Figure (III–11) Dependence of Sophora japonica Galactomannan Viscosity on Temperature at Varying Concentrations

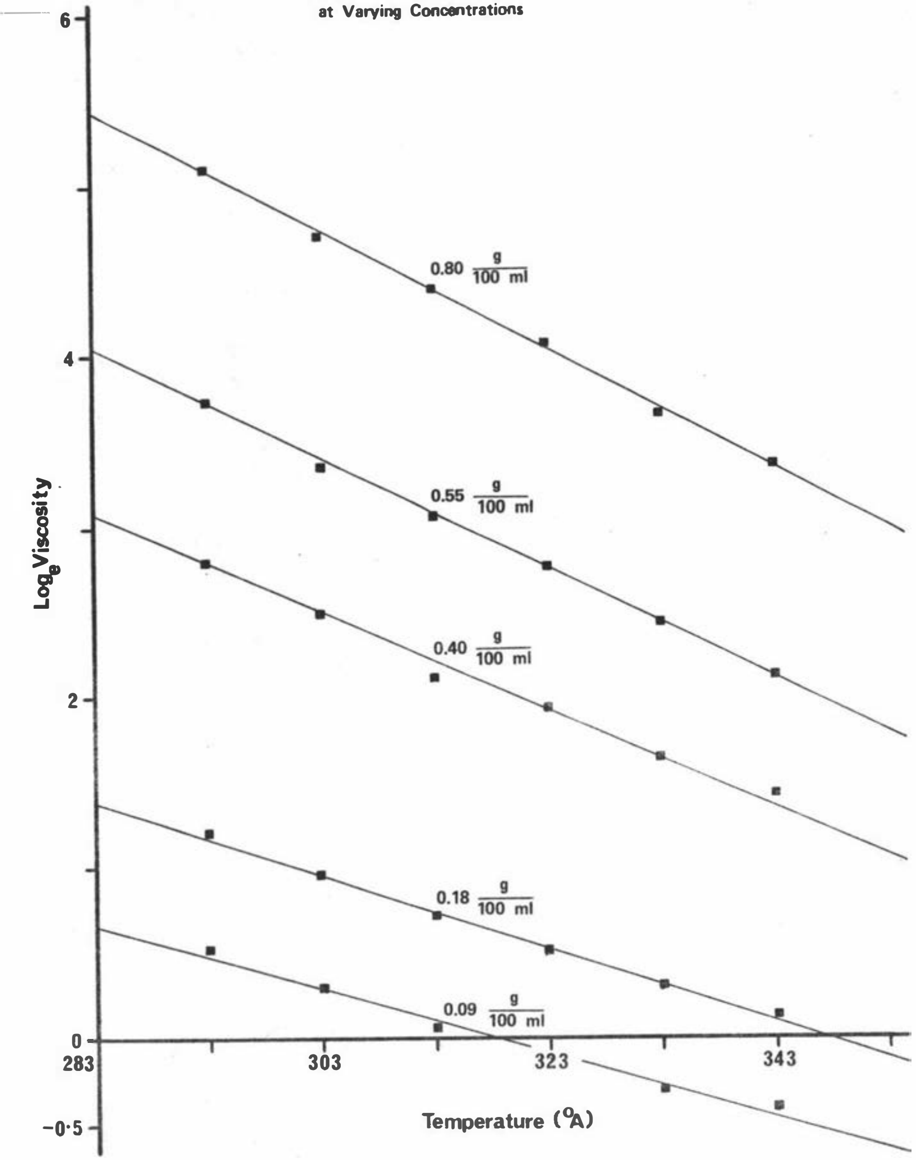
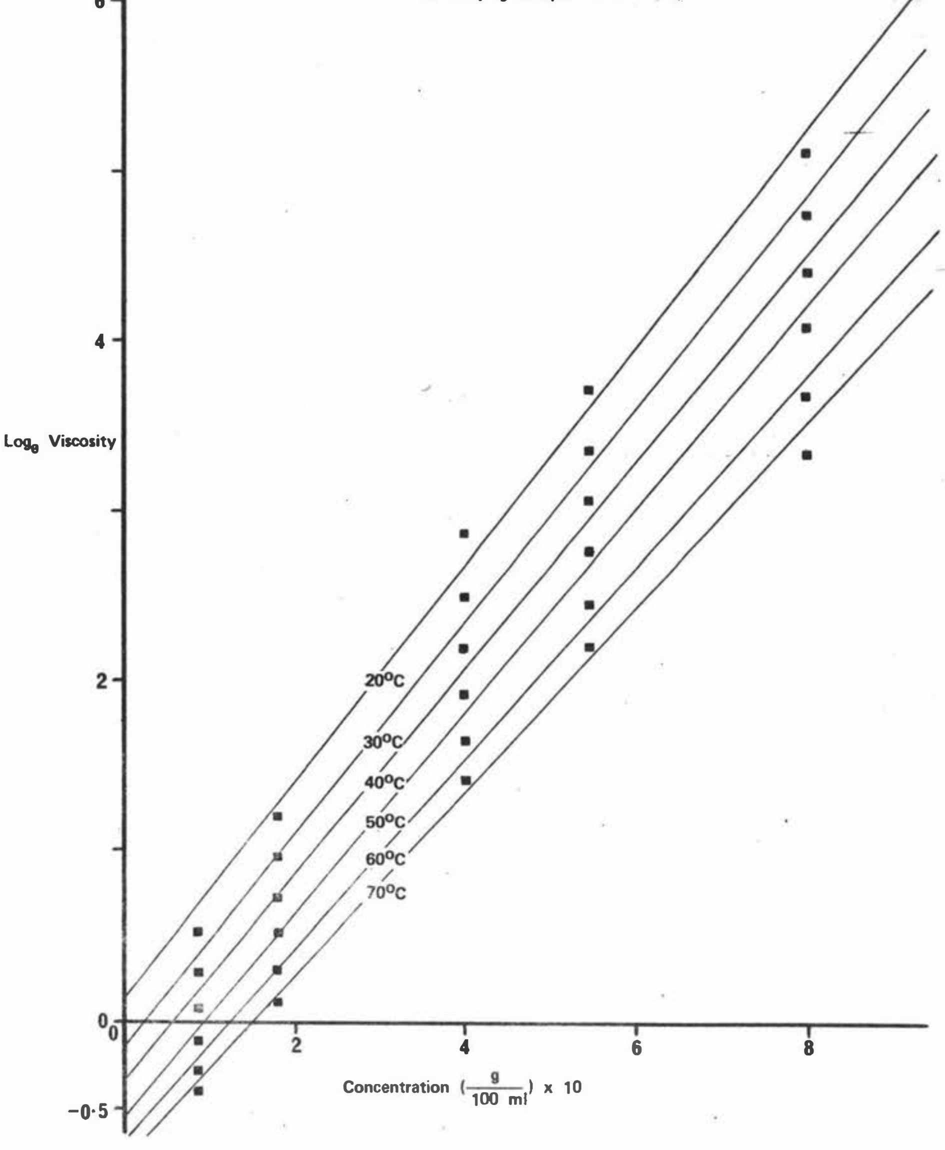


Figure (III–10):–Dependence of Sophora japonica Galactomannan Viscosity on Concentration at Varying Temperatures



governed by the Power Law. It can also be noticed in table (III-6) that both $(\frac{b}{a})$ and K' decrease with temperature. In equation (III-20) the value of K' was fixed by

$$K' = kK_c^{1/a}$$

$$\text{i.e. } \log_e K' = \log_e k + \frac{1}{a} \log_e K_c$$

Since both k and 'a' are constant by definition, it must be K_c which decreases with increasing temperature.

Similarly, in equation (III-21) 'a' is constant so that b must decrease with increasing temperature in order for the value of $(\frac{b}{a})$ to decrease. Therefore equation (III-20) should be rewritten as:

$$\eta = k\ K_c(T)\ e^{b(T)c^{1/a}} \qquad \text{(III-33)}$$

where (T) implies conditions of constant temperature.

This leads to the modified form of equation (III-21):

$$\log_e \eta = \log_e K'(T) + \frac{b}{a}(T) \qquad \text{(III-34)}$$

(c) <u>Temperature Dependence of Viscosity</u>

The temperature dependence of the galactomannan solution viscosity was examined by fitting the experimental results to equation (III-23):

$$\log_e \eta = \log_e K'' + \frac{D}{aT} \qquad \text{(III-23)}$$

Some typical plots are shown in figs. (III-9) and (III-11). The parameters K" and $\frac{D}{a}$ were obtained by the method of least squares, and these and the correlation coefficients to the relationship shown in equation (III-23) are given in table (III-7). Good correlation coefficients ($r > 0.995$) were obtained in all cases.

The values of K" and $\frac{D}{a}$ in table (III-7) can be seen to be concentration dependent. K" increases with increasing concentration for Guar, Carob, Lotus pedunculatus I and II, Lucerne and Red Clover galactomannans, and decreases for those of Sophora japonica and Soybean. The reason for this anomolous behaviour of K" with concentration is not known.

Table (III-7)

Comparison of Experimental Results with an Equation of the Form $\log_e \eta = \log_e K'' + (\frac{D}{a}) \frac{1}{T}$ at a Series of Galactomannan Concentrations

Galactomannan	Concentration (g / 100 ml)	Coeff. of $\frac{1}{T}$ $(\times 10^{-3})(\frac{D}{a})$	Constant $\log_e (K'')$	Correlation Coefficient
Carob	0.100	2.12	-6.44	1.000
	0.219	2.52	-6.77	1.000
	0.430	3.15	-7.20	1.000
	0.563	3.38	-7.08	1.000
	0.861	3.50	-5.85	1.000
Guar	0.100	2.32	-6.67	0.999
	0.203	2.69	-6.35	0.998
	0.480	3.26	-5.99	0.999
	0.547	3.29	-5.51	1.000
	0.755	3.53	-4.52	0.999
Lotus pedunculatus (I)	0.100	1.79	-6.09	0.998
	0.250	1.78	-6.06	0.999
	0.500	1.80	-6.09	0.999
	0.750	1.80	-6.02	0.999
	1.000	1.81	-6.02	0.999
Lotus pedunculatus (II)	0.107	1.95	-6.09	0.999
	0.221	2.11	-6.06	1.000
	0.437	2.37	-5.99	1.000
	0.639	2.63	-6.01	0.999
	0.840	2.86	-5.99	0.997

(to be cont)

Table(III-7)(cont)

Galactomannan	Concentration (g / 100 ml)	Coeff. of $\frac{1}{T}$ $(x10^{-3})(\frac{D}{a})$	Constant $\log_e$ (K")	Correlation Coefficient
Lucerne	0.094	2.04	-6.26	0.999
	0.206	2.27	-6.18	1.000
	0.290	2.43	-5.88	1.000
	0.324	2.40	-6.04	1.000
	0.424	2.52	-5.67	1.000
Red Clover	0.075	2.06	-6.65	0.998
	0.271	2.40	-6.47	1.000
	0.360	2.47	-6.18	1.000
	0.460	2.69	-6.35	0.998
	0.554	2.78	-6.20	0.999
Sophora japonica	0.087	1.90	-5.97	0.998
	0.177	2.15	-6.12	1.000
	0.404	2.79	-6.72	1.000
	0.546	3.06	-6.72	1.000
	0.803	3.53	-6.90	0.998
Soybean	0.100	1.82	-6.08	0.999
	0.245	1.87	-6.06	0.999
	0.413	1.95	-6.15	1.000
	0.634	2.01	-6.11	1.000
	0.832	2.09	-6.20	1.000

In equation (III-22) the value of K" was fixed by:

$$K'' = kK_T^{1/a}$$

$$\text{i.e.}\ \log_e K'' = \log_e k + \frac{1}{a}\log_e K_T$$

Since k and 'a' are constant by definition, K_T must be changing with concentration. Similarly since $\frac{D}{a}$ varies with concentration, the variation must be due to changes in the value of D. Consequently equation (III-22) should be rewritten:

$$\eta = k\,K_T(c)\;{}_{e}D(c)/T\quad 1/a \qquad \text{(III-35)}$$

where (c) implies conditions of constant concentration.

This leads to the modified form of equation (III-23):

$$\log_e \eta = \log_e K''(c) + \frac{D(c)}{aT} \qquad \text{(III-36)}$$

(d) Calculation of Intrinsic Viscosity

(i) Definition of Intrinsic, Specific, and Relative Viscosities:

The definitions of these various types of viscosity appear in table (III-8).

Table(III-8)

Definition of Viscometry Terms (Onyon, 1959)

Definition	Old Symbol	Old Name	New Symbol	Recommended Name
η/η_o	η_r	Relative Viscosity	η/η_o	Viscosity Ratio
$(\eta/\eta_o)-1$	η_{sp}	Specific Viscosity	-	-
$(\eta/\eta_o)-1 \quad \frac{1}{c}$	$\frac{100\eta_{sp}}{c}$	-	-	Viscosity Number
$\left[\log_e(\frac{\eta}{\eta_o})\right] \frac{1}{c}$	-	-	-	Logarithmic Viscosity Number
$\lim_{c\to 0} \left[(\frac{\eta}{\eta_o})-1\right] \frac{1}{c}$	$100\,[\eta]$	100 x Intrinsic Viscosity		Limiting Viscosity Number
$\lim_{c\to 0} \left[\frac{\log_e(\frac{\eta}{\eta_o})}{c}\right]$	"	"	"	"

(η is the solution viscosity, η_o is the solvent viscosity.) (Although the International Union of Pure and Applied Chemistry introduced the new names and symbols in 1953, current practice is still to use the old terms, and that useage has been followed in this thesis. The factors of 100 in $\frac{\eta_{sp}}{c}$ and $[\eta]$ are used to correlate units between the old and new systems due to a change in the concentration unit from g/100 ml (old) to g/ml (new).)

(ii) Calculations:

For the dependence of viscosity on concentration, a number of equations have been used. Simha (1950) derived the general equation for polymer solutions:

$$\eta = \eta_o(1 + a_1c + a_2c^2 + a_3c^3 \ldots\ldots) \qquad \text{(III-37)}$$

(where η is the solution viscosity, η_o is the solvent viscosity, c is the concentration of solute, a_1, a_2, a_3, ... etc. are constants.)

If terms higher than c^2 are neglected, the equation becomes:

$$\eta = \eta_o(1 + a_1c + a_2c^2)$$

i.e. $$\frac{\eta}{\eta_o} - 1 = a_1c + a_2c^2$$

i.e. $$\frac{\eta_{sp}}{c} = a_1 + a_2c$$

$$\lim_{c \to 0} \frac{\eta_{sp}}{c} = a_1$$

$$= [\eta] \text{ by definition.}$$

i.e. $$\frac{\eta_{sp}}{c} = [\eta] + k[\eta]^2 c \qquad \text{(III-38)}$$

(where k is a constant.)

This equation is identical to that developed by Huggins (1942), and is applied to systems at low concentration.

Empirically, Martin (1942) found that:

$$\log_e \frac{\eta_{sp}}{c} = \log_e [\eta] + k'[\eta]c \qquad \text{(III-39)}$$

(where k' is a constant.)

Martin's equation can be applied over a wider range of concentration than that of Huggins, although Huggins' is obviously much easier to use as it does not require logarithmic plots.

Intrinsic viscosity can thus be calculated by a numerical or graphical extrapolation of a plot of $(\frac{\eta_{sp}}{c})$, $\log_e (\frac{\eta_{sp}}{c})$ or $\log_{10} (\frac{\eta_{sp}}{c})$ against c. (A full discussion of the physical meaning of intrinsic viscosity is given later in part 2 of the viscometry results (for Cannon-Fenske viscometers.)

Initially, to interpret the results from galactomannan solutions, plots of $\frac{\eta_{sp}}{c}$ against c were used, but these were found to be non-linear and therefore difficult to extrapolate to zero concentration. Plots of $\log_{10} (\frac{\eta_{sp}}{c})$ against c (based on the Martin equation (III-30)) however were linear for all but the Soybean and Lotus pedunculatus I galactomannans. The plots for these two cases were non-linear. In accordance with current practice (see, for example Eliezer and Silberberg, 1967) extrapolations were made to zero concentration from the linear portions of the curves in the case of Soybean galactomannan. The plots for Lotus pedunculatus I galactomannan solutions showed no evidence of concentration dependence as a scatter of values were obtained which ranged to $\pm$ 10% of the mean value of $\log_{10} (\frac{\eta_{sp}}{c})$. An estimate of the intrinsic viscosity was calculated by averaging the values of $\log_{10} \frac{\eta_{sp}}{c}$.

From the graphs of $\log_{10} [\eta]$ against temperature (fig.(III-12), it can be seen that at 20°C the order of magnitude of intrinsic viscosity is Guar > Carob > Lucerne > Red Clover > Sophora japonica > Lotus pedunculatus II > Soybean > Lotus pedunculatus I. The order for Guar and Carob is confirmed by the findings of Hui and Neukom (1964), who compared the viscosity of Guar and Carob galactomannan solutions at the same concentration. Lotus pedunculatus II galactomannan was found by Beveridge (1965) to have an intrinsic viscosity of 5dl/g, slightly less than the value obtained for the present sample at 20°C. Whistler and Saarnio (1957) reported a low value for the intrinsic viscosity of Soybean galactomannan, as was found here. Intrinsic viscosity data for the other galactomannans have not been reported.

(iii) Variation of Galactomannan Intrinsic Viscosity with Temperature:

From the plots of $\log_{10}[\eta]$ against temperature (fig. (III-12)) a good linear correlation is obtained between $\log_{10}[\eta]$ and temperature over the range 20° - 60°C, but not at higher temperatures. A more rapid decrease in $\log_{10}[\eta]$ (apart from Soybean and Lotus pedunculatus I and II galactomannans) is evident as 70°C is reached. The reason for the anomolous increase in $\log_{10}[\eta]$ at 70°C for Sophora japonica galactomannan is not known, but on the basis of the dependence of $\log_{10}[\eta]$ on temperature for the other galactomannans, this result does not appear to constitute a real effect.

The temperature dependence of intrinsic viscosity arises from the nature of the galactomannan molecule, and its interaction with water. Possible effects could be due to changes in the state of aggregation in the polymer, changes of solvation of the polymer molecule, and changes in the molecular conformation (Vink, 1971).

If it can be assumed that galactomannans behave as flexible long-chain molecules, then the temperature effect may be interpreted in terms of Flory's (1953) theory from:

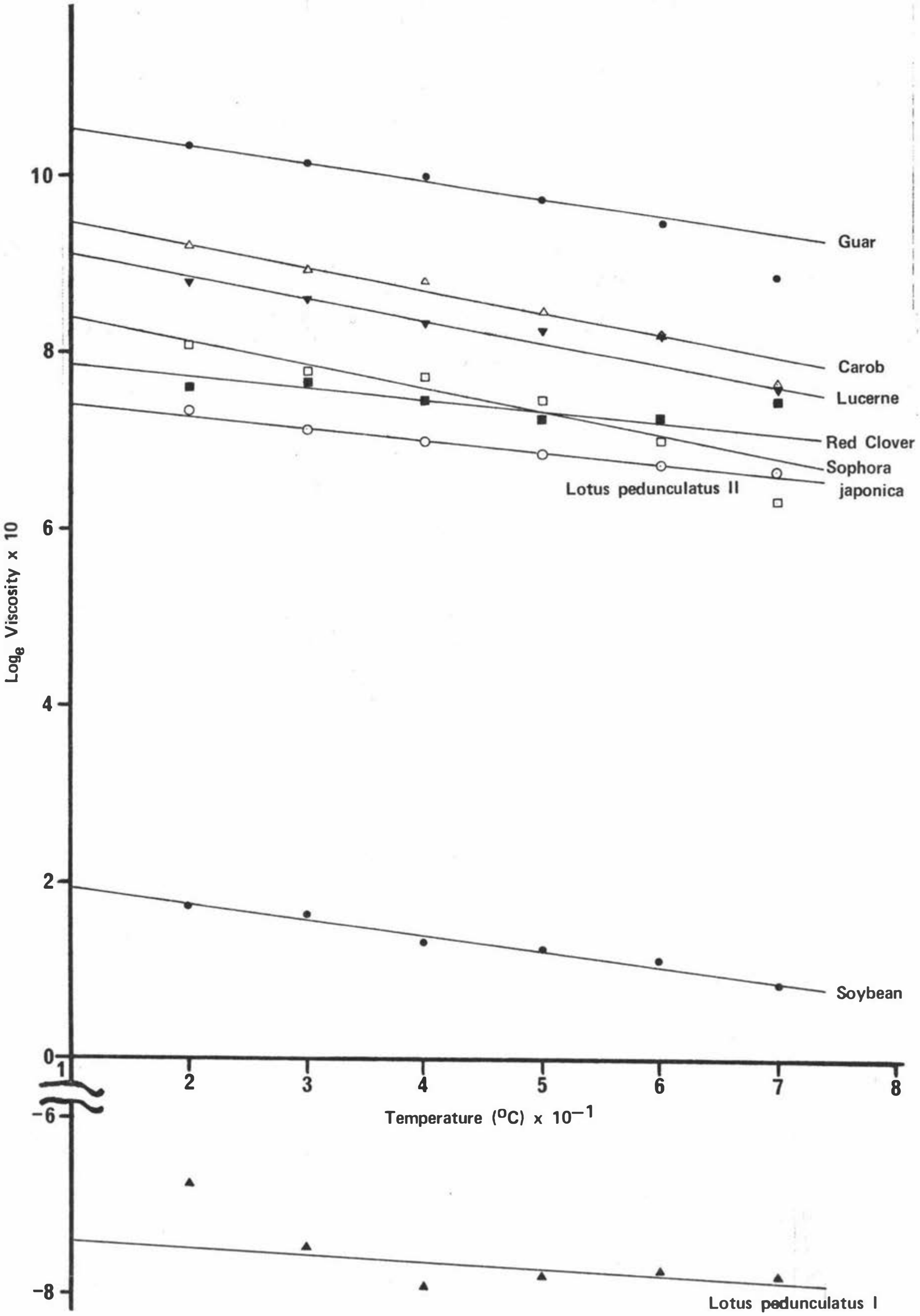
$$\alpha^5 - \alpha^3 = 2C_m\psi_1(1 - \Theta/T)M^{\frac{1}{2}} \qquad \text{(III-40)}$$

(where α is the intramolecular expansion factor.

ψ_1 is an entropy parameter.

C_m is a parameter arising from the intermolecular theory of the thermodynamic properties of dilute

Figure (III–12) :— The Dependence of Galactomannan Intrinsic Viscosity on Temperature



solutions.

Θ is the "ideal" or "theta" temperature (where the chemical potential due to segment-solvent interactions is zero).

T is the temperature of measurement.

M is the molecular weight.)

According to Flory (1953, P.622), a change in the temperature may affect the intrinsic viscosity through alteration of the expansion factor, α, and the unperturbed dimensions of the molecule $(\bar{r}_o^2)^{\frac{1}{2}}$ (which is part of the parameter C_m), with the change in α generally predominating. The sign of the temperature dependence is therefore essentially determined by the polymer/solvent interaction.

For a very good solvent Θ is negative and α should decrease with increasing temperature; in athermal solvents Θ is zero, and hence α should be independent of temperature; and in poor solvents Θ is positive and α should increase with temperature.

The case of galactomannans in water would be expected to correspond to that of a solute in a good solvent. This would be expected because galactomannans are essentially long chain polyhydroxy compounds, with short side-chains which prevent close association of the molecules over long lengths of their chains resulting in cellulose-like insolubility. There would thus be expected to be a large degree of interaction between a galactomannan molecule and water.

It is obvious from the negative gradients of the plots of $\log_{10} [\eta]$ against temperature (fig. (III-12)) that the temperature dependence of galactomannan intrinsic viscosity does correspond to the case of a solute in a good solvent.

Although only a change in α has been considered above, it is also possible that an increase in temperature could result in an increase in chain flexibility. This would decrease the unperturbed dimensions $(\overline{r_o^2})^{\frac{1}{2}}$ of the molecule by increasing rotation about the bonds and hence also the viscosity. Some contribution to change in $[\eta]$ from change in $(\overline{r_o^2})^{\frac{1}{2}}$ would seem quite feasible, especially as the temperature dependence of $[\eta]$ seems to be quite high (as Vink, 1971, found for cellulose).

(2) Cannon-Fenske Viscometers

(a) General

The viscosity of all the galactomannans in solution was found to be shear-rate dependent, even for those galactomannans (e.g. Lotus pedunculatus I) which gave solutions of very low viscosity. Shear rate dependence of viscosity was highest for the most viscous solutions, such as those of Guar and Carob, and decreased with decreasing viscosity. Viscosity/shear rate data appears in Appendix V.

(b) Limitations of the Apparatus

A serious limitation of the apparatus was that for samples of low viscosity, a mean shear rate below about 200 sec.$^{-1}$ could not be obtained. This was due to the limitation on the external opposing pressure ($-h_e$) that could be applied to the filling arm of the viscometer (see fig. (III-2)) opposing solution flow. This pressure was found to be limited by the approximate relation:

$$-h_e \leqslant -h_o + 3 \qquad \text{(III-41)}$$

(where h_o is the mean head of **the viscometer**)

The limitation was due to the surface tension of the solution in the viscometer capillary. If external opposing pressures greater than (h_o + 3) were applied to the filling arm, the solution would flow from the top etched mark of the viscometer through the bulb until it emptied, but flow would stop at the top of the capillary before the solution meniscus reached the bottom etched mark. This effect is not nearly so pronounced for solutions of low surface tension such as cupriethylenediamine, which was used as a cellulose solvent in the determination of the shear rate dependence of cellulose solution viscosity, using an identical method (e.g. Browning and Sell, 1956).

Equation (III-30) gave the relationship between flow time, external pressure, and solution and manometer fluid density:

$$\eta = A(h_o d_o + h_e d_e)t \qquad \text{(III-30)}$$

Values for most terms in this equation were known:

d_o = density of galactomannan solution = approx. 1.0 g/ml

d_e = density of dibutylphthallate = 1.043 g/ml

A = constant of proportionality (previously determined.)

h_o = mean viscometer head (previously determined).

If a flow time of 600 seconds is considered as a reasonable maximum for observation, the viscosity at this flow time is dependent only on the external pressure head h_e. In order to calculate the minimum measurable viscosity at this flow time, h_e must take on its minimum value aiding solution flow. This will be identical to its maximum value opposing flow which was shown by inequality (III-41) to be $(-h_o + 3)$. Substitution of this value for h_e in equation (III-30) for each viscometer gives the values of the minimum measureable viscosity in each, tabulated in table (III-9).

Similarly, the maximum measureable value of viscosity in each viscometer can be obtained by taking a flow time of 200 seconds as a reasonable minimum and measuring the maximum possible external pressure aiding solution flow. This latter was found to be h_e = 60 cm. of dibutylphthallate, due to the limitations of the apparatus. The calculated maximum value of measureable viscosity for each viscometer is also presented in table (III-9).

Although t = 600 seconds may appear an unreasonable value for the

Table (III-9)

Maximum and Minimum Values of Viscosity and Shear Rate Obtainable with a Given Viscometer

Viscometer No.	Minimum Viscosity (cP) (t=600 secs)	Maximum Viscosity (cP) (t=200 secs)	Mean Shear Rate (Sec^{-1})	
			Minimum (t=600 secs)	Maximum (t=200 secs)
25	0.414	3.31	540	1620
75	1.67	13.4	214	652
100	2.83	22.5	147	441
150	7.26	58.7	68.2	205
200	19.8	158	33.3	100
300	43.3	347	18.1	54.3
350	94.4	753	10.2	30.6
400	228	1470	5.34	16.0
450/2	318	2530	3.95	11.9
500	1540	12200	1.16	3.48

calculation of minimum viscosity, it must be remembered that since mean shear rate is inversely proportional to time (Kroeplin's equation (III-1)) t = 600 seconds gives the minimum value of shear rate. This minimum shear rate value (also tabulated in table (III-9)) combined with the minimum viscosity value for the same viscometer represents one extreme point obtainable on a flow curve using that particular viscometer.

Likewise, t = 200 seconds gives the maximum value of the mean shear rate, which when combined with the maximum viscosity value represents the other extreme point of a flow curve obtainable with the same viscometer. (N.B. Viscosity in equation (III-30) is directly proportional to the external pressure as well as to time, and since the former predominates over the latter in the above cases this leads to the initially incongruous-seeming result of a maximum viscosity occurring at a minimum flow time, and vice-versa.)

From examination of the table there is an approximately eight-fold range between the maximum and minimum measureable viscosities at their respective mean shear rates.

The plots of Soybean galactomannan solution viscosity against shear rate (fig. (III-13)) are an example of the limitations imposed by the surface tension effect. In no case, even for galactomannan concentrations as high as 0.80% was it possible to measure viscosity at a mean shear rate below 200 sec.$^{-1}$ due to the low viscosity of Soybean galactomannan solutions. Sophora japonica galactomannan solutions (fig. III-14)) provide an intermediate case, with low shear rates accessible at medium to high concentrations (0.5 - 0.8%), but not at lower ones; while the plots of Guar galactomannan viscosity against mean shear rate (fig. (III-15) shows that low mean shear rates are accessible at all but the lowest of concentrations.

Figure (III–13):–The Dependence of Soybean Galactomannan Viscosity on Mean Shear Rate

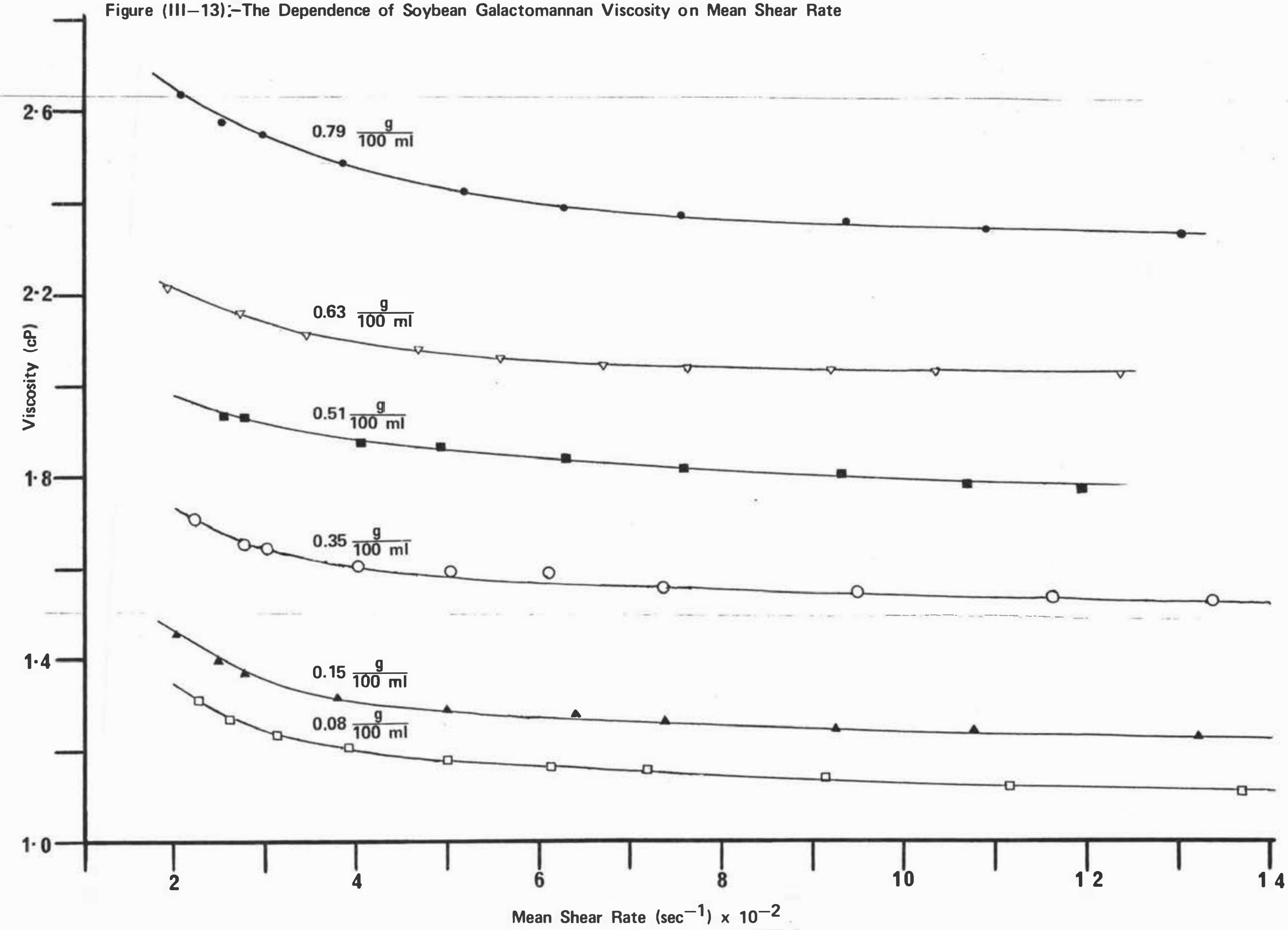


Figure (III–14) :– The Dependence of Sophora japonica Galactomannan Viscosity on Mean Shear Rate

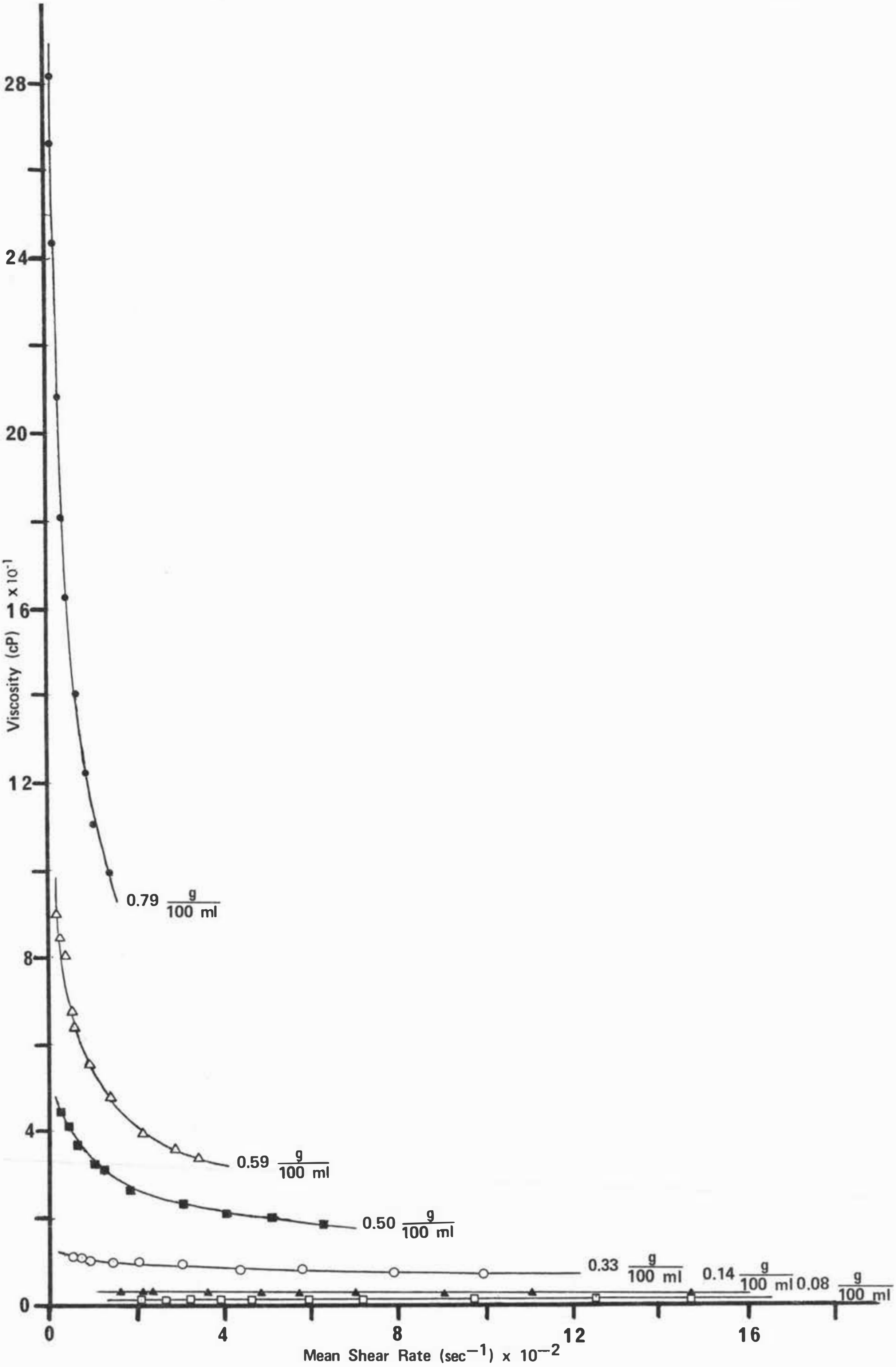
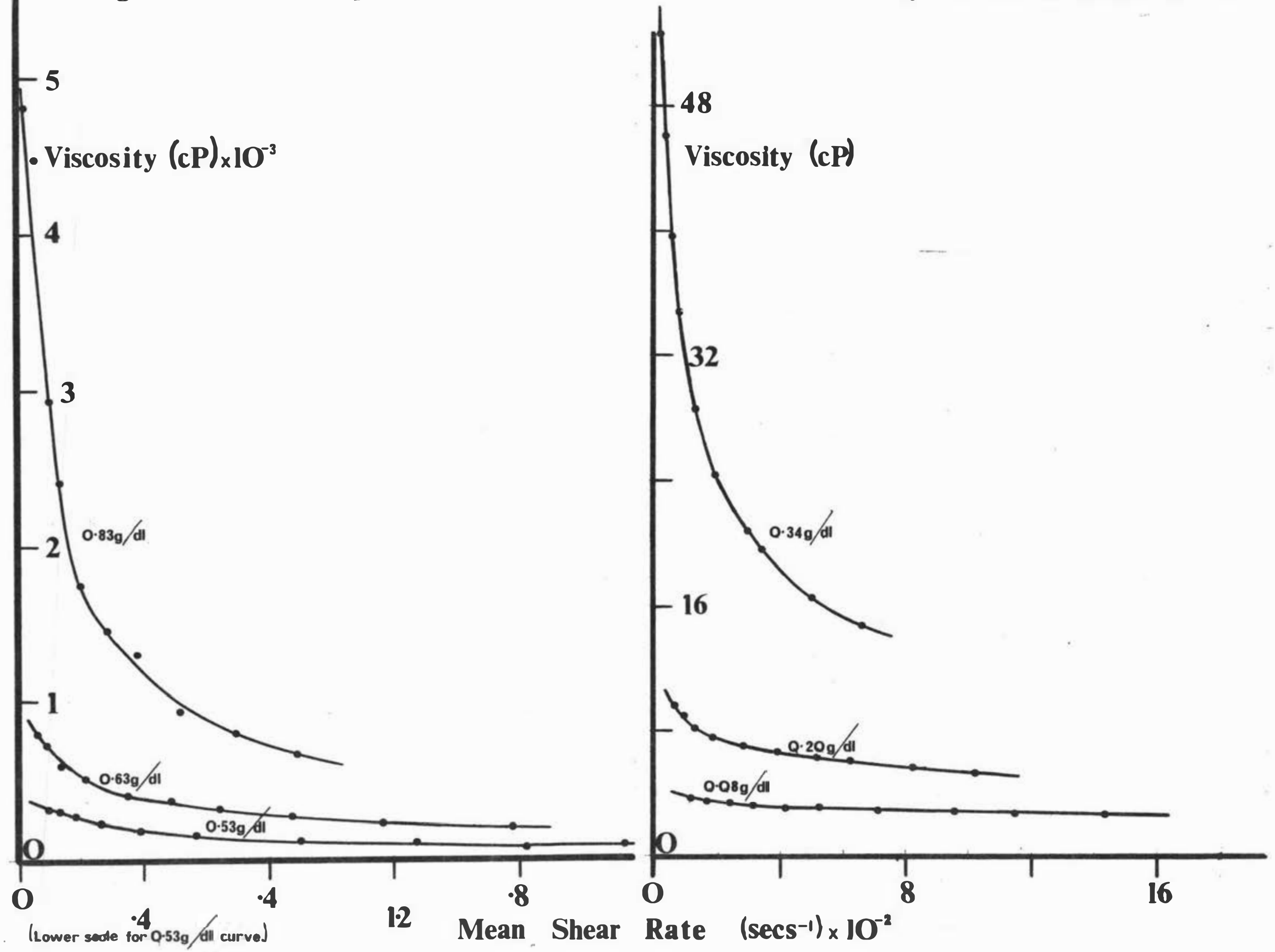


Fig. III-15 : The Dependence of Guar Galactomannan Viscosity on Mean Shear Rate.



For the higher concentrations of Guar and Carob galactomannans the reverse problem to that outlined above for Soybean is encountered. Because of the high viscosity of the solutions, application of the maximum external driving pressure (60 d.b.p.) produced a mean shear rate of less than 100 sec.$^{-1}$. Since an extrapolation of viscosity to zero shear rate was required, this low value of maximum shear rate was considered acceptable.

(c) Comparison of Measured Viscosity and Mean Shear Rate Values with the Schutz Equation.

(i) Comparison:

The Cross equation is $\eta = \eta_{\infty} + \left[\dfrac{\eta_o - \eta_{\infty}}{1 + \alpha \bar{G}^{2/3}}\right]$ (III-4)

and it was shown that by assuming that $\eta_{\infty} \ll \eta_o$ and η, this reduced to the Schutz equation,

$$\eta = \frac{\eta_o}{1 + \alpha \bar{G}^{2/3}} \qquad \text{(III-5)}$$

By rearranging the above equation, the form

$$\frac{1}{\eta} = \frac{1 + \alpha \bar{G}^{2/3}}{\eta_o} \qquad \text{(III-42)}$$

can be obtained, and from this (Schutz, 1970)

$$\frac{1}{\eta} = \frac{1}{\eta_o} + \frac{\alpha}{\eta_o} \bar{G}^{2/3} \qquad \text{(III-43)}$$

Thus a plot of reciprocal viscosity against mean shear rate to the power of two-thirds should yield a straight line of slope $\dfrac{\alpha}{\eta_o}$ and intercept $\dfrac{1}{\eta_o}$.

The viscosity and mean shear rate data at each concentration for all the galactomannans were plotted in the above form. A least squares line was fitted to the points, and a correlation coefficient between the

Table (III-10)

Comparison of the Viscosity and Mean Shear Rate Data of Galactomannans with the Schutz Equation $\eta = \dfrac{\eta_o}{1 + \alpha \bar{G}^{2/3}}$ (for those cases with correlation coefficient > 0.99)

Galactomannan	Concentration (% =g/100ml)	Viscosity at Zero Shear Rate η_o (cP)	Constant (α)	Correlation Coefficient (r)	Mean Shear Rate Range (Sec^{-1})
Carob	0.351	41.2	0.0229	0.993	31 - 616
	0.538	255	0.0869	0.999	12 - 227
	0.631	420	0.0994	0.999	4 - 102
	0.875	2520	0.292	0.998	2 - 55
Guar	0.201	10.50	0.0103	0.991	65 - 1018
	0.343	63.2	0.0446	0.997	18 - 655
	0.524				8 - 194
	0.631	1334	0.334	0.999	2 - 78
	0.828	3010	0.352	0.998	1 - 45
Lucerne	0.134	2.76	0.00236	0.997	244 - 888
	0.254	7.99	0.00577	0.995	106 - 500
	0.484	19.5	0.0106	0.995	62 - 472
	0.596	34.9	0.0167	0.993	43 - 441
	0.732	65.7	0.0272	0.998	22 - 201
Red Clover	0.368	19.3	0.0129	0.990	61 - 487
	0.422 (Max. concentration used)	103	0.0466	0.998	19 - 331
Sophora japonica	0.331	14.1	0.0108	0.990	51 - 988
	0.500	52.0	0.0264	0.995	30 - 622
	0.591	113	0.0491	0.995	16 - 341
	0.785	422	0.124	0.998	8 - 139

ten values of $(\frac{1}{\tau})$ and $(\bar{G}^{2/3})$ obtained. Results appear in table (III-10) for those galactomannans whose data gave correlation coefficients better than 0.99.

In all cases, for low viscosity galactomannan solutions the fit of the data to the Schutz equation (III-5) is poor. This is due to the breakdown of the assumption that $\eta_{\infty} \ll \eta_0$ or η used in the derivation of equation (III-5), as for solutions of low viscosity η_0 is not much greater than the viscosity of the solvent, water, and η_{∞} will certainly be no less than the solvent viscosity. Thus a good fit to Schutz's (1970) modified form of the Cross equation could not be expected for data from the solutions of Lotus pedunculatus I and II or Soybean galactomannans; or for solutions containing low concentrations of the other galactomannans.

Generally, as can be seen from the correlation coefficients, for the other galactomannans the fit of the data to the Schutz equation improves with increasing solution concentration, and consequent viscosity increase. Schutz found (1970) that his equation gave "satisfactory" results for Guar galactomannan solutions in the concentration range 0.3% to 1.0% and his results are verified by those for Guar in table (III-10). About the same lower limit of concentration appears to apply for Carob, Red Clover, and Sophora japonica galactomannans. The much lower limiting value in the case of Lucerne seems exceptional but the reason for it is unknown.

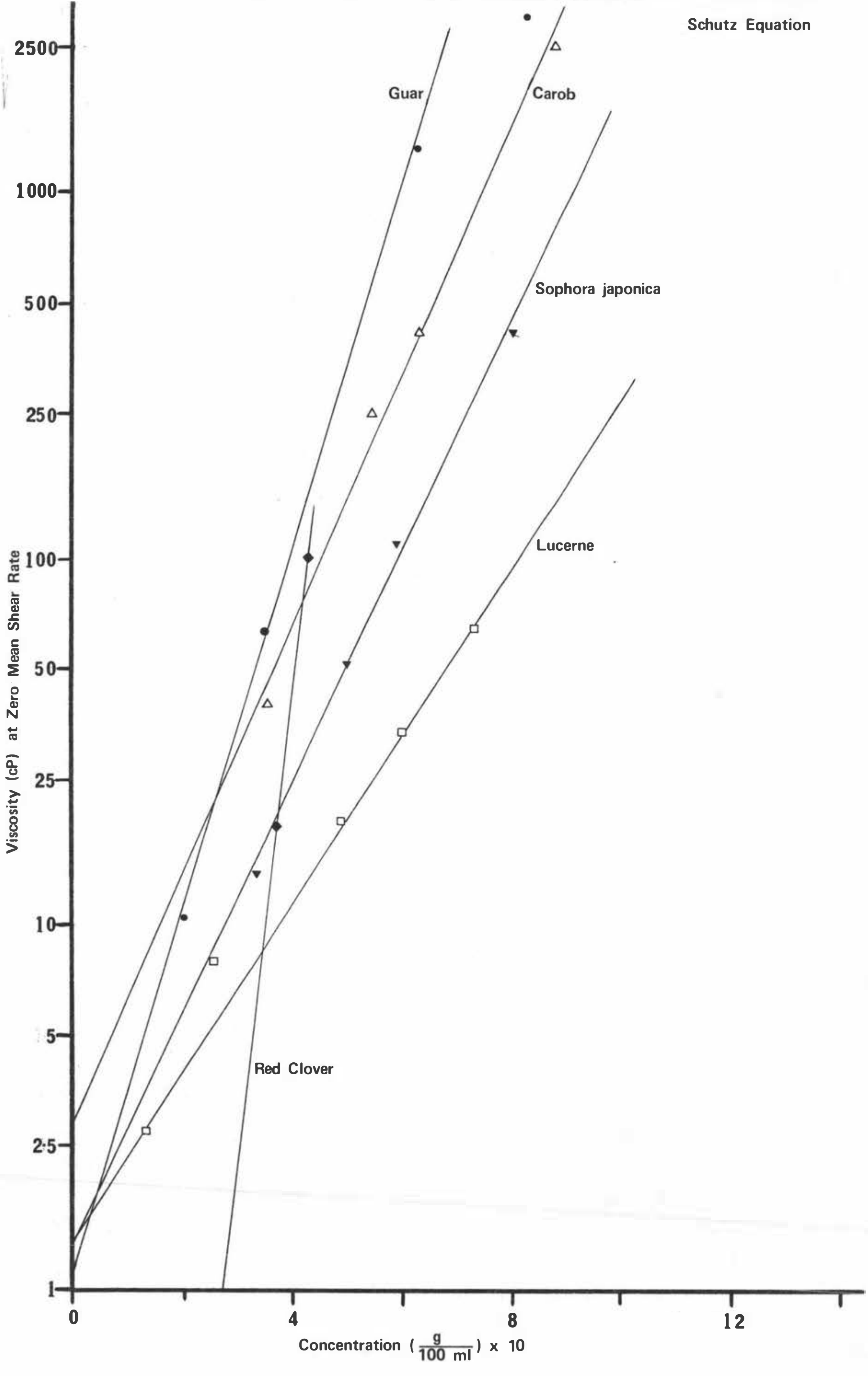
(ii) Concentration Dependence of Viscosity for Galactomannan Solutions Following Schutz Equation Behaviour:

Schutz (1970) found empirically for carbohydrate solutions that

$$\eta_0 = \eta_0' \, e^{Bc} \qquad \text{(III-9)}$$

where η_0 is the viscosity of the solution at zero shear rate η_0' and B are constants, and c is the concentration (g/100 ml)

Figure (III–16):– Plot of the Logarithm of the Viscosity at Zero Shear Rate against Concentration for the Schutz Equation



It follows that a plot of $\log_{10}\eta_{sp}$ against concentration should be linear, with slope B and intercept $\log_{10}\eta_o$. Such a plot is shown in fig.(III-16), using the data from table (III-10) for Carob, Guar, Lucerne, Red Clover and Sophora japonica galactomannans. In all cases an approximately linear relationship was found, as in Schutz's work. The abnormal slope of the Red Clover plot is unexplained. It seems extremely likely that one or both of the experimental points is in error since the slopes of the other plots are not nearly as large.

(d) Comparison of Measured Viscosity and Mean Shear Rate Values of Galactomannan Solutions with the Power Law Equation.

(i) Log Shear Stress/Log Shear Rate Plots:

The Power Law was shown earlier to be expressed by:

$$\tau = K\,G^{a} \qquad \text{(III-6)}$$

A plot of $\log_{10}\tau$ against $\log_{10}G$ should therefore give a straight line of slope 'a' and intercept $\log_{10}K$ if the Power Law is obeyed.

The viscosity/shear rate data of the galactomannan solutions was plotted in this form by using equation (III-2) to obtain the value of τ.

$$\tau = \eta G \qquad \text{(III-2)}$$

Because η and G are the mean viscosity and mean shear rate respectively, τ represents the mean shear stress applied to the solutions.

A least squares line was fitted to each plot so that K and 'a' values could be obtained, and a correlation coefficient (r) was also calculated for the ten pairs of points on each plot. Results appear in table (III-11).

The high correlation coefficients obtained for all concentrations of Soybean and Lotus pedunculatus I and II galactomannans show a good fit of their data to the Power Law equation over the experimentally accessible range of shear rate values. For Guar, Carob, Sophora japonica, Red Clover and Lucerne the viscosity/shear rate data follows the Power Law

Table (III-11)

Comparison of the Viscosity and Mean Shear Rate Values of Galactomannans with the Power Law Relationship ($\tau = KG^a$)

Galactomannan	Concentration (g/100 ml)	Coefficient(a) of $\text{Log}_{10}\bar{G}$	Constant ($\text{Log}_{10}K$)	Correlation Coefficient
Carob	0.126	0.866	0.889	1.000
	0.196	0.838	1.22	1.000
	0.351	0.728	1.97	1.000
	0.538	0.659	2.62	0.997
	0.631	0.679	2.82	0.997
	0.875	0.610	3.41	0.992
Guar	0.084	0.866	0.839	1.000
	0.201	0.787	1.37	1.000
	0.343	0.641	2.20	0.999
	0.524	0.499	3.03	0.995
	0.631	0.570	3.12	0.997
	0.828	0.431	3.79	0.978
Lotus pedunculatus I	0.105	0.922	0.250	1.000
	0.184	0.972	0.128	0.995
	0.333	0.945	0.200	0.999
	0.525	0.932	0.253	1.000
	0.659	0.959	0.179	0.995
	0.908	0.941	0.210	0.999
Lotus pedunculatus II	0.159	0.956	0.342	1.000
	0.314	0.952	0.540	1.000
	0.498	0.940	0.795	1.000
	0.608	0.942	0.927	1.000

(to be cont.)

Table (III-11)
(cont)

Galactomannan	Concentration (g/100 ml)	Coefficient (a) of $\mathrm{Log}_{10}\bar{G}$	Constant ($\mathrm{Log}_{10}K$)	Correlation Coefficient
Lotus pedunculatus II (cont)	0.757	0.924	1.13	1.000
	0.804	0.920	1.23	1.000
Lucerne	0.083	0.955	0.359	1.000
	0.134	0.902	0.645	0.999
	0.254	0.879	1.10	1.000
	0.484	0.825	1.55	1.000
	0.596	0.787	1.82	1.000
	0.732	0.780	2.05	1.000
Red Clover	0.050	0.933	0.373	1.000
	0.095	0.904	0.649	1.000
	0.180	0.835	1.01	0.995
	0.317	0.829	1.33	1.000
	0.368	0.806	1.56	1.000
	0.422	0.679	2.33	0.999
Sophora japonica	0.079	0.921	0.434	1.000
	0.142	0.902	0.683	1.000
	0.331	0.787	1.49	1.000
	0.500	0.706	2.09	1.000
	0.591	0.662	2.39	0.999
	0.785	0.622	2.82	0.999

(to be cont)

Table (III-11)
(cont)

Galactomannan	Concentration (g/100 ml)	Coefficient(a) of $\text{Log}_{10}\bar{G}$	Constant ($\text{Log}_{10}K$)	Correlation Coefficient
Soybean	0.078	0.917	0.304	1.000
	0.153	0.890	0.423	0.997
	0.354	0.943	0.361	1.000
	0.507	0.924	0.480	0.999
	0.626	0.931	0.505	0.999
	0.788	0.932	0.574	1.000

relationship closely at low concentrations, but deviations become evident as the galactomannan concentration increases. At these higher concentration levels the plots of $\log_{10}\bar{\tau}$ against $\log_{10}\bar{G}$ become non-linear at low values of $\bar{G}$.

The fit of all the galactomannan data is still good, however, except for the highest concentration of Guar galactomannan.

From a comparison of the Power Law equation (III-6) with the equation defining viscosity (III-2) it is evident that a solution showing Newtonian behaviour constitutes a special case of the Power Law, with the exponent 'a' equal to one. Consequently the more Newtonian the behaviour of the galactomannan solutions, the closer their values of 'a' would be expected to be to one. An examination of the values of 'a' in table (III-11) shows that Lotus pedunculatus I and II and Soybean galactomannans do not deviate greatly from Newtonian behaviour; solutions of Lucerne, Red Clover and Sophora japonica galactomannans deviate more, and those of Guar and Carob deviate most.

(ii) <u>Log Viscosity/Log Shear Rate Plots</u>:

These plots were made to amplify the non-linearity of the $\log_{10}\bar{\tau}$ / $\log_{10}\bar{G}$ plots mentioned above. As normally $\bar{G} > \eta$, the deviations from linearity caused by η are less evident in the product

$$\bar{\tau} = \eta\bar{G} \qquad \text{(III-2)}$$

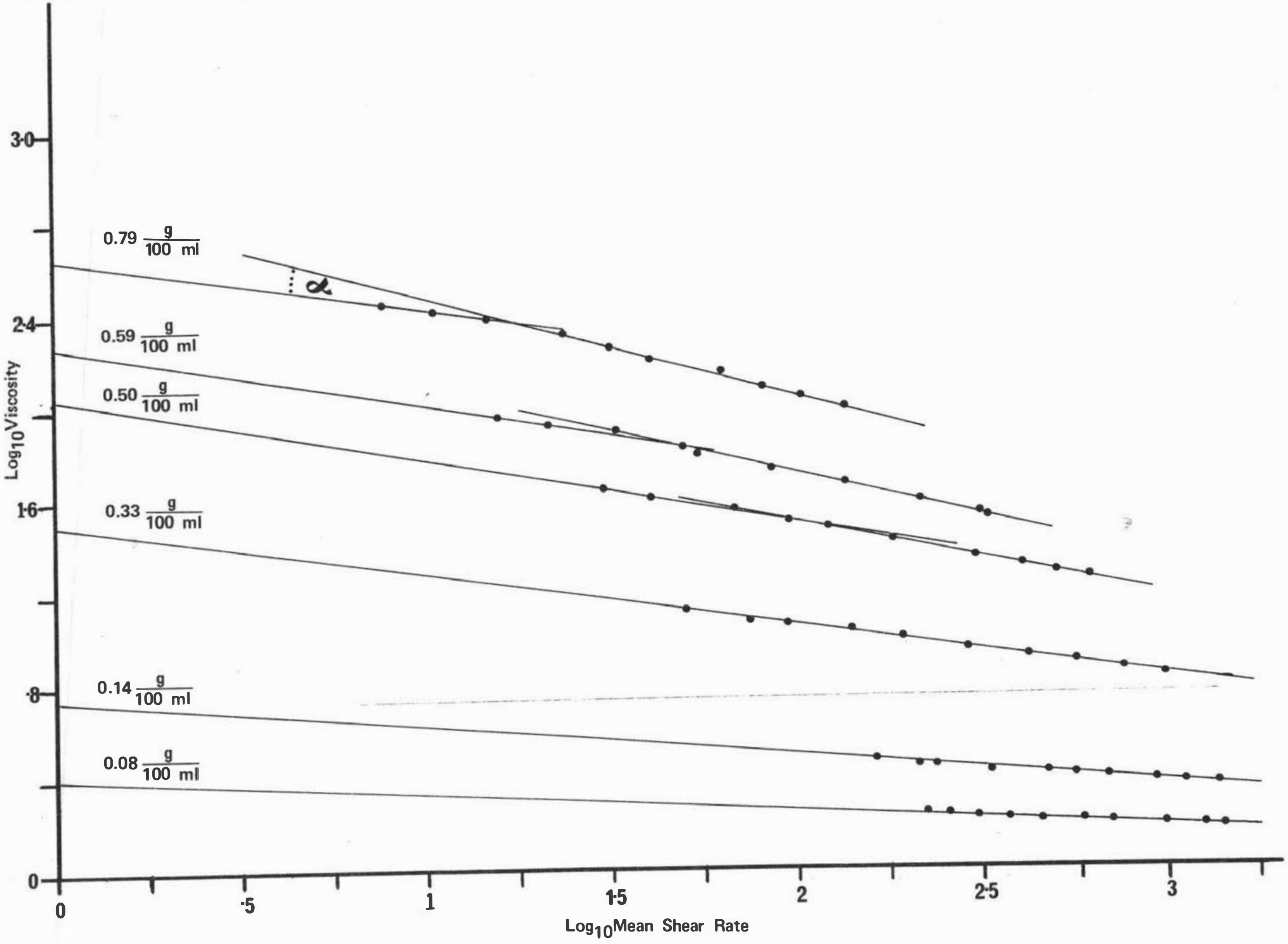
If the Power Law is obeyed, then the form of the Power Law connecting η and $\bar{G}$ (equation (III-7)) predicts that a plot of $\log_{10}\eta$ against

$$\eta = K\bar{G}^{(a-1)} \qquad \text{(III-7)}$$

$\log_{10}\bar{G}$ will be linear with slope (a-1) and intercept K.

The viscosity/mean shear rate data for all the galactomannans was accordingly plotted in this form. A typical plot for Sophora japonica is shown in fig. (III-17). As would be expected, at all concentrations

Figure (III–17) :— Log–Log Plot of Viscosity against Mean Shear Rate for Sophora japonica Galactomannan



of Lotus pedunculatus I and II and Soybean galactomannans a linear relationship between $\log_{10} \eta$ and $\log_{10} \bar{G}$ was found. A linear relationship was also observed in the case of the remaining galactomannans below a concentration of about 0.5% (see fig. (III-17)). Above this concentration however, the plots of Guar, Carob, Sophora japonica, Lucerne and Red Clover galactomannans consisted of a second linear portion of differing slope to the first (see fig. (III-17)). The linear portion of the graph between $\log_{10}\bar{G} = 0$ and the intersection point was less steep (i.e. $\log_{10} \eta$ decreased less with increasing $\log_{10}\bar{G}$) than the linear portion after the intersection point.

The intersection point occurred at successively lower values of $\bar{G}$ with successive increases in concentration of galactomannan. Additionally, not only do the slopes of the two parts of each line increase with increasing concentration, but the difference in slope between them (which can conveniently be expressed in terms of the acute angle α between them,) also increases.

The effect observed above could be attributed to a change in the mechanism of structural breakdown in the galactomannan solutions caused by increasing the shear rate. One type of breakdown could have been completed at the intersection point of the plots, after which a different type commences. This idea is in line with Schutz's (1970) suggestion that there is a successive breakdown of solute aggregates with increasing shear rate (see P. 27 of this thesis). There are two possible reasons for the effect not being observed at lower galactomannan concentrations. One is that the change in the mechanism occurs at higher shear rates than were used in the experiments. The second, more likely reason, is indicated by the decrease in the angle between the two linear portions (α) of the plots with decreasing galactomannan concentration. This suggests that as the concentration decreases, the difference between the types of structural breakdown eventually becomes so small that it cannot be observed.

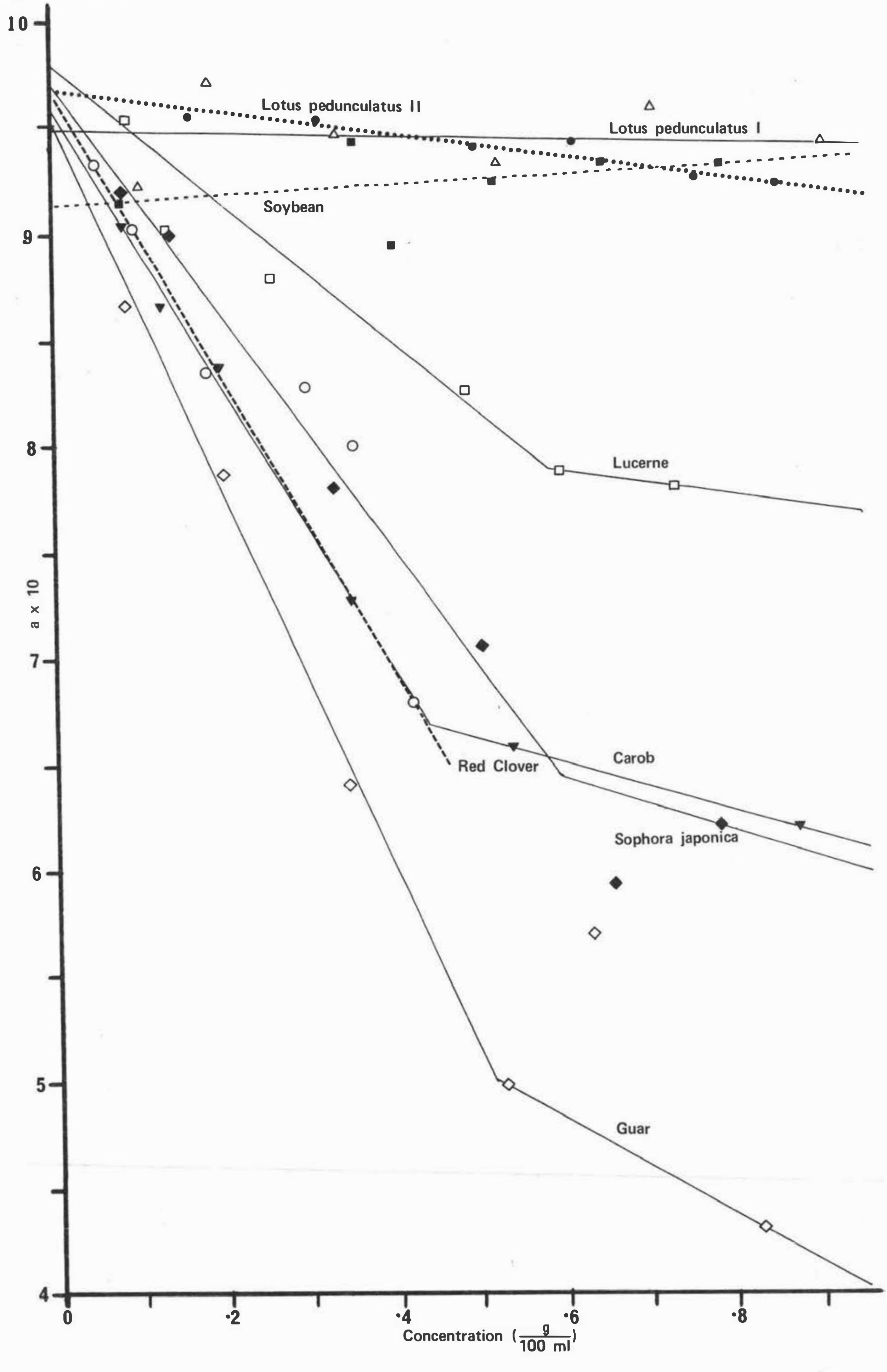
(iii) Plot of Power Law Exponent 'a' Against Galactomannan Solution Concentration:

In the introduction to this section on viscometry, the change of the Power Law exponent 'a' with concentration as determined empirically by Schutz (1970) was discussed. He found that 'a' changed over wide ranges of concentration (decreasing with increasing concentration), and was a function of the structure of the system. If a change in the structure of the galactomannan system is causing the bilinearity of the $\log_{10}\eta/\log_{10}\overline{G}$ plots, then a plot of 'a' against solution concentration should be non-linear above a galactomannan concentration of 0.5%, the concentration at which the bilinearity of the plots becomes noticeable. Plots of 'a' against galactomannan solution concentration appear in fig. (III-18).

In all cases except that of Soybean galactomannan 'a' was either constant or decreased with increasing concentration of galactomannan as predicted by Schutz. The exception in the case of Soybean may be due to experimental error, as the curve is almost parallel to the concentration axis. The magnitude of the Soybean galactomannan viscosity, similar to that of Lotus pedunculatus I, would have been expected to yield a similar linear plot of constant 'a'.

Lotus pedunculatus I and II and Soybean galactomannans, which had linear $\log_{10}\eta/\log_{10}\overline{G}$ plots, also had simple linear plots of 'a' against concentration. With the exception of Red Clover, the remaining galactomannans have bilinear plots, with the intersection point of the two linear portions occurring at solution concentrations between 0.45 and 0.65%. Although plots of $\log_{10}\eta/\log_{10}\overline{G}$ for Red Clover galactomannan solutions showed a slight bilinearity at higher concentrations (0.35 - 0.45%), a

Figure (III–18):— Plot of Power Law Exponent "a" against Galactomannan Solution Concentration



change in the slope of the graph of 'a' against concentration does not become apparent in this region. From the magnitude of the initial slope of the plot of 'a' against concentration, which is similar to that of Guar, Carob, Lucerne and Sophora japonica galactomannans, a change in slope would be expected, but at higher concentrations of Red Clover galactomannan than were used in the present experiments.

The approximate linearity of the bilinear plots of 'a' against concentration above the point of intersection must be regarded as fortuitous, as at this stage 'a' is obtained from an average line fitted over both linear regions of the plot of $\log_{10}\eta$ against $\log_{10}\bar{G}$.

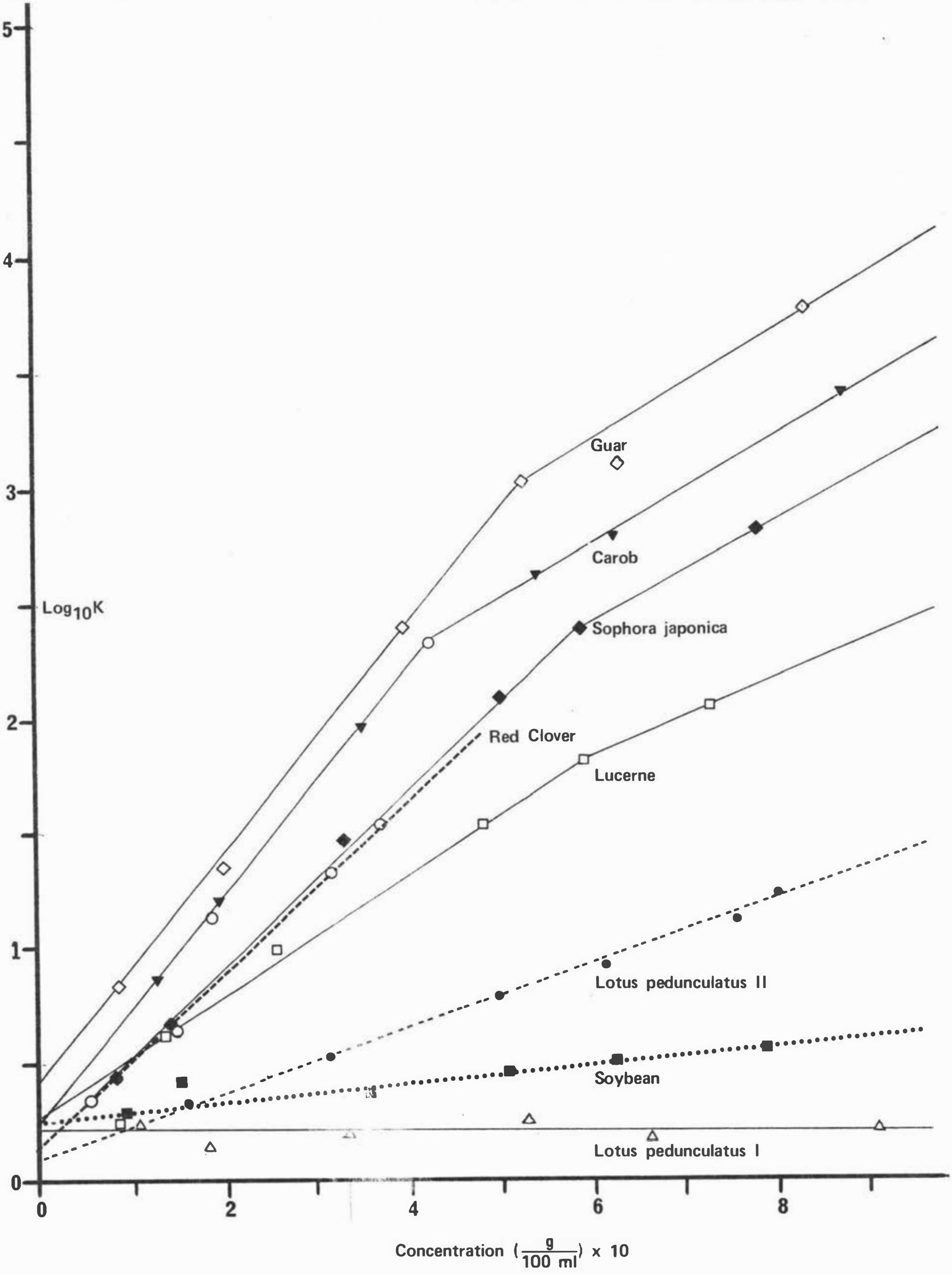
It would appear that the conclusion that the change in the slopes of the $\log_{10}\eta/\log_{10}\bar{G}$ plots is due to a change in the structure of the galactomannan solution is valid, based on Schutz's deduction of the structural dependence of 'a'. This is despite the averaging effect on 'a' outlined above which would render it less sensitive to changes in solution structure than if the two portions of a bilinear plot were considered separately.

(iv) Plot of Power Law Constant, K, Against Galactomannan Solution Concentration:

The premise of Schutz's remaining to be tested is that the Power Law constant K follows a constant dependence on concentration for a constant value of the Power Law exponent 'a'. If this is so, then a plot of $\log_{10}K$ (since $K = K_c e^{bc}$; K_c,b are constants (III-10)) against concentration should be linear where 'a' is constant. A plot of $\log_{10}$ K against concentration for all the galactomannans is shown in fig. (III-19).

The plots are found to be linear over the same regions of concentration as the plots of 'a' against concentration were. Where bilinear plots

Figure (III–19):– Logarithm of the Power Law Constant (K) Plotted against Galactomannan Concentration



occur, the intersection of the linear portions is found at the same value of concentration on both the $\log_{10}K$/concentration and 'a'/concentration plots.

For the case of galactomannans in solution it is therefore necessary to modify Schutz's (1970) statement of the dependence of K upon 'a'. This modification takes the form that equation (III-10) holds where a plot of 'a' against concentration has constant slope. Where the slope of a plot of 'a' against concentration changes, the values of K_c and b in equation (III-10) will also change.

(e) Calculation of the Intrinsic Viscosity of Galactomannans at $(\bar{G}=1)$.

(i) Definition of Intrinsic Viscosity:

For a Newtonian fluid, such as water, which flows slowly along a tube without obstacles, the flow pattern observed is essentially that of streamline, or laminar, flow. The viscosity of the liquid is constant and defined by:

$$\eta = \frac{\bar{\tau}}{\bar{G}} \tag{III-2}$$

When solid particles much larger than the solvent molecules, but still much smaller than the dimensions of the experimental apparatus (such as a capillary tube) are suspended in a liquid, the viscosity on a microscopic scale remains the same (i.e. the viscosity of the solvent still governs the frictional force between adjacent volume elements). If any method of macroscopic measurement of viscosity is used, however, a change in the measurement is observed. This change arises from the distortion of the pattern of flow, caused by a suspended particle much larger than a solvent molecule stretching across a number of flow lines (Tanford, 1961, P.333).

Einstein (1906) calculated the effect of suspended spherical particles and he concluded that, for any number of such particles suspended far enough apart as to prevent overlap of the distortion of the flow lines produced by each individual particle, the macroscopic viscosity of the suspension (η) is given by:

$$\eta = \eta_s(1 + 2.5\phi) \tag{III-44}$$

(where η_s is the viscosity of the solvent, and ϕ the volume fraction of the particles in the suspension.)

If Einstein's treatment is extended to assymetric particles, then it can be shown that the formal viscosity depends on the orientation of the particles. Orientation parallel to the streamlines (as would be the case at large velocity gradients) would give a smaller viscosity increment than if the particles were randomly oriented so that some of them were perpendicular to the streamlines.

At sufficiently low viscosity gradients the fluid is hardly moving, and the contribution to the total viscosity from the particles distorting the streamlines becomes very small. This means that the effect of particle orientation will be negligible. For this situation, Simha (1940) extended the Einstein treatment to include particles which are ellipsoids of revolution. He obtained

$$\eta = \eta_s (1 + \gamma\phi) \tag{III-45}$$

(where γ, which in Einstein's equation is 2.5 for suspended spheres, is larger than this for ellipsoids.)

Rearrangement of the above equation gives:

$$\frac{\eta - \eta_s}{\eta_s} = \gamma\phi \tag{III-46}$$

The left hand side of equation (III-46) is defined as the specific viscosity, η_{sp}. The volume fraction of the solute, ϕ, will be proportional to the concentration, c, of the solute molecules in solution, and thus η_{sp} will be concentration dependent. To remove this effect, the term intrinsic viscosity, $[\eta]$, is defined by:

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} \qquad \text{(III-47)}$$

The intrinsic viscosity represents the capacity of the solute to enhance the viscosity of the system (Flory, 1953, P.308). Intrinsic viscosity is normally calculated by measuring η_{sp} at a number of concentrations and extrapolating to zero concentration.

The effect of orientation of assymetric particles in the velocity gradient on viscosity has been shown to be finite unless very low velocity gradients are employed. For such non-Newtonian cases it is therefore desirable to eliminate this effect, and this is achieved by extrapolating a plot of viscosity against shear rate back to zero shear rate (i.e. zero velocity gradient) to obtain the viscosity at zero shear rate. This value is substituted back into equation (III-46), and the series of specific viscosities used to calculate the intrinsic viscosity as previously outlined.

(ii) Calculation of Galactomannan Intrinsic Viscosities:

Unfortunately the plots of galactomannan solution viscosity against mean shear rate are difficult to extrapolate at low values of $\overline{G}$ (e.g. figs. (III-14), and (III-15)), and therefore it was impossible to obtain viscosity values at $\overline{G} = 0$. From the linear form of the dependence of $\log_{10}\eta$ on $\log_{10}\overline{G}$ however, it is possible to obtain values of viscosity when $\log_{10}\overline{G} = 0$ (i.e. when $\overline{G} = 1$). The resulting viscosity values were used in the calculation of galactomannan intrinsic viscosities. As was found for the Ubbelohde viscometers, it was necessary to use the Martin equation:

$$\log_e \frac{\eta_{sp}}{c} = \log_e [\eta] + k'[\eta]c \qquad \text{(III-39)}$$

but in the form

$$\log_{10} \frac{\eta_{sp}}{c} = \log_{10} [\eta] + \frac{k'[\eta]c}{2.303} \qquad \text{(III-48)}$$

for ease of calculation.

Graphs of $\log_{10}(\frac{\eta sp}{c})$ were extrapolated to zero concentration in order to obtain $[\eta]$. The curved plots for Soybean and Lotus pedunculatus I galactomannans were extrapolated as described earlier (P. 58). The value of intrinsic viscosity for each galactomannan is given in table (III-12).

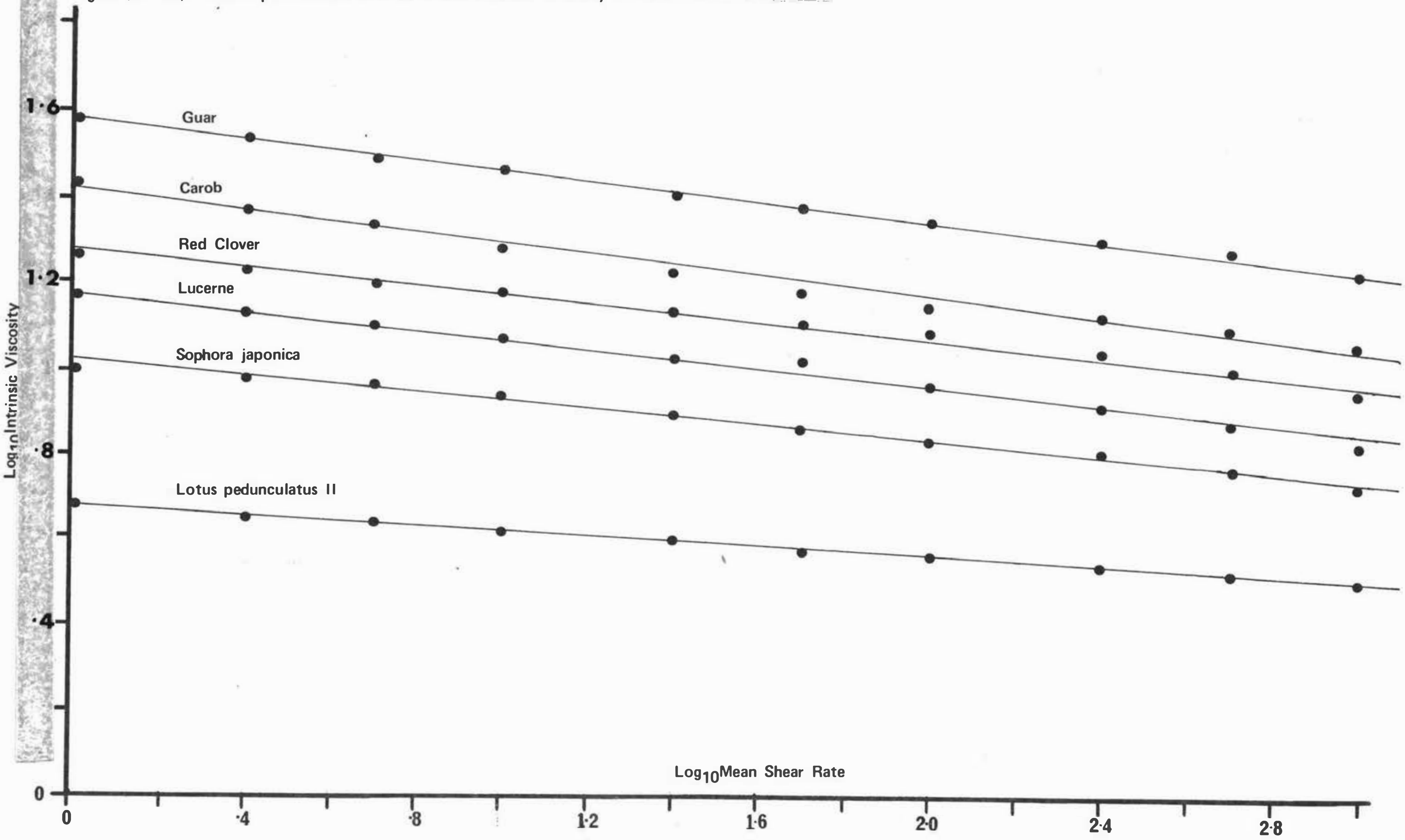
Table (III-12)

Values of $[\eta]_o$ and D for $\log_{10}[\eta] = \log_{10}[\eta]_o\ (1-D \log_{10}\bar{G})$

Galactomannan	$[\eta]_o$ Units = $\frac{dl}{g}$ ($\bar{G}$=1)	D
Guar	38.5	0.076
Carob	27.1	0.091
Lotus pedunculatus I	0.69	-
Lotus pedunculatus II	4.74	0.087
Lucerne	14.7	0.089
Red Clover	19.2	0.054
Sophora japonica	10.3	0.094
Soybean	1.76	-

From this table the value of $[\eta]_o$ can be seen to follow the order Guar > Carob > Red Clover > Lucerne > Sophora japonica > Lotus pedunculatus II > Soybean > Lotus pedunculatus I. Again, as earlier found in the Ubbelohde viscometers (P. 58), the order of intrinsic viscosity value Guar > Carob is as predicted by Hui and Neukom (1964), with $[\eta]_o$ for Lotus pedunculatus II now slightly below the value of 5 dl/g found by Beveridge (1965). The intrinsic viscosity of Soybean galactomannan is still low as predicted by Whistler and Saarnio (1957).

Figure (III–20):– The Dependence of Galactomannan Intrinsic Viscosity on Mean Shear Rate



The values of galactomannan intrinsic viscosity (at 20°C) are lower when calculated with data obtained with the Ubbelohde viscometers than those obtained from the Cannon-Fenske viscometers, except for Lotus pedunculatus II galactomannan. The general difference between the Ubbelohde viscometer and Cannon-Fenske viscometer results can be explained by the fact that the measurements in the Ubbelohde viscometers were carried out at finite shear rates and not extrapolated to $\bar{G} = 1$.

The reason for the different behaviour of Lotus pedunculatus II galactomannan was that two batches of material were used, as the first batch was lost between measurements on the different viscometers. The mannose:galactose ratio of both batches was the same, but the viscosity of the first was higher than the second. It would thus appear that the second sample is of lower molecular weight (and hence lower viscosity) than the first.

(f) The Dependence of Galactomannan Intrinsic Viscosity on Mean Shear Rate.

Values of $[\eta]$ were determined at successively greater values of $\bar{G}$ by obtaining values of viscosity for a given $\bar{G}$, and extrapolating the resulting $\log_{10} \frac{\eta_{sp}}{c}$ against concentration plot to zero concentration. In this way the effect of mean shear rate on the intrinsic viscosity was determined for each galactomannan solution. Plots of $\log_{10} [\eta]$ against $\log_{10}\bar{G}$ for all the galactomannans except those of Soybean and Lotus pedunculatus appear in fig. (III-20). Data for the latter two becomes complex at values of $\bar{G} > 1$.

Although Vink (1971) states that the dependence of intrinsic viscosity on rate of shear may be expressed by:

$$[\eta] = [\eta]_o + a_1 G + a_2 G^2 + \ldots\ldots \qquad \text{(III-49)}$$

(where $[\eta]_o$ is the intrinsic viscosity at zero rate of shear) and that in most cases this simplified to:

$$[\eta] = [\eta]_o(1 - BG) \qquad \text{(III-50)}$$

this relationship was not found to hold for galactomannans in solution. Instead, the linear nature of the plot of $\log_{10}[\eta]$ against $\log_{10}\bar{G}$ shows that a relationship of the form:

$$\log_{10}[\eta] = \log_{10}[\eta]_o(1 - D\log_{10}\bar{G}) \qquad \text{(III-51)}$$

(where D is a constant)

is valid. The form of equation (III-51) could possibly be inferred from the dependence of $\log_{10}\eta$ on $\log_{10}\bar{G}$. The equations of the straight lines in fig. (III-20) are given in table (III-12), along with the values of $[\eta]_o$ at mean shear rate $\bar{G}$=1 sec^{-1}.

SECTION IV : THE EFFECT OF ADDITIVES (IONIC AND NON-IONIC) ON THE VISCOSITY OF GALACTOMANNAN SOLUTIONS

A. Introduction

As was noted in the introduction to this thesis, the galactomannans of Guar and Carob are widely employed in industry, especially the food industry. Under normal conditions of use, these two galactomannans would contain varying types of additive to enhance flavour, colouring etc. Two widely employed such additives are sucrose and sodium chloride, which can be respectively categorized as non-ionic and ionic in solution character.

It was considered desirable to investigate the effects of these two common additives on the viscosity of galactomannan solutions, and also to determine whether these effects were the same for all galactomannans.

B. Experimental

(1) Determination of the Stability of Stored Galactomannan Solutions Containing Sucrose or Sodium Chloride

Previous workers (Carlson et. al. (1962); Carlson and Zeigenfuss (1965); Kassem and Mattha (1969) have noted that the viscosity of Guar galactomannan solutions containing additives changes with time.

In order to compare the effects of the additives on the different galactomannan solutions under identical conditions, it was necessary to examine the change in viscosity with time of a galactomannan solution containing either sucrose or sodium chloride.

A fresh solution of guar galactomannan (0.5g/100ml) was prepared and its final concentration determined as described elsewhere (see page 39). The viscosity of the solution was measured in the normal manner in a Cannon-Fenske viscometer at 20°C. Sufficient sucrose (A.R.) was added to give a final concentration of 25% by weight in the final solution. (This was the mid-point of the concentration range of added sucrose to be studied). The change in viscosity of the solution was measured at hourly intervals at 20°C. In addition, a sample was held in the refrigerator and its viscosity at 20°C was measured every 24 hours. The results are plotted in fig (IV-1)

To the same initial solution of guar galactomannan was added sufficient sodium chloride (A.R.) to give a final concentration of 15% by weight. (Again this was the mid-point of the concentration range to be studied).

The viscosity of this solution was determined under the same conditions as sucrose above, i.e. hourly at 20°C and also 24 hourly at 20°C after refrigerated storage.

The change in viscosity at hourly intervals is shown graphically in

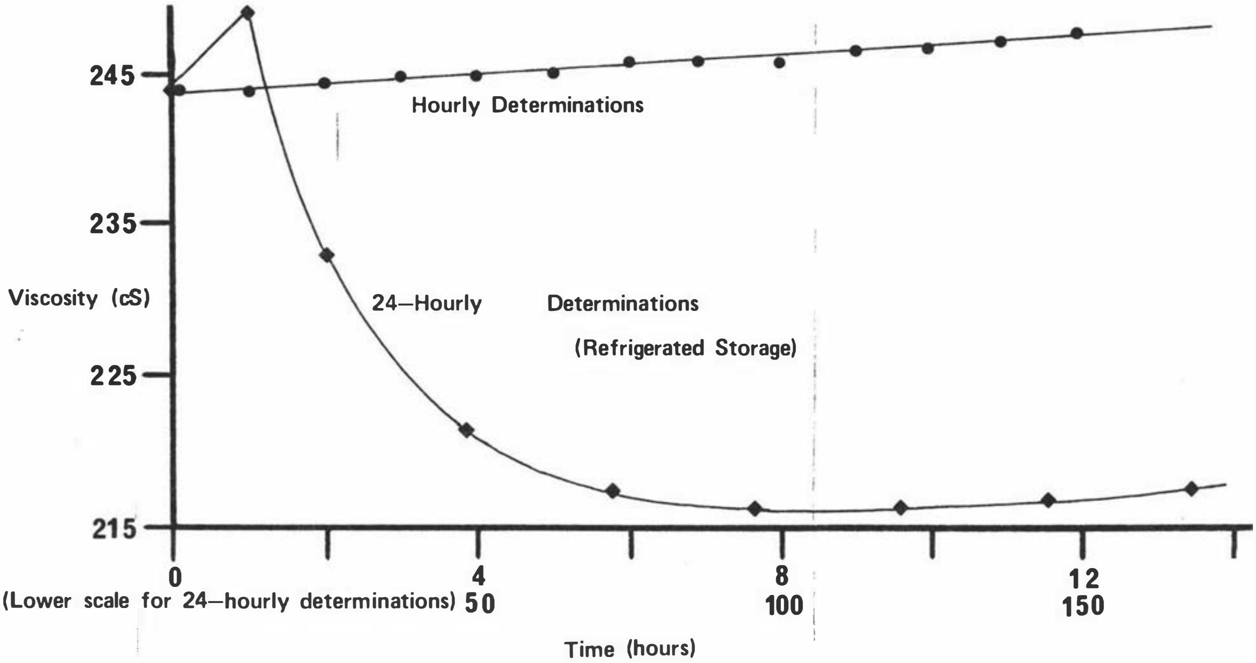


Figure (IV–1) :– The Effect of Aging on the Viscosity of a Guar Galactomannan containing Sucrose

Guar (0.39 $\frac{g}{100\ ml}$) + Sucrose ($\frac{25\,g}{100\ ml}$ galactomannan solution)

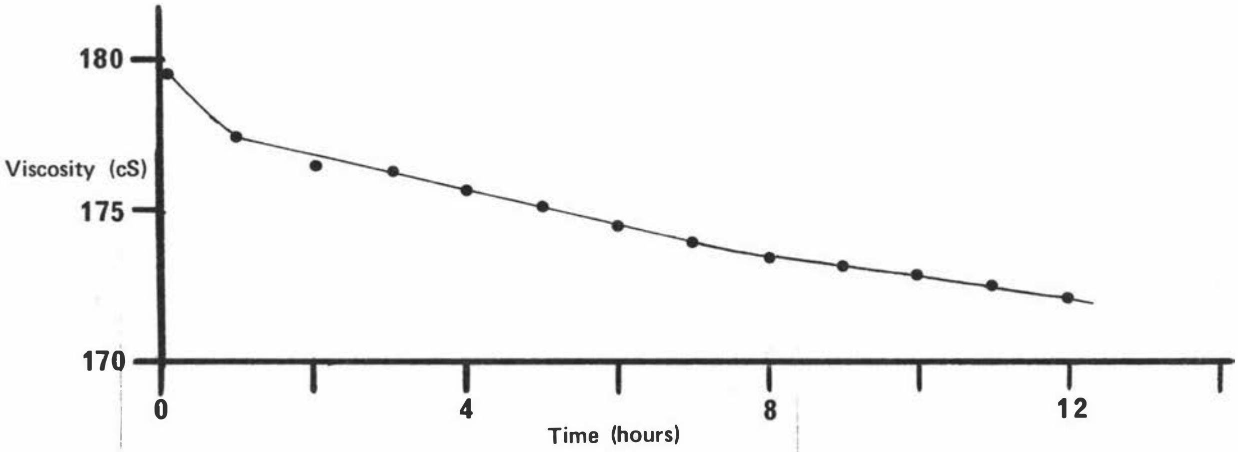


Figure (IV–2) :– Effect of Aging on the Viscosity of a Guar Galactomannan Solution containing Sodium Chloride

Guar (0.39 $\frac{g}{100\ ml}$) + Sodium Chloride ($\frac{15\ g}{100\ g}$ galactomannan solution) fig (IV-2). Those measured at 24-hour intervals decreased very steadily, and the results are not shown graphically.

(2) Measurement of the Effects on Galactomannan Solution Viscosity of Different Concentrations of Added Sucrose or Sodium Chloride

The effects of storage on solution viscosity determined in the preceding section and shown in figs (IV-1) and (IV-2) led to the adoption of the following procedure.

(i) The galactomannan was dissolved in water as previously described (page 39) to give a solution containing approximately 0.5 g / ml.

(ii) This solution was stored under refrigeration until required (no longer than 72 hours).

(iii) For sucrose added to galactomannan solutions:-

The aging at 20°C of the guar galactomannan containing sucrose is shown in fig (IV-1). As the change in viscosity with time is minimal between three and six hours after the sucrose was added, it was decided to measure all the experimental viscosities of galactomannan solutions containing sucrose in this region of the time / viscosity curve.

Three hours before the viscosity measurements were due to be made, sufficient sucrose to give a final concentration of 1, 10, 20, 30, 40 or 50% by weight was added to the galactomannan solution (12.5g). The suspension was shaken in a mechanical shaker until the sucrose dissolved, and then degassed at the vacuum pump. The resulting solution was kept at room temperature until required.

(iv) For sodium chloride added to galactomannan solutions:-

The aging at 20°C of guar galactomannan solution containing sodium chloride is shown in fig (IV-2). The decrease in viscosity is uniform between one and seven hours after adding the sodium chloride to the galactomannan solution. Accordingly, the first section of this portion of the aging curve was where all the experimental viscosity determinations were carried out.

One hour before the viscosity measurements were due to be made, sufficient sodium chloride to give a final concentration of 5, 10, 15, 20 or 25% was added to the galactomannan solution (12.5g). This suspension was treated as for sucrose.

The addition of solid sodium chloride or sucrose (rather than solutions) to the galactomannan solutions was desirable in order to keep the amount of water in the system constant.

Only one concentration of each galactomannan was used for each concentration of additive.

All measurements were carried out at 20°C ± 0.02°C using the Cannon-Fenske viscometers and applied external pressure. One viscometer only was used for each galactomannan / additive series.

Viscometry determinations were carried out over a small mean shear rate range so that viscosity values for each series of solutions could be obtained at a common rate of shear by interpolation. Solution densities were measured by the method already described (page 44), and viscosities calculated from equation (III-30)

$$\eta = A\ (hodo + hede)\ t \qquad \text{(III-30)}$$

C. Results

(1) Presentation

The results are presented in Appendix VI. Typical plots appear in fig (IV-3), for the effect on viscosity of the addition of sucrose to Red Clover galactomannan solution and fig (IV-4), for the effect on viscosity of the addition of sodium chloride to Sophora japonica galactomannan solution. In both the above plots, the following common features may be noted:-

(a) The additive changes the viscosity of the galactomannan solution, reducing it slightly for low concentrations of additive and increasing it very considerably for higher concentrations of additive, except for solutions of very low initial viscosity such as Lotus pedunculatus I galactomannan. In the latter cases, a viscosity increase only is noted.

(b) The curves are parallel at lower concentrations of additive, and only become markedly non-parallel at high concentrations of added sucrose or sodium chloride.

(2) Discussion of Results

(a) Examination of the Effect of Additive Concentration on each Galactomannan at Constant Shear Rate.

On the plots in figures (IV-3) and IV-4), one value of mean shear rate was chosen, and the corresponding viscosity for each concentration of additive was obtained. The effect of each additive was then calculated by plotting the relative viscosity (η_r),

where $\eta_r = \dfrac{\eta}{\eta_o}$ (IV-1)

η = viscosity of galactomannan solution plus additive.

η_o = viscosity of galactomannan solution without additive

against the additive concentration (%, w/w) in the final solution. These plots are presented in fig (IV-5 a, b and c) for added sucrose, and fig

Figure (IV–3):– Effect of Varying Added Sucrose Concentration on the Viscosity of Red Clover Galactomannan Solution (0.57 $\frac{g}{100\ ml}$)

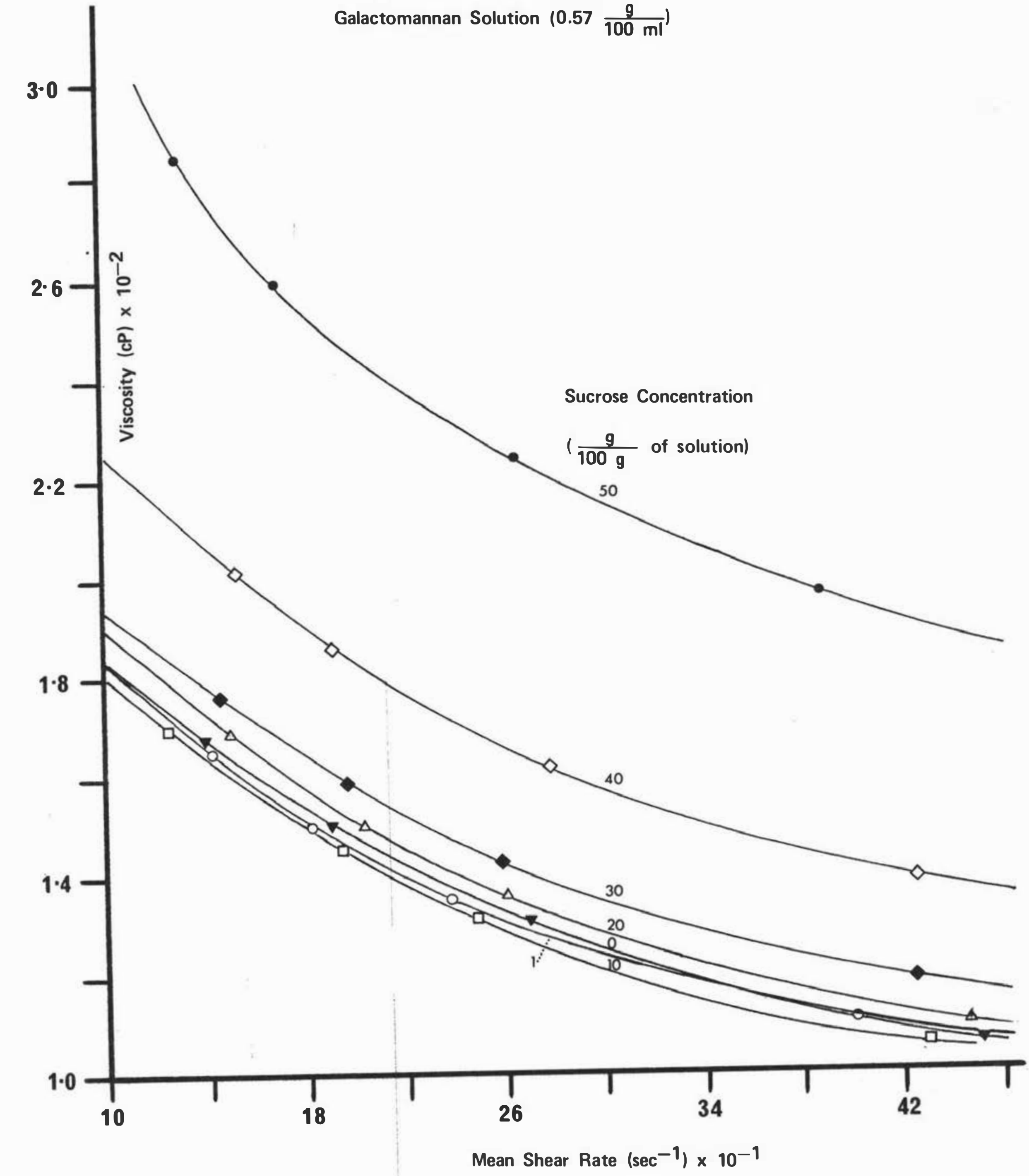
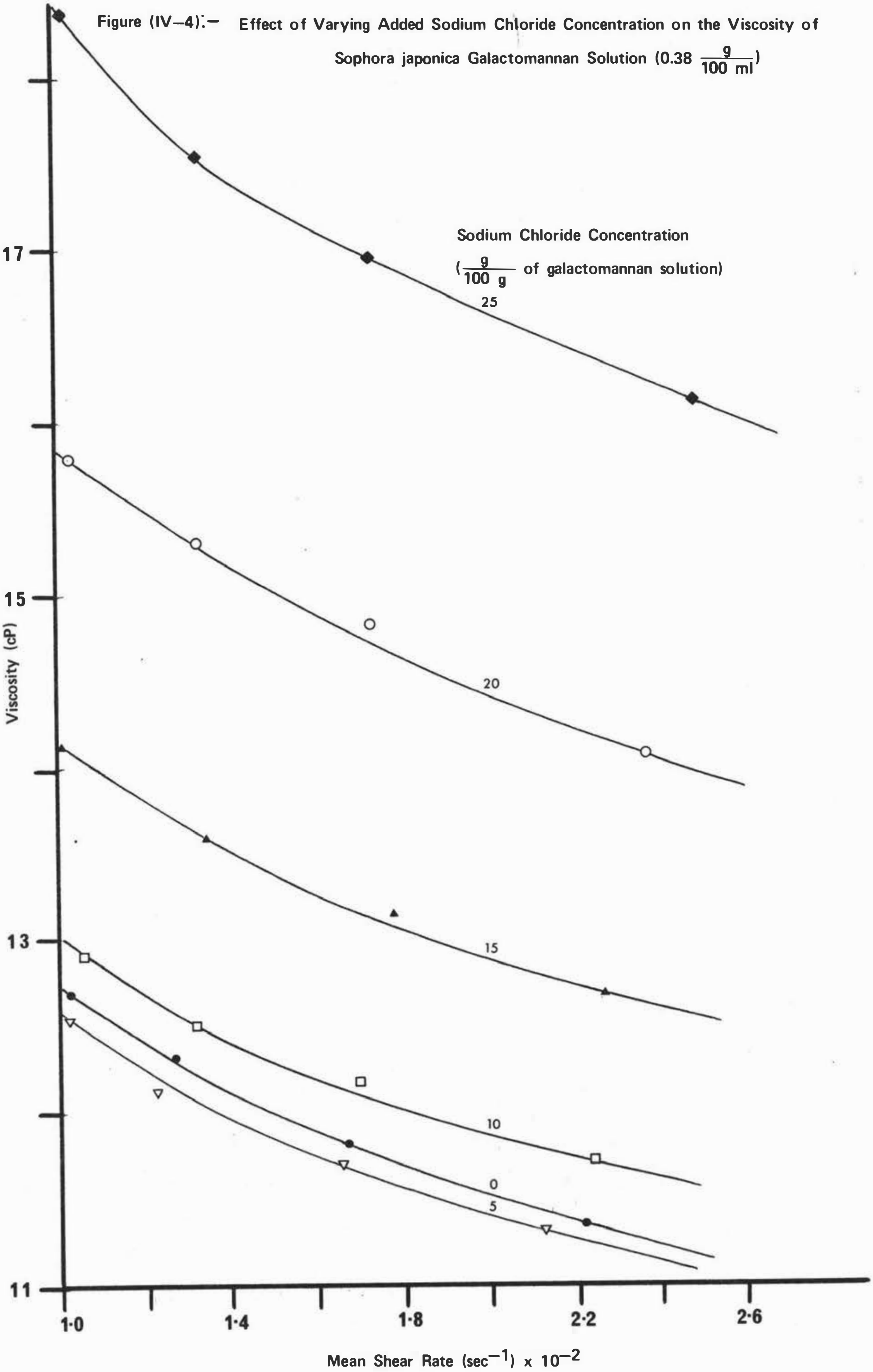


Figure (IV–4):– Effect of Varying Added Sodium Chloride Concentration on the Viscosity of Sophora japonica Galactomannan Solution (0.38 $\frac{g}{100\ ml}$)



(IV-6) a, b and c for added sodium chloride.

(i) Sucrose Solutions: The plots of η_r against concentration of Lotus pedunculatus I and II and Soybean galactomannans (fig ~~VI~~ IV-6a) show increasing viscosity with increasing concentration of added sucrose.

The plots for the remaining galactomannans (fig IV-6 b and c) show a decrease in viscosity on the initial addition of sucrose, followed by an increase in viscosity upon the addition of further sucrose. It is noteworthy that the galactomannan solutions which do not exhibit an initial decrease in viscosity on the addition of sucrose are those which have very low viscosities. These low viscosities are in fact of the same order as the viscosities of pure aqueous sucrose solutions, so that it is possible that in these cases the initial decrease in viscosity of the galactomannan solution is concealed by the actual viscosity of the sucrose solutions.

The relative effect of sucrose on galactomannan solution viscosity (at a level of 50% additive) decreases in the order Lotus pedunculatus I > Soybean > Lotus pedunculatus II > Lucerne > Sophora japonica > Red Clover > Carob > Guar. In the case of Guar galactomannan solutions the addition of sucrose changed the viscosity only very slightly until high concentrations of sucrose were reached.

(ii) Sodium Chloride Solutions: The plots of η_r against concentration of sodium chloride for all the galactomannans except that of Lotus pedunculatus I show the same features as were observed on the addition of sucrose, namely an initial decrease in viscosity followed by a subsequent increase at higher concentrations of additive.

As the viscosity of the added sodium chloride is comparable with that of Lotus pedunculatus I galactomannan solution, it would appear that again any initial decrease in the viscosity of the solution is being swamped by the viscosity of the additive.

Figure (IV–5):-Variation of the Relative Viscosity of Galactomannan Solutions with the Concentration of Added Sucrose

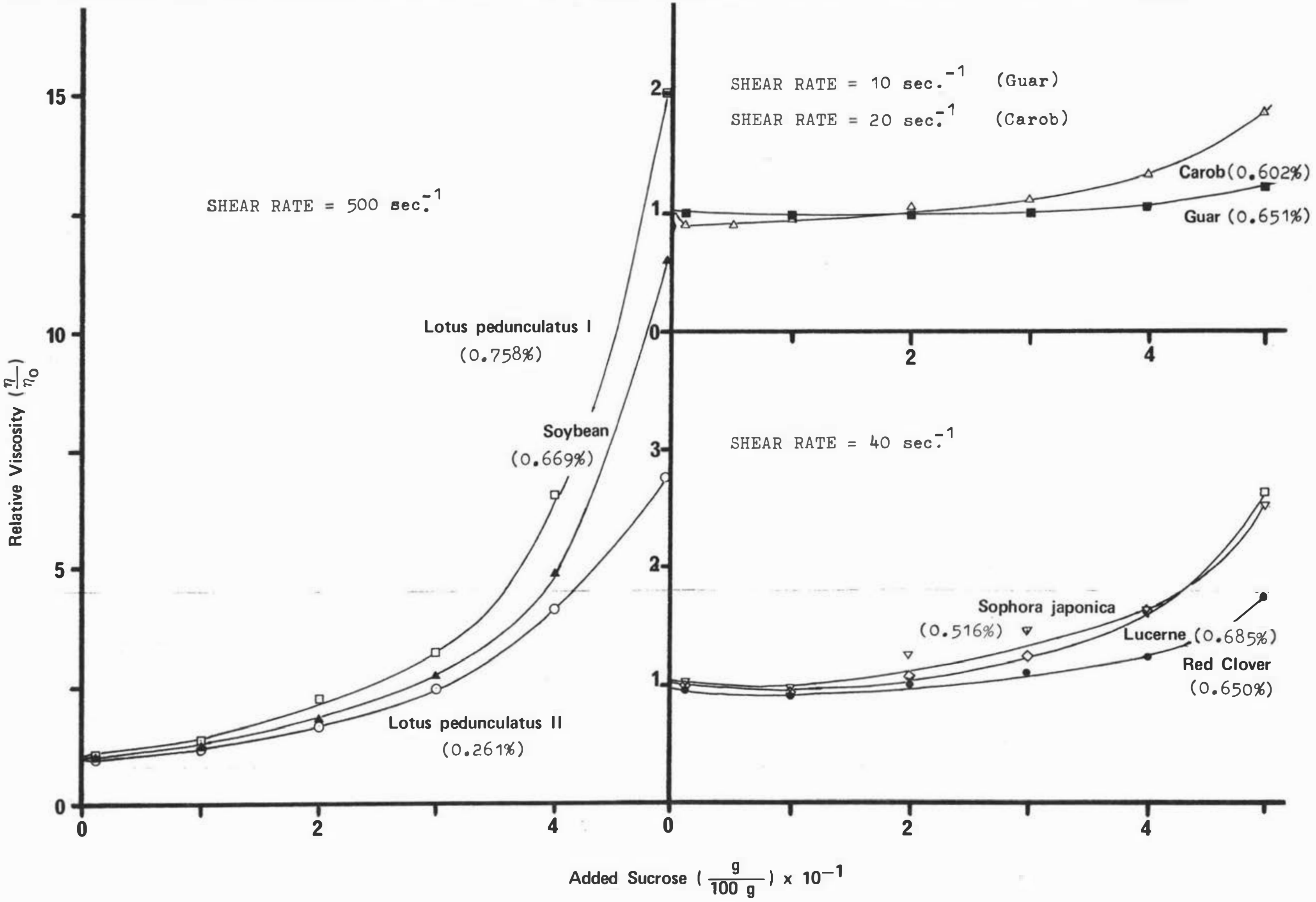
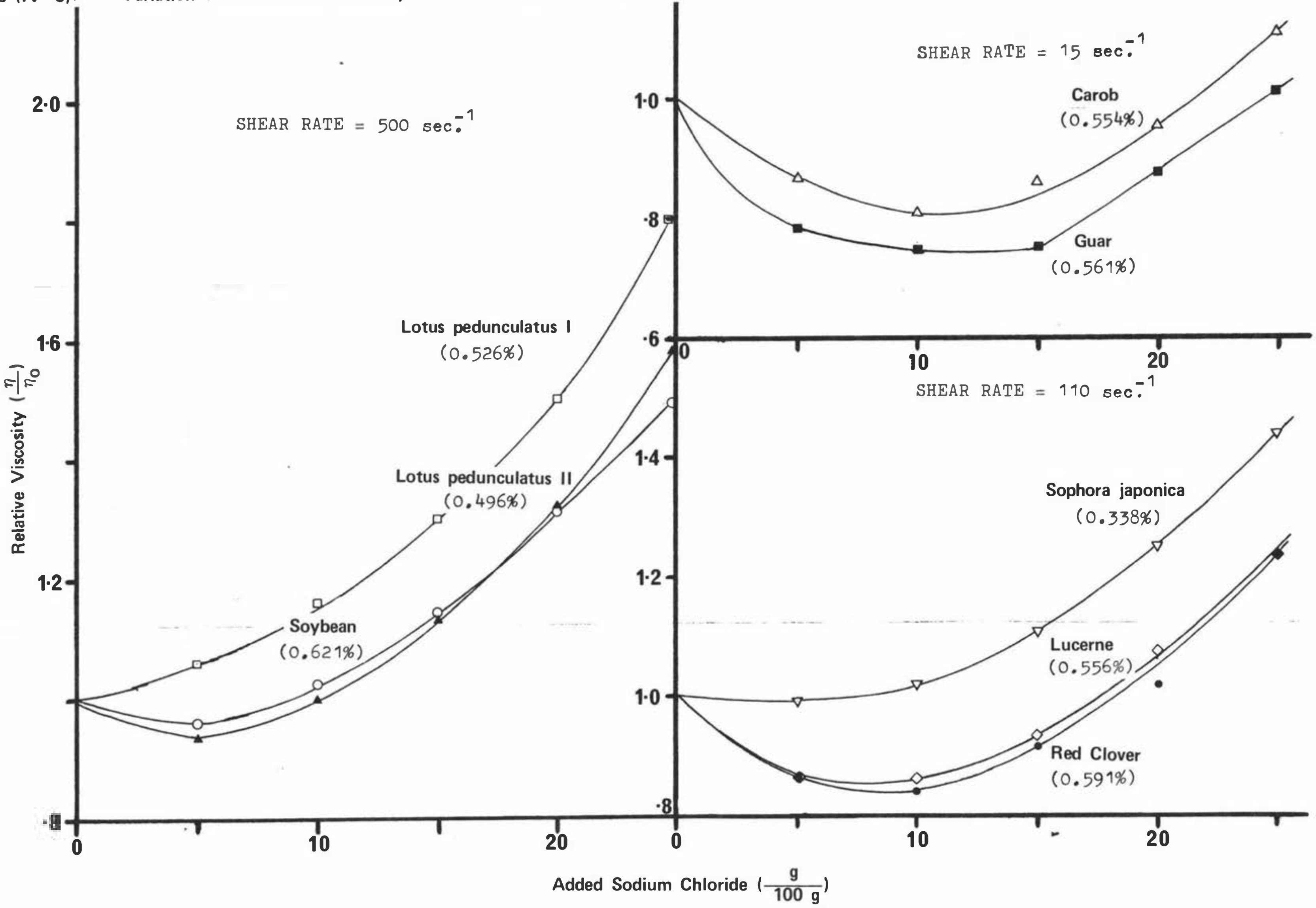


Figure (IV–6):– Variation of the Relative Viscosity of Galactomannan Solutions with the Concentration of Added Sodium Chloride.



The relative effect of sodium chloride on galactomannan solution viscosity (at a level of 25% additive) decreases in the order Lotus pedunculatus I > Lotus peducnulatus II > Soybean > Sophora japonica > Lucerne > Red Clover > Carob > Guar. This order is almost identical to that observed for sucrose addition.

3. Interpretation of the Observed Effects

(a) Magnitude of the Effects

From the plots of η_r against concentration of additive in the previous section, it was noted that the order of the magnitude of viscosity change upon the addition of either sucrose or sodium chloride was almost identical.

The effect of the additive on solution viscosity can be correlated with the original viscosity of the galactomannan solution.

The viscosities of the solutions in the order of decreasing effect of sucrose on viscosity are:- 1.13 cP, 1.98 cP, 3.50 cP, 57.8 cP, 73.3 cP, 113 cP, 244 cP, 567 cP.

The viscosities of the solutions in order of decreasing effect of sodium chloride are 1.18 cP, 1.23 cP, 2.07 cP, 12.6 cP, 20.4 cP, 25.9 cP, 191 cP, 299 cP.

It is evident that the magnitude of the effect of either additive is dependent on the viscosity of the original galactomannan solution. The magnitude of the effect is also dependent on the viscosity of the additive in solution, since sucrose which has a higher pure aqueous solution viscosity than sodium chloride, had a greater effect on galactomannan solution viscosity.

(b) Proposed Mechanism of the Effects

There have been three earlier studies of the effects of additives on the viscosity of galactomannan solutions. In all cases experiments were carried out using Guar galactomannan.

The effect of sucrose in solution on the hydration of guar galactomannan was studied by Carlson and Zeigenfuss (1965), and a similar study of the effect of sodium chloride in solution on guar galactomannan hydration was carried out by Carlson et. al. (1962).

In these studies the galactomannan was hydrated in solutions containing varying concentrations of either sucrose or sodium chloride, and the effect of this on the hydration time and final viscosity of the solution was noted.

Carlson and Zeigenfuss found that successive increments of sucrose caused an increasing delay in the time of hydration of the galactomannan, and the resulting solution viscosities were successively lower than for the pure galactomannan solutions.

They interpreted this effect as competition between sucrose and the galactomannan for "available water" in the solution, since the normal galactomannan solution viscosity could be obtained by diluting the sucrose / galactomannan mixture with water.

Carlson et. al. (1962) found that hydration of guar galactomannan in sodium chloride solution resulted in a solution whose final viscosity increased with the concentration of sodium chloride. Since the galactomannan was hydrated in 100 mls of <u>solution</u> for each NaCl covered, the increased viscosity was attributed to decreased amount of water in the system.

Neither of the above results are similar to the effects described in this thesis for the galactomannans. It must be remembered, however, that Carlson's work involves the hydration of guar galactomannan in NaCl or sucrose solutions, and not the addition of sucrose or sodium chloride to an already hydrated galactomannan in solution. In Carlson's case, the hydration of the galactomannan is so slow that the ultimate equilibrium state may not have been attained in the time of the experiment.

Kassem and Mattha (1969) investigated the effect on guar galactomannan solution (0.8% w/w) of ethanol, propylene glycol, glycerol, and sorbitol. Ethanol and propylene glycol, at maximum additive concentrations of 10% and 20% respectively, precipitated the galactomannan, while glycerol and sorbitol were completely miscible with the guar galactomannan solution over the concentration range (5 - 40%). It appears that a larger number of hydroxyl groups on the additive molecule ensures better miscibility with the solution.

It was noted by Kassem and Mattha that the guar galactomannan solutions showed an initial decrease in viscosity when a small amount of an alcohol was added, followed by an increase when the concentration of additive was increased. These effects are the same as were observed in the present work for the addition of sucrose, which can be considered as an alcohol.

Just before precipitation occurred (for ethanol and propylene glycol), a decrease in viscosity was again seen.

Kassem and Mattha also investigated the effects of the addition of sodium, calcium or aluminium chlorides to a guar galactomannan solution (0.8% w/w).

The effect was similar to that of sucrose, with an initial lowering of viscosity at a low concentration of additive followed by an increase at higher additive concentrations. The magnitude of the effect was very similar for sodium and aluminium chlorides and was much greater for calcium chloride.

To interpret the effect of both the alcohols and the ionic additives on guar galactomannan viscosity, Kassem and Mattha compared the behaviour of guar galactomannan solutions on the addition of additives to that of methyl cellulose. They postulated that dehydrating agents, such as alcohols, or ionic salts initially induce dehydration of the molecules or aggregates of galactomannan. (This is supported by Carlson and Zeigenfuss'(1965) interpretation of the effect of sucrose on guar galactomannan hydration.)

The dehydrated units, at higher concentrations of the dehydrating agent, tend to aggregate into micelles, thereby increasing the relative viscosity of the solution. At still higher concentrations of the dehydrating agent, the micelles are dehydrated further, lowering the relative viscosity of the system again, and eventually with increasing concentration of dehydrating agent the micelles agglomerate and precipitate.

This interpretation of the effect appears to be a good one, combining as it does the idea of Schutz (1970) that the carbohydrates exist as aggregates in solution, with Carlson and Zeigenfuss' (1965) concept of competition for available water.

Presumably increasing concentrations of sucrose over 50% (w/w) should eventually precipitate the galactomannan from solution, however this could not be tested because of the practical difficult of dissolving sufficient sucrose in the galactomannan solution. The effect of sodium chloride was also limited by its solubility.

SECTION $\bar{V}$:

Molecular Weight Determinations

A. Introduction

a) Molecular Weight Averages

Unless polymers (except proteins) have been prepared or isolated under exceptional circumstances, they do not contain molecules having an identical degree of polymerisation. Instead, variations in the degree of polymerisation are found, giving rise to a distribution of molecular weights. The average molecular weight calculated for such a distribution depends on the statistical treatment used, and this in turn depends on the relationship between the measured physical quantity and the molecular weight.

The molecular weight averages normally used are defined below (Tanford (1961), p. 145):-

i) The number-average molecular weight is

$$\bar{M}_n = \frac{\sum N_i M_i}{\sum N_i} = \sum X_i M_i \qquad (\underline{\bar{V}}\text{-}1)$$

N_i = no. of molecules of type i in the mixture

M_i = molecular weight of molecules of type i.

X_i = mole fraction of number of molecules of type i.

$\bar{M}_n$ represents the total weight of the polymer sample divided by the total number of moles in the sample. It is sensitive to a small weight fraction of low molecular weight molecules, but insensitive to a similar fraction of molecules of high molecular weight. (Flory (1953), p. 273). $\bar{M}_n$ is normally determined by colligative methods such as osmosis or cryoscopy.

ii) The weight-average molecular weight is

$$\bar{M}_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum X_i M_i^2}{\sum X_i M_i} \qquad (\underline{\bar{V}}\text{-}2)$$

$\bar{M}_w$ represents the sum of the weights of polymer i with molecular weight M_i divided by the total weight of polymer present. It is insensitive to a small weight fraction of low molecular weight, but sensitive to one of high molecular weight. (Flory (1953), p. 292). $\bar{M}_w$ may be determined by methods such as light-scattering or ultracentrifugation.

iii) Although the weight average and number average molecular weights are those most frequently encountered, higher averages have been defined, namely the Z- average, (Z + 1)- average etc. where

$$\bar{M}_z = \frac{\sum_i N_i M_i^3}{\sum_i N_i M_i^2} \qquad (V-3)$$

$$\bar{M}_{(z+1)} = \frac{\sum_i N_i M_i^4}{\sum_i N_i M_i^3} \qquad (V-4)$$

(Tanford (1961), p. 147).

iv) For a completely homogeneous sample of polymer, then

$$\bar{M}_n = \bar{M}_w = \bar{M}_z = \bar{M}_{(z+1)}$$

If the polymer is not homogeneous (as is usually the case), then

$$\bar{M}_n < \bar{M}_w < \bar{M}_z < \bar{M}_{(z+1)}$$

The ratio of $\frac{\bar{M}_w}{\bar{M}_n}$ is used as an index of polydispersity, the higher the value of $\frac{\bar{M}_w}{\bar{M}_n}$, the greater the polydispersity of the sample.

v) It is possible to define number-average and weight-average degrees of polymerisation analogous to (V-1) and (V-2) above, i.e. the number-average degree of polymerisation, $X_n = \sum_{x=1} xX_x$ (V-5) where X_x = mole fraction of molecules of degree of polymerisation x.

Similarly, the weight average degree of polymerisation is

$$X_w = \sum_{x=1} xW_x \qquad (V-6)$$

where W_x is the weight fraction of molecules of degree of polymerisation x.

MOLECULAR WEIGHTS

b) Ultracentrifuge Methods

i) General

The galactomannan molecular weights were determined using an analytical ultracentrifuge. There are a number of molecular weight determining methods available using this machine, among them are:-

(i) The Equilibrium Method.
(Schachman 1959, Bowen 1970)

(ii) The High Speed Equilibrium (or Yphantis) Method.
(Bowen 1970)

(iii) The Low Speed Equilibrium Method.
(Bowen 1970)

(iv) The Approach to Equilibrium Method (Archibald Method).
(Schachman 1959, Bowen 1970)

(v) The Sedimentation and iffusion (or Transport) Method.
(Schachman 1959, Bowen 1970)

The theoretical basis of methods (i), (iv) and (v) has been extensively covered by Fujita (1962). Method (i) was eliminated because of the long time involved in making measurements. Methods (ii) and (iii) were eliminated due to a fault in the interference optics of the ultracentrifuge used. Method (iv) was eliminated by low speed instability of the rotor. This left the Transport Method (v), which is based on Swedburg's (1925) equation:-

$$M = \frac{RTs}{D(1-\bar{v}p)} \qquad \text{(V-7)}$$

where R = Universal gas constant

T = Absolute temperature

s = Sedimentation coefficient of solute

D = Diffusion coefficient of solute

$\bar{v}$ = Partial specific volume of the solute

p = Density of the solvent.

Under normal conditions, when solute density is greater than that of the solvent, sedimentation is the process always favouring the movement of the solute to the bottom of the cell, while diffusion is an opposing process that attempts to redistribute the solute evenly through the cell. Thus sedimentation and diffusion work in opposition to each other, and the magnitude of the centrifugal field determines which is dominant.

The Svedburg equation can be derived in several different ways, each of which illustrates different features (Schachman 1959, p. 215 ff), but for ideal systems at zero concentration, each derivation gives the same result. Separate determinations of s, D and $\bar{v}$ are necessary, all under the same conditions.

Creeth and Pain (1967) qualified the use of sedimentation and diffusion data for the calculation of molecular weights. They assumed that the calculation would not be applied to solutes exhibiting gross heterogeneity, and then applied the transport method to mixed solutes as for single solutes. Only a single value of sedimentation or diffusion coefficient is obtained, even if the solute is polydisperse, but although these are each a well defined average for the whole non-redistributed system at the concentration used, on combination of these values the resulting molecular weight is not a simple average analagous to the number, weight, or Z-average molecular weights.

ii) <u>Calculation of Sedimentation Coefficients</u>

When the sedimentation peak in the Schlieren Diagram is nearly symmetrical, as it is for some polydisperse solutes (including galactomannans, (Kubal and Gralen, 1948)), the assumption that $r_p = r^*$ is still valid (where r_p is the distance of the maximum ordinate from the axis of rotation, and r^* is the square root of the second moment of the concentration gradient curve.)

r* is given by

$$r^* = \left[\int_{r_a}^{r_x} r^2 \left(\frac{dn}{dr}\right) dr \Bigg/ \int_{r_a}^{r_x} \left(\frac{dn}{dr}\right) dr \right]^{\frac{1}{2}} \qquad \text{(V-8)}$$

(Creeth and Pain, 1967).

where r_a is the distance of the upper limit of the solution column from the axis of rotation,

r_x is the distance of an arbitrary cylindrical surface in the cell from the axis of rotation.

$\frac{dn}{dr}$ is the change in refractive index gradient.

Sedimentation coefficients may therefore be calculated as for single solutes, i.e. as s_w (or weight-average) coefficients.

iii) Calculation of Diffusion Coefficients.

For diffusion coefficients the situation is more complicated. In polydisperse systems such as galactomannans in solution, different methods of calculation result in a different average of the diffusion values and hence different molecular weight averages. The height/area method (giving D_A, the apparent diffusion coefficient), and the second moment method (giving D_m, the weight-average diffusion coefficient) can be respectively obtained from

$$D_A = \frac{A^2}{4\pi t h^2} \qquad \text{(V-9)}$$

(Kubal & Gralen (1948)).

$$\text{and} \quad D_m = \frac{M_2}{2At} \qquad \text{(V-10)}$$

where A = area under the diffusion curve (Schlieren optics)

h = maximum height of the curve

t = time (seconds) elapsed.

M_2 = the second moment of the curve about the vertical axis through its arithmetic mean.

The value of D_A lies between the weight- and number-average diffusion coefficient values for a polydisperse system, whereas D_m should correspond to the weight-average value, i.e. D_m should have an equal or greater value than D_A. The value of the ratio $\frac{D_m}{D_A}$ can be used as a qualitative indication of polydispersity (Henley, 1962).

iv) Combination of Sedimentation and Diffusion Data

If the value of D_A is substituted into the Svedburg equation (equation V-7), then the average molecular weight thus obtained is denoted as $\bar{M}_{s,D}$ while if D_m is substituted, the weight-weight average molecular weight $\bar{M}_{w,w}$ is obtained.

Jullander (1945) showed that $\bar{M}_n < \bar{M}_{s,D} < \bar{M}_w$, with the actual position of $\bar{M}_{s,D}$ dependent on the molecular weight distribution. Similarly, Henley (1962) set the boundaries $\bar{M}_n < \bar{M}_{w,w} < \bar{M}_w$. Since by definition $D_A < D_m$, then

$$\bar{M}_n < \bar{M}_{s,D} < \bar{M}_{w,w} < \bar{M}_w \qquad \text{(V-11)}$$

The difference between $\bar{M}_{w,w}$ and $\bar{M}_{s,D}$ will not be great however, unless the molecular distribution is broad. As Henley has also stated that $\bar{M}_{w,w}$ normally lies closer to $\bar{M}_w$ than $\bar{M}_n$, this would also be expected to apply to $\bar{M}_{s,D}$.

B: EXPERIMENTAL

a) Materials

i) Phosphate Buffer (0.025 mol/l): KH_2PO_4 (3.40g) and Na_2HPO_4 (3.55g) (previously dried for two hours at 120°C) dissolved in deionised distilled water (1l). Final pH 6.88-6.92 (Vogel, 1961).

ii) Galactomannan: All samples purified by normal isolation method.

iii) Photographic Plates: Ilford G.30 chromatic backed 5 x 25.4 cm thin glass plates. Normal exposure time was 10-15 seconds.

b) Methods

Preparation of Solutions)

The galactomannan (0.5g, finely divided) was suspended in phosphate buffer (0.025 mol/l, exactly 50 mls) according to Beveridge (1965), and the suspension stirred in a waterbath (70°C) for 90 minutes, cooled, and filtered (sintered glass). The volume of the solution was adjusted to exactly 50.0 mls by the addition of distilled deionised water. The solution was dialysed overnight against a large excess of 0.025 mol/l phosphate buffer and portions of the approximately 1% solution (with respect to galactomannan) diluted (by weight (after Kawahara, 1969)) with the buffer to give solutions (10 ml) containing between 0.1% and 0.7% galactomannan.

The real concentration was determined by freeze drying known weights of the approximately 1% galactomannan solution in duplicate, weighing the freezedried material and subtracting the KH_2PO_4 and Na_2HPO_4 content. All solutions were stored in the refrigerator when not in use.

C. SEDIMENTATION COEFFICIENT DETERMINATIONS

i) Theoretical

The properties of the sedimentation coefficient, s, have been extensively described by Schachman (1959). It is the velocity of the solute molecules divided by the centrifugal field. If x is the distance of the maximum peak ordinate from the centre of rotation and w the angular velocity, then sedimentation coefficient is given by

$$s = \frac{dx/dt}{xw^2} = \frac{d \log_e x}{w^2 \, dt} \qquad \text{(V-12)}$$

provided s is independent of x, and w is constant.

Hence a plot of the logarithm of the distance x, against time should give a straight line from the slope of which s (in seconds) can be obtained. This value of s is corrected to a standard temperature and solvent. The sedimentation coefficient must also be corrected for concentration effects, as in general s decreases with increasing solute concentration. A plot of s against concentration (c) can be expressed over a small concentration range by either

$$s = \frac{s^0}{1+k_s c} \qquad \text{(V-13)}$$

$$\text{or} \quad s = s^0 \, (1-K_s c) \qquad \text{(V-14)}$$

(Schachman 1959, Bowen 1970).

where K_s, are constants.

This plot is extrapolated to zero concentration to obtain s_o.

Because it is impossible entirely to free any biological colloid of small ions by dialysis it is necessary to carry out the sedimentation and diffusion analyses in a swamping excess of neutral electrolyte to neutralise any effect from these small ions. It is usually assumed that the solvent plus added salts can be considered as a single component.

ii) Experimental Methods

Most of the sedimentation velocity runs were carried out at 20.0°C in 12 mm single or double sector cells in an A.N.D. rotor at 59,780 r.p.m.

For two dilute ($<$ 0.1%) samples of guar galactomannan 30 mm single sector cells were used in an A.N.J. rotor at 50,740 r.p.m.

In order to determine the zero time, the first photograph of a run was taken when the centrifuge rotor had reached two-thirds the desired operating speed. Successive photographs were then taken at regular intervals, with the rotor running at full speed. Photographs were enlarged, and traced onto graph paper for measurement. A typical sedimentation Schlieren Pattern appears in fig. (V-1a). This was interpreted as follows:-

The distance (x_e) between the reference edges was measured. This distance had previously been measured on the Schlieren counterbalance and a series of photographic plates using a travelling microscope, and found to be 1.594 ± 0.004 cm for the counterbalance, and 3.024 ± 0.004 cm on the photographic plates. The ratio $\frac{X_e}{1.594}$ gave the magnification factor between the cell and the enlargement on graph paper.

The values x, x', x'' (representing the distance of the maximum ordinate of the refractive index gradient from the inner reference edge at times t, t', and t'' minutes) were measured, and corrected using the magnification factor. The distance between the inner reference edge and the air/solvent meniscus was checked for each exposure to ensure that no leakage from the cell had occurred.

A graph was plotted of log (x + 5.72) (where 5.72 cm is the distance between the inner reference edge and the axis of rotation at 59,780 r.p.m.) against time t (minutes). This gave a straight line from which s (the sedimentation coefficient) was obtained from equation (V-12).

The sedimentation coefficient is usually expressed in a corrected form, $s_{20,w}$, which is the value of the coefficient when pure water at 20°C is used as a solvent. This correction is made by substituting in the equation (Chervenka 1969):

$$s_{20,w} = s_{app} \left(\frac{\eta_T}{\eta_{20}}\right) \left(\frac{\eta}{\eta_o}\right) \left(\frac{1 - \bar{v}p_{20,w}}{1 - \bar{v}p_T}\right) \qquad (\underline{\bar{V}} - 15)$$

where s_{app} = apparent sedimentation coefficient at temperature T^o

η_T = viscosity of solvent at T^o

η_{20} = viscosity of solvent at 20^oC

η = viscosity of solvent at a specified temperature

η_o = viscosity of water at the same specified temperature

$\bar{v}$ = partial specific volume of the solute

$p_{20,w}$ = density of water at 20^oC

p_T = density of solvent at T^o

As all runs were carried out at 20^oC,

$$\eta_T = \eta_{20}, \quad p_T = p_{20}$$

substituting back into equation ($\underline{\bar{V}}$ - 15) gives:-

$$s_{20,w} = s_{app} \left(\frac{\eta}{\eta_o}\right) \left(\frac{1 - \bar{v}p_{20,w}}{1 - vp_{20}}\right) \qquad (\underline{\bar{V}} - 16)$$

The viscosity and density of water were obtained from physical data tables (Kaye and Laby, 1966). The density of the 0.025 mol/l phosphate buffer was measured using a 1000 µl micropipette as a pycnometer, and its viscosity measured at 20.0 ± 0.05°C in an Ubbelohde viscometer. Substituting these values back into Equation ($\underline{\bar{V}}$ - 16) gave:-

$$s_{20,w} = s_{app} \left(\frac{1.021}{1.0038}\right) \left(\frac{1-0.99821\,\bar{v}}{1-1.0059\,\bar{v}}\right) \qquad (\underline{\bar{V}} - 17)$$

The sedimentation coefficient at zero concentration (s_o) was obtained by plotting reciprocal $s_{20,w}$ values against concentration, according to equation ($\underline{\bar{V}}$ - 13), and extrapolating to zero concentration to give s_o.

Figure (V–1a):–Typical Schlieren Diagram Obtained During a Sedimentation Velocity Experiment

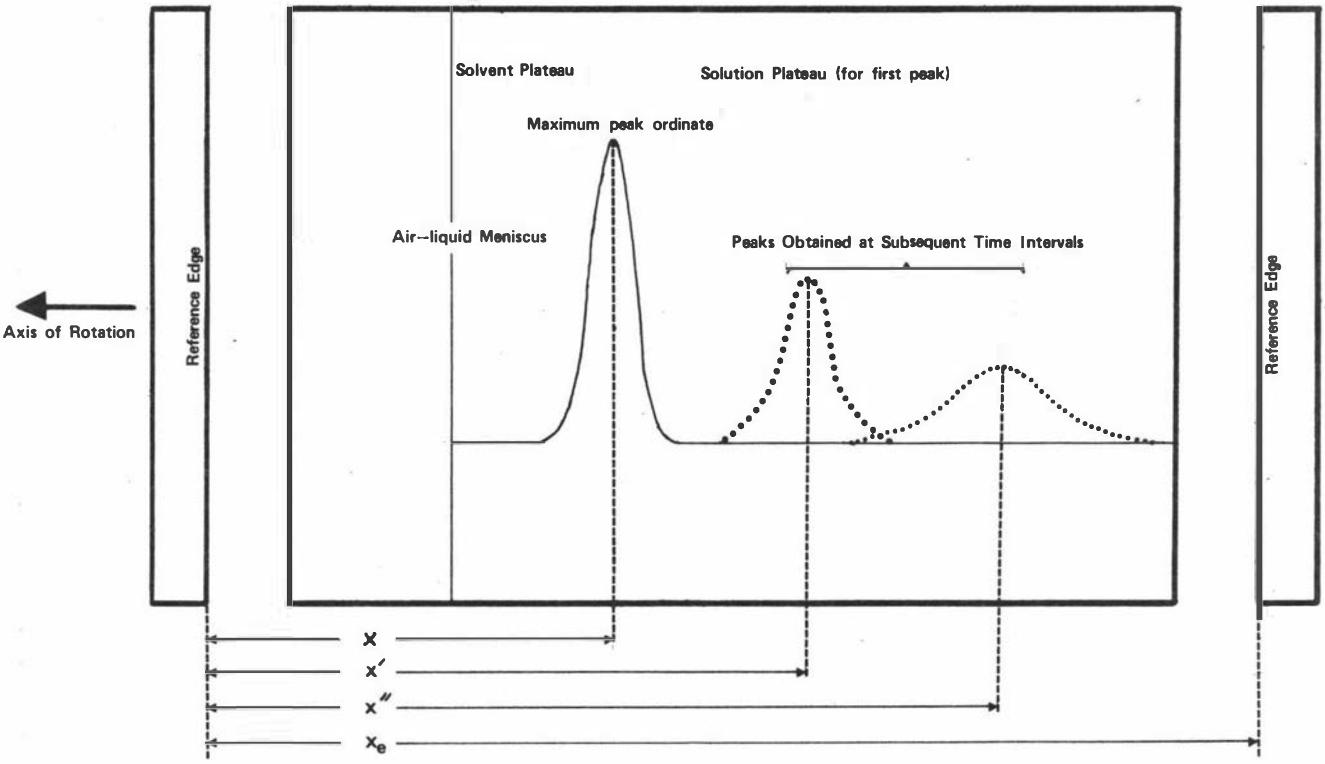
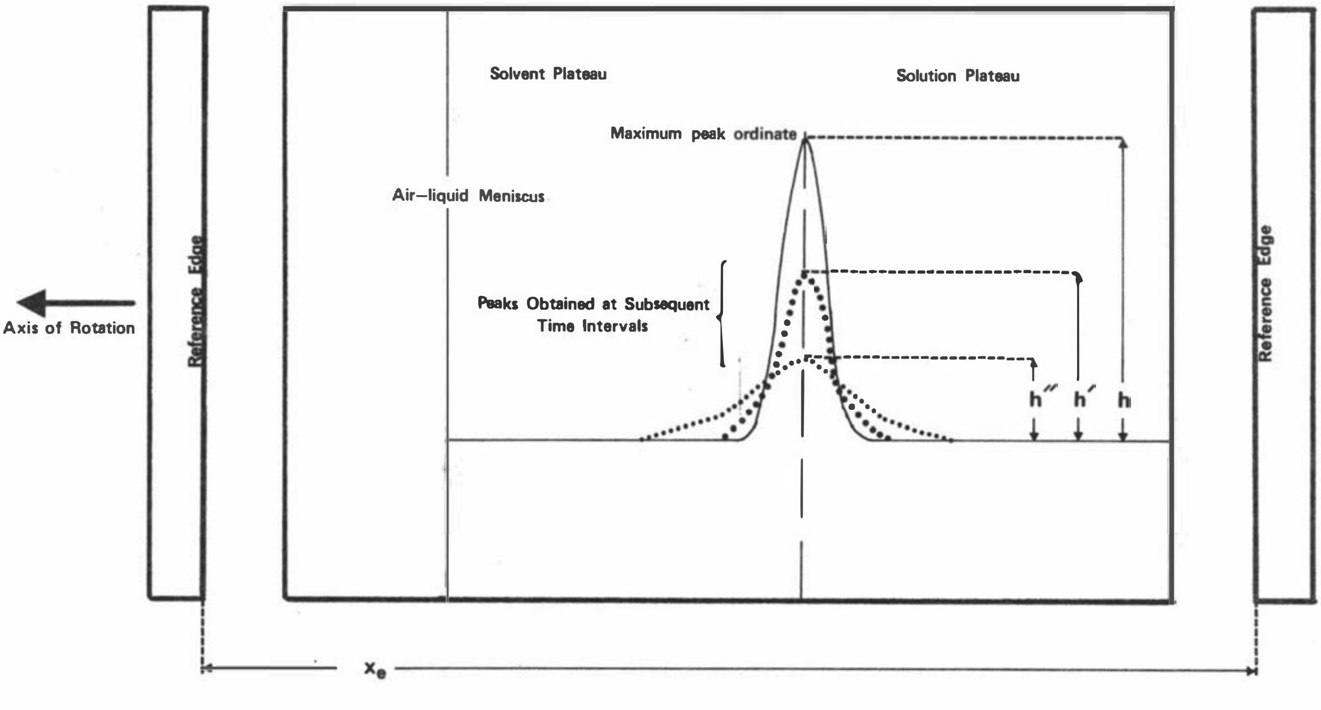


Figure (V–1b) :– Typical Schlieren Diagram Obtained During a Diffusion Experiment



d. DIFFUSION COEFFICIENT DETERMINATIONS

i) Theoretical

The two average diffusion coefficients (D_A and D_m) required were calculated using the formulae introduced earlier (p. 97).

$$D_A = \frac{A^2}{4\pi t h^2} \qquad (\bar{V} - 9)$$

and $$D_m = \frac{M_2}{2At} \qquad (\bar{V} - 10)$$

where A = area under the diffusion curve

h = maximum height of curve

t = time elapsed

M_2 = second moment of the curve about the vertical axis through its arithmetic mean.

Hence a plot of $\frac{A^2}{4\pi h^2}$ against time should give a straight line plot from which D_A can be calculated. A similar plot of $\frac{M_2}{2A}$ against time should yield D_m.

Diffusion coefficients, like sedimentation coefficients, must be corrected to a standard solvent and temperature (normally water at 20°C). The $D_{20,w}$ values thus obtained are then extrapolated to zero concentration to eliminate concentration effects and obtain D_o. This extrapolation normally follows the equation:-

$$D_g = D^o(1 - k_D c) \quad (k_D \text{ constant}) \quad (\bar{V} - 18)$$

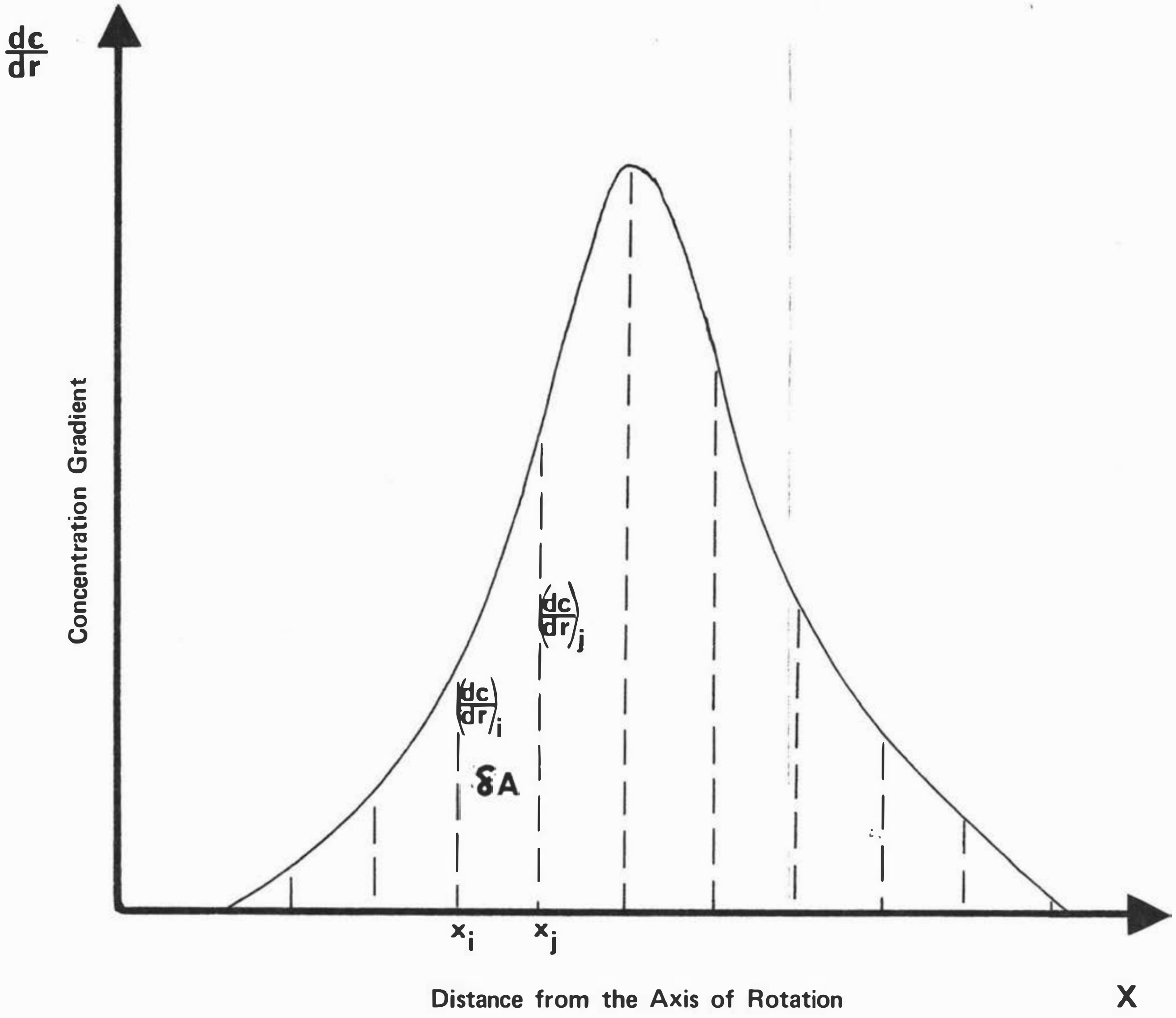
ii) Experimental Methods

Diffusion experiments were carried out in a synthetic boundary cell of the cup and depressable rubber valve type, using solutions prepared as for the sedimentation velocity experiments.

All experiments were at 20°C and at 12,590 r.p.m. (as at speeds much below this instability of the rotor made results difficult to interpret.) No sedimentation of the Schlieren peaks was detectable.

Photographs were taken at regular intervals after the formation of the boundary. They were enlarged, and traced onto graph paper for measurement

Figure (V–2) :– Calculation of the Area under the Diffusion Boundary



Area of one trapezoidal section under the curve

$$= \left(\frac{x_j - x_i}{2}\right) \left[\left(\frac{dc}{dr}\right)_i + \left(\frac{dc}{dr}\right)_j\right]$$

$$= \delta A$$

$\therefore$ Total Area = $\Sigma \delta A$

(allowing for magnification) similary to those from the sedimentation velocity experiments.

A typical Schlieren diffusion curve appears in fig. ($\overline{\underline{V}}$ - 16)

The <u>Apparent Diffusion Coefficient</u>, D_A, was calculated from

$$D_A = \frac{A^2}{h^2}\left(\frac{1}{4\pi t}\right) \qquad (\overline{\underline{V}} - 9)$$

The area, A, under the curve was measured by trapezoidal approximation while the maximum height, h, was measured directly. For any one experiment the area A under the curve should remain constant. The average area obtained from all the photographs (between 5 and 8 in number) was thus used to calculate D_A at any one concentration of galactomannan from the slope of a plot of $\frac{A^2}{h^2} \times \frac{1}{4\pi}$ against time t (in seconds).

The <u>Weight-Average Diffusion Coefficient</u>, D_m, was calculated from

$$D_m = \frac{M_2}{2At} \qquad (\overline{\underline{V}} - 10)$$

The total area under the diffusion curve was determined as above. The arithmetic mean of the curve ($\bar{x}$) was obtained by calculating

$$\bar{x} = \frac{\Sigma\, x\delta A}{\Sigma\, \delta A} \qquad (\overline{\underline{V}} - 19)$$

where x is the distance from the centre of the trapexoidal section of area (δA) under the diffusion curve to the origin (see fig. ($\overline{\underline{V}}$ - 2)). x is chosen to increase at uniform intervals.

The second moment of the diffusion curve was then calculated from

$$M_2 = \frac{\Sigma\, \delta A(x-\bar{x})^2}{\Sigma\, \delta A} - \left[\frac{\Sigma\, \delta A(x-\bar{x})}{\Sigma\, \delta A}\right]^2 - 0.083$$

(Chambers (1958), p. 29) ~~-0.083~~ ($\overline{\underline{V}}$ - 20)

For each series of exposures corresponding to one concentration of galactomannan, D_m was calculated from the slope of a plot of $\frac{M_2}{2A}$ against time (in seconds).

Corrections to D_A and D_m were applied as in the case of sedimentation coefficients, i.e. the diffusion coefficients were corrected to 20°C in pure water as solvent, according to:-

$$D_{20,w} = D_g \left(\frac{293.2}{T}\right) \left(\frac{\eta_t}{\eta_{20}}\right) \left(\frac{\eta_{sol}}{\eta_w}\right) \qquad (\underline{\overline{V}} - 21)$$

(Chervenka (1969))

where T = temperature, in degrees absolute

η_t = viscosity of solvent at T°A

η_{20} = viscosity of solvent at 20°C

η_{sol} = viscosity of solvent at a specified temperature

η_w = viscosity of water at the same temperature

D_g = Diffusion Coëfficient obtained from graph.

As all the diffusion experiments were carried out at 20°C, T = 293.2°A, and $\eta_t = \eta_{20}$ i.e. $D_{20,w} = D_g \left(\frac{\eta_{sol}}{\eta_w}\right)$ $(\underline{\overline{V}} - 22)$
substituting for 0.025M phosphate buffer gives

$$D_{20,w} = 1.017\ D_g \qquad (\underline{\overline{V}} - 23)$$

The $D_{20,w}$ values obtained were plotted against galactomannan concentration and extrapolated to zero to obtain D_o.

e. PARTIAL SPECIFIC VOLUME DETERMINATION

A micropipette (1000 μl) was used as a pycnometer. It was calibrated by filling it with freshly distilled water from an all glass apparatus, and weighing it on a Mettler H20 balance. The weight of water was corrected for air buoyancy and the volume of the micropipette at 20.0°C calculated from the appropriate tables (Kaye and Laby 1966). The volume of the micropipette used was the mean of five determinations.

Solutions (approx. 1%) of galactomannan in 0.025 mol/l phosphate buffer were prepared as for the sedimentation and diffusion coefficient determinations, except that the amounts of red clover and lucerne galactomannan to be dissolved were increased to 1.0 g / 50 ml of buffer. After dialysis and adjustment to 50.0 mls, the solution was immersed in a waterbath at 20.00 ± 0.05°C. Samples were removed into the tared micropipette and weighed as quickly as possible. This was repeated until three weights within a 6×10^{-5}g range were obtained. The mean of these weights divided by the volume of the micropipette gave the density, and galactomannan concentration was determined as normal.

The partial specific volume was calculated from

$$\bar{v} = \frac{1}{p_s} - \frac{1}{C_{solute}} \frac{(p_{sol} - p_s)}{p_s} (\underline{\bar{V}} - 24)$$

(Chervenka 1969)

where p_s = density of solvent (in this case phosphate buffer) (g/ml)

p_{sol} = density of solution (g/ml)

C_{solute} = concentration of solute (g/ml)

(It is normal practice for the purposes of this calculation to assume that a plot of concentration of $\bar{v}$ against concentration is linear for low concentration (≤1.0%), this was shown for Carob galactomannan concentrations ≤0.4% by Kubal and Gralen (1948).

C. RESULTS AND DISCUSSION

a) SEDIMENTATION COEFFICIENTS

Some typical sedimentation velocity photographs (fig. V-3), show that the boundaries consist of a single, approximately Gaussian, peak with no evidence of gross heterogeneity. This form of sedimentation boundary was found for all the galactomannans at all concentrations.

Creeth and Pain (1967) list in their review on Ultracentrifugation four systems which may give rise to symmetrical peaks which remain fairly sharp. These are:-

(i) a single, homogeneous solute

(ii) a concentration dependant polydisperse solute with a continuous distribution of sedimentation coefficients.

(iii) a mixture of two solutes with similar values of sedimentation coefficient.

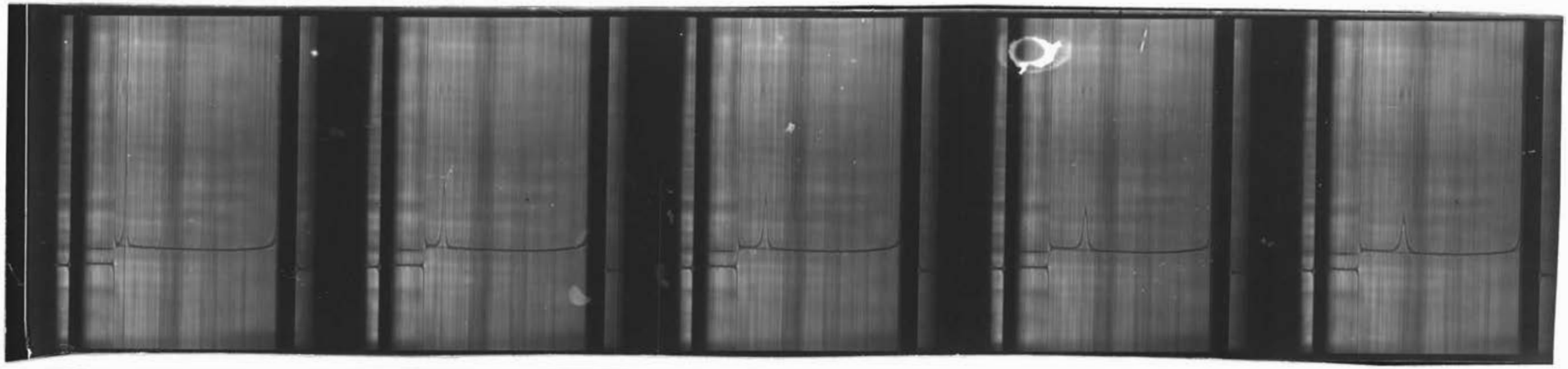
(iv) some systems in rapid equilibrium.

As the galactomannans are natural polysaccharides formed in legume seeds, a broad spectrum of molecular weights would be expected. Evidence for the polydispersity of galactomannans was provided by Kubal and Gralen (1948) who estimated the degree of polydispersity of carob galactomannan from both sedimentation and diffusion photographs. Galactomannans have been fractionated on the basis of water solubility (Hui & Neukom, 1964) and by alcohol precipitation (Richards et al, 1968). On the basis of the above evidence it seems reasonable to assume that galactomannans correspond to the second system (polydisperse) of Creeth and Pains' classification.

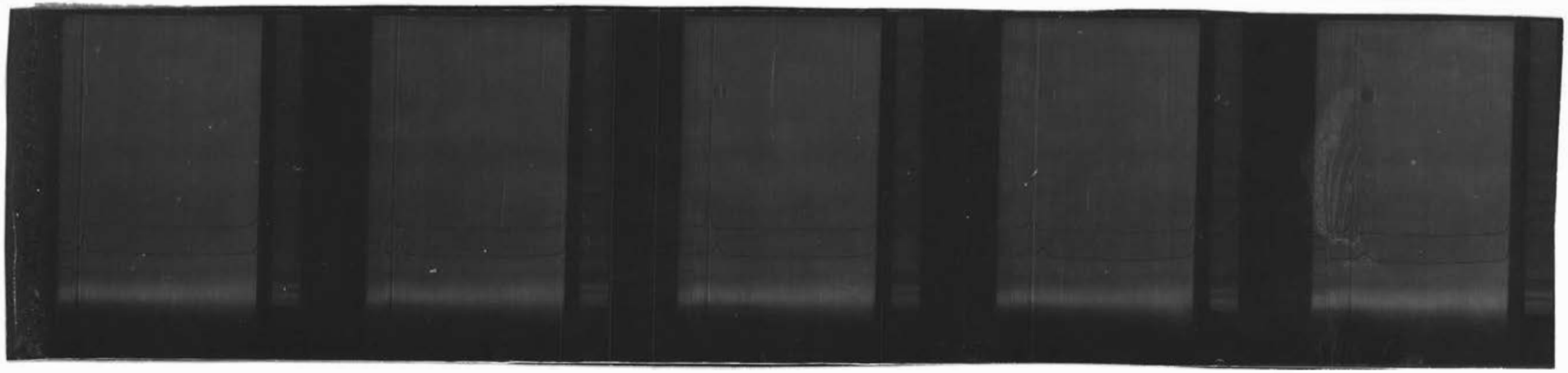
The sedimentation coefficient at zero concentration (s_o) of each galactomannan was obtained by extrapolation of a plot of $^1/s_{20,w}$ (where $s_{20,w}$ is the corrected form of the apparent sedimentation coefficient) against concentration (c).

The value of k_s (the concentration dependance parameter) in the

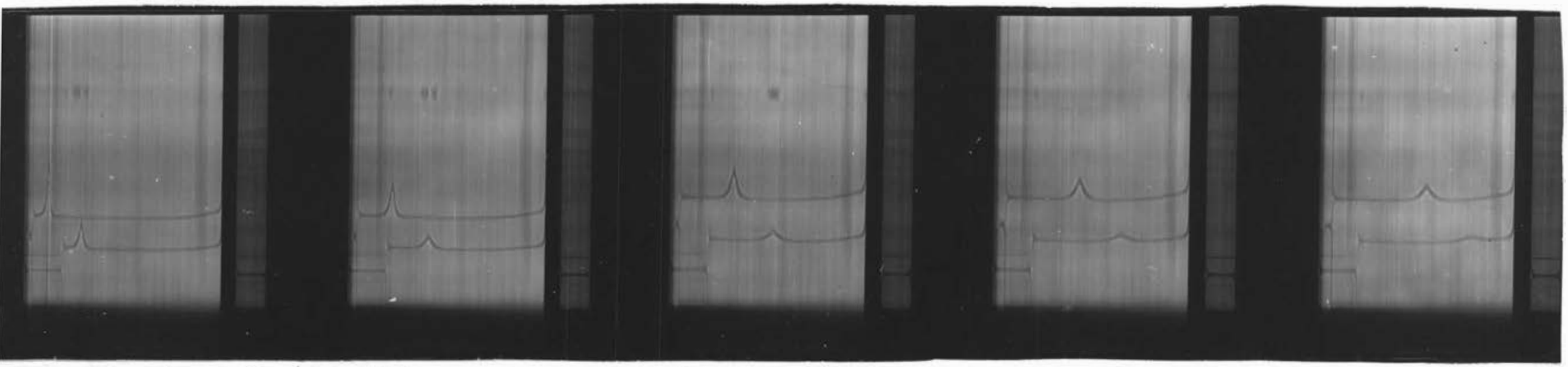
Fig. (V-3) Typical Galactomannan Schlieren Patterns Obtained During Sedimentation Velocity Experiments



Red Clover
(0.364g/100ml)
59,780 r.p.m.
Successive Exposures
at 16 minute intervals.



Sophora japonica
(0.375g/100ml
0.157g/100ml)
59,780 r.p.m.
Successive Exposures
at 16 minute intervals.



Lucerne
(0.264g/100ml
0.180g/100ml)
59,780 r.p.m.
Successive Exposures
at 32 minute intervals.

equation $$s = \frac{s_o}{1 + k_s c} \qquad (\overline{\underline{V}} - 13)$$

rearranging $$\frac{1}{s} = \frac{1}{s_o} + \left(\frac{k}{s_o}\right)c \qquad (\overline{\underline{V}} - 25)$$

was obtained from the slope of the graph. Values of s, s_o, and k_s are given in table ($\overline{\underline{V}}$ - 1).

By writing in the form

$$1 + k_s c = \frac{s_o}{s} \qquad (\overline{\underline{V}} - 26)$$

it can be seen that the smaller the value of k_s, the less the concentration dependance of s. This can be verified by inspection of table ($\overline{\underline{V}}$ - 1).

TABLE (V-1)

Values of s, s_o and k_s for Galactomannans

Galactomannan	Concentration $(\frac{g}{100ml})$	s x 10^{13} (sec^{-1})	k_s $(\frac{100ml}{g})$	Literature values and Source
Lotus pedunculatus I	1.00	1.09		
	0.751	1.21		
	0.502	1.28		
	0.250	1.32		
	0.134	1.34		
	0.000	s_o=1.35	non-linear	
Soybean	0.504	1.19		
	0.370	1.29		
	0.250	1.41		
	0.152	1.63		
	0.000	s_o=1.72	0.89	
Sophora japonica	0.681	1.24		
	0.335	1.75		
	0.262	1.95		
	0.157	2.27		
	0.097	2.49		
	0.000	s_o=3.02	2.15	
Carob	0.552	1.37		
	0.394	1.72		s_o=3.6) Kubal and
	0.321	1.98		k_s=2.8) Gralen (1948)
	0.203	2.42		
	0.134	2.95		
	0.000	s_o=4.65	4.32	
Red Clover	0.679	1.96		
	0.364	2.71		
	0.242	3.15		
	0.213	3.40		
	0.172	3.48		
	0.130	3.76		
	0.000	s_o=4.81	3.08	
Guar	0.487	1.81		
	0.407	2.02		
	0.293	2.47		s_o=9.0 Hui & Neukom (1964) (interpolation of their results)
	0.163	3.20		
	0.036	4.59		
	0.023	4.96		
	0.000	s_o=5.33	4.00	
Lotus pedunculatus II	0.310	2.67		
	0.218	3.15		
	0.168	3.41		s_o=3.7 (Richards et al 1968)
	0.121	3.89		
	0.000	s_o=7.69	4.49	

Table (V-1) continued:-

Galactomannan	Concentration $(\frac{g}{100\ ml})$	$s \times 10^{13}$ (sec^{-1})	k_s $(\frac{100\ ml}{g})$	Literature values and Source
Lucerne	0.427	2.70		
	0.379	2.78		
	0.282	3.29		
	0.264	3.53		
	0.180	4.27		
	0.115	5.18		
	0.000	s_o=7.69	4.49	

b) DIFFUSION COEFFICIENTS

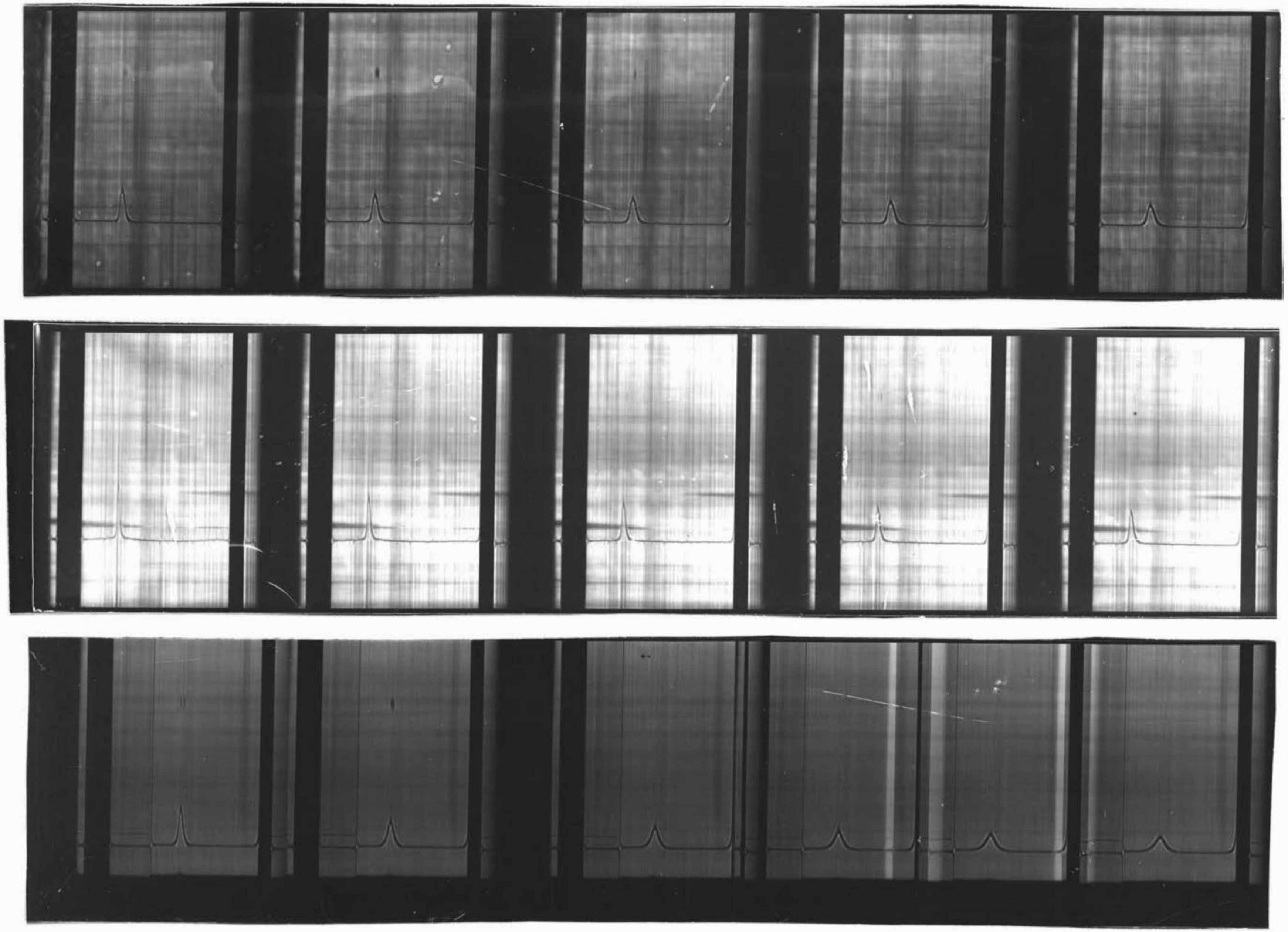
Photographs of some typical diffusion experiments are shown in fig. (V - 4). All the exposures were measured as described earlier, and the values of D_A and D_m at zero concentration were obtained.

Values of D_A and D_m at different galactomannan concentrations and zero concentration are given in table (V - 2). It is evident from this table that in some cases identical sets of galactomannan concentrations have not been used. This was caused by difficulty in calculating D_m, which resulted in a wide scatter of points. In order to obtain meaningful plots of D_m against concentration it was necessary to carry out additional diffusion experiments.

It was also difficult to carry out diffusion experiments on Soybean and Lotus pedunculatus I galactomannans below concentrations of 0.25 (g/100ml), as the diffusion boundaries spread extremely rapidly.

The use of $\frac{D_m}{D_A}$ as an index of polydispersity was indicated earlier, and a discussion of $\frac{D_m}{D_A}$ values at zero concentration of the galactomannans is included in the section on Molecular Distribution (section VI, p. 130).

Fig. (V-4) Typical Galactomannan Schlieren Patterns Obtained During Diffusion Experiments



Red Clover
(0.364g/100ml)
12,590 r.p.m.
Successive Exposure
at 16 minute
intervals

Lucerne
(0.485g/100ml)
12,590 r.p.m.
Successive Exposure
at 16 minute
intervals

Soybean
(0.504g/100ml)
12,590 r.p.m.
Successive
Exposures at 16
minute intervals.

TABLE: (V-2)

Calculation of Diffusion Coefficients D_Λ and D_m of Galactomannans

Galactomannan	Concentration $\frac{g}{100ml}$	$D_\Lambda \times 10^7$ $\frac{cm^2}{sec}$	$D_m \times 10^7$ $\frac{cm^2}{sec}$	Literature Values of D_Λ or D_m at Zero Concentration
Lotus pedunculatus I	0.751	8.4	6.4	
	0.600	10.6	9.2	
	0.502	12.0	12.5	
	0.433	12.5	14.8	
	0.250	16.0	18.2	
	0.000	19.2	24.2	
Soybean	1.00	3.15		
	0.754	3.27	2.39	
	0.504	3.28	3.64	
	0.370	3.56	4.81	
	0.250	3.65	5.43	
	0.000	3.84	6.90	
Sophora japonica	0.681	0.59		
	0.551		0.35	
	0.444	0.78	0.52	
	0.289	0.96	0.81	
	0.152	1.06	1.05	
	0.000	1.13	1.32	
Lotus pedunculatus II	0.605	0.70		
	0.488	0.74		
	0.327		0.54	
	0.250	0.85	0.69	
	0.168	0.89	0.78	
	0.107	0.90	0.91	
	0.000	0.95	1.09	
Lucerne	0.485	0.37	0.51	
	0.427		0.85	
	0.293	0.49	1.16	
	0.282		1.22	
	0.264	0.52		
	0.170	0.59	1.57	
	0.115	0.63		
	0.000	0.79	2.14	
Red Clover	0.583	0.41		
	0.483	0.42	0.29	
	0.364	0.49	0.46	
	0.242	0.56	0.53	
	0.158		0.59	
	0.135	0.61	0.64	
	0.000	0.69	0.77	

Table (V-2) continued:-

Galactomannan	Concentration $\frac{g}{100ml}$	$D_A \times 10^7$ $\frac{cm^2}{sec}$	$D_m \times 10^7$ $\frac{cm^2}{sec}$	Literature Values of D_A or D_m at Zero Concentration
Carob	0.394	0.41	0.40	D_A = 0.49) (Kubal and D_m = 0.85) Gralen, 1948)
	0.283	0.46	0.54	
	0.203	0.48	0.66	
	0.151	0.51	0.75	
	0.134	0.54		
	0.000	0.58	0.97	
Guar	0.487	0.31	0.24	
	0.294	0.35	0.34	
	0.163	0.41	0.44	
	0.122	0.38		
	0.077	0.42	0.49	
	0.000	0.44	0.54	

c) Partial Specific Volume

Results appear in Table ($\bar{\bar{V}}$-3)

TABLE ($\bar{\bar{V}}$-3)

Galactomannan Partial Specific Volumes

Galactomannan	Partial Specific Volume, $\bar{v}$ $\frac{ml}{g}$	Error (%)	Reported Value
Lotus pedunculatus I	0.628	± 1.4	0.625 (Richards et al, 1968)
Carob	0.629	± 1.3	0.66 (Kubal & Gralen, 1948)
Sophora japonica	0.629	± 1.5	
Lotus pedunculatus II	0.630	± 1.6	0.625 (Richards et al, 1968)
Red Clover	0.630	± 1.3	
Soybean	0.634	± 1.4	
Guar	0.635	± 1.5	
Lucerne	0.636	± 1.3	

The main error in these calculations arises from the subtraction of the solvent density from that of the solution. This is illustrated below, in the case of Sophora japonica galactomannan. Knowing that $\bar{v}$

$$\bar{v} = \frac{1}{p_{solvent}} - \frac{1}{C_{solute}} \left(\frac{p_{solution} - p_{solvent}}{p_{solvent}}\right) \qquad (\bar{\bar{V}}\text{-}24)$$

where $p_{solvent}$ = density of solvent = 1.00591 ± 0.003% g/ml for phosphate buffer (0.025 moles/l)

$p_{solution}$ = density of galactomannan solution = 1.00923 ± 0.003% g/ml

C_{solute} = concentration of galactomannan = 0.00904 ± 0.5% g/ml.

Substituting gives:-

$$\bar{v} = \frac{1}{1.00591} - \frac{1}{0.00904 \pm 0.5\%} \left[\frac{1.00923(+0.00003) - 1.00591(+0.00003)}{1.00591 \pm 0.003\%} \right]$$

$$= \frac{1}{1.00591} - \frac{1}{0.00904} (\pm 0.5\%) \left[\frac{0.00332 \pm (0.00006)}{1.00591 \pm 0.003\%} \right]$$

$$= 0.9941 - 110.6(\pm 0.5\%) \left[\frac{0.00332}{1.00591} (\pm 2.0\%) \right]$$

$$= 0.9941 - 0.3651 \pm 2.5\%$$

$$= 0.9941 - 0.3651 \pm 0.0091$$

$$= 0.629 \pm 0.0091$$

$$= 0.629 \pm 1.5\% \text{ ml/g.}$$

Granath (1958) obtained values of 0.611 and 0.603 for the partial specific volumes of two dextran samples. As dextrans are neutral, water-soluble polysaccharides they could be expected to have partial specific volumes similar to those of other neutral, water-soluble polysaccharides such as galactomannans. Kubal and Gralen (1948) found $\bar{v}$ for Carob galactomannan to have a partial specific volume of 0.66.

d) Calculation of Molecular Weights

Molecular weights of the galactomannans were calculated by substitution of the appropriate values into Svedburg's (1940) equation:-

$$M = \frac{sRT}{D(1-vp)} \qquad (\overline{V} - 7)$$

introduced earlier (p. 95). By substitution of both the apparent diffusion coefficient (D_A) and the weight-average diffusion coefficient (D_m) respectively into Svedburg's equation, the sedimentation-diffusion average molecular weight ($\overline{M}_{s,D}$) and the weight-weight average molecular weight ($\overline{M}_{w,w}$) were obtained.

These molecular weight values are presented in table ($\overline{V}$ - 4), together with literature values where available of either $\overline{M}_N$, $\overline{M}_w$, $\overline{M}_{(s,D)}$, or $\overline{M}_{w,w}$. (These latter averages are as defined on p. 93 and 98).

Specific comments on the galactomannan molecular weights are:-

(i) Soybean: The $\overline{M}_N$ of Whistler and Saarnio (1957) was determined on a sample of galactomannan extracted in water at 40°C. Since the extracting power of water would be expected to be greater at 40°C than 20°C, a galactomannan extracted at 40°C would be expected to contain more high molecular weight material than one extracted at 20°C. This explains the observed order of molecular weights $\overline{M}_N$ (40°C) $>$ $\overline{M}_{s,D}$ (20°C) whereas $\overline{M}_{s,D}$ (20°C) $>$ $\overline{M}_N$(20°C) would be expected.

(ii) Sophora japonica: The high value of $\overline{M}_{s,D}$ and $\overline{M}_{w,w}$ compared to $\overline{M}_N$ would appear to reflect a large degree of polydispersity in the sample. This conclusion is not supported by the value of the $\frac{D_m}{D_A}$ ratio which can also be taken as an index of polydispersity implying that the difference may be due in part to the maturity of the galactomannan (Reid and Meier, 1970). Further discussion of the reasons for the widely differing values of $\overline{M}_{s,D}$ and $\overline{M}_{w,w}$ will be delayed until the section on molecular distribution (p. 121).

TABLE (V-4)

Galactomannan Molecular Weights

Galactomannan	$M_{s,D}$ x10^{-3}	$M_{w,w}$ x10^{-3}	Literature Molec.Wt(x10^{-3})	Method	Source
Lotus pedunculatus I	4.7	3.7			
Soybean	30.1	16.6	32($\bar{M}_n$)	Osmotic	Whistler & Saarnio (1957)
Sophora japonica	177	152	6($\bar{M}_n$)	End Group Analysis	Kooiman (1971)
Lotus pedunculatus II	379	332	100($\bar{M}_w$)	Ultracentrifuge (Archibald)	Richards et al (1968)
Red Clover	461	416			
Carob	532	319	520($\bar{M}_{s,D}$) 300($\bar{M}_{w,w}$) 650($\bar{M}_n$)	Ultra-centrifuge End Group Analysis	Kubal & Gralen (1948) Hui & Neukom (1964)
Lucerne	731	239			
Guar	817	657	200 1900($\bar{M}_w$) 250($\bar{M}_n$) 1720($\bar{M}_w$)	Ultracentrifuge (Method unknown) Ultracentrifuge (Method unknown) End Group Determination Light Scattering	Boggs (quoted by Hui & Neukom, 1964) Hui & Neukom (1964) Hui & Neukom (1964) Deb & Mukherjee (1963)

$\bar{M}_n$ = Number-average molecular weight

$\bar{M}_w$ = Weight-average molecular weight

$\bar{M}_{w,w}$ = Weight-weight average molecular weight

$\bar{M}_{s,D}$ = Sedimentation Diffusion average molecular weight.

iii) <u>Carob</u>: The values of $\bar{M}_{s,D}$ and $\bar{M}_{w,w}$ are in reasonable agreement with those of Kubal and Gralen (1948).

The extremely high value of $\bar{M}_n$ (compared to $\bar{M}_{s,D}$ and $\bar{M}_{w,w}$ determined here and by Kubal and Gralen) obtained by Hui and Neukom (1964) seems questionable, especially since endgroup analysis becomes rather inaccurate over $\bar{M}_n = 20{,}000$ (Price, 1959). Unfortunately Hui and Neukom have not elaborated on their modified analytical procedure with sodium chlorite.

iv) <u>Lotus pedunculatus (II)</u>: The order of $\bar{M}_{s,D}$ and $\bar{M}_{w,w} > \bar{M}_w$ is not predicted, and can be explained only on the basis of differences in the maturity of the legume seeds before the galactomannan was extracted (see Reid and Meier, 1970).

v) <u>Guar</u>: The order $\bar{M}_w > \bar{M}_{w,w} > \bar{M}_{s,D} > \bar{M}_n$ is as would be expected. From the value of $\frac{\bar{M}_w}{\bar{M}_n}$, a moderate amount of polydispersity is evident, a although the accuracy of the $\bar{M}_n$ value determined by the sodium chlorite analysis (of Hui and Neukom (1964)) is again open to question.

e) THE RELATIONSHIP BETWEEN GALACTOMANNAN MOLECULAR WEIGHTS, DIFFUSION COEFFICIENTS, AND SEDIMENTATION COEFFICIENTS.

It was indicated in the introduction (p. 10) that the short single unit galactose side-chains of galactomannans might be expected to have little or no influence on the behaviour of the molecule in solution. If this is so, then the differing galactose:mannose ratios of different galactomannans will have no effect on solution properties, and galactomannans can be treated as a series of linear molecules differing only in molecular weight.

For such a series it has been shown that definite relationships exist between the molecular weight (M) and the diffusion coefficient (D), and the molecular weight and the sedimentation coefficient (s).

Tanford (1961, p.362) predicts that if the molecule is randomly coiled, then Diffusion coefficient $D \sim M^{-0.5}$ in a poor solvent, and $D \sim M^{-0.55}$ in a good solvent. Similarly, sedimentation coefficient $s_o \sim M^{0.5}$ in a poor solvent and $s_o \sim M^{0.45}$ in a good solvent (Tanford, (1961,p.382)).

If the molecule is rod-shaped then $D \sim M^{-0.8}$.

Using the D_A, D_m, s_o, $\bar{M}_{s,D}$ and $\bar{M}_{w,w}$ values obtained, plots of $\log_{10} D_A$ against $\log_{10} \bar{M}_{s,D}$, $\log_{10} s_o$ against $\log_{10} \bar{M}_{s,D}$, $\log_{10} D_m$ against $\log_{10} \bar{M}_{w,w}$ and $\log_{10} s_o$ against $\log_{10} \bar{M}_{w,w}$ were made (see fig. ($\overline{\underline{V}}$-5) and ($\overline{\underline{V}}$-6).

A correlation coefficient was calculated and a least squares line fitted to each plot. Results are presented in table ($\overline{\underline{V}}$-5).

From the table it can be seen that good correlation was obtained in all cases except the plot of $\log_{10} s_o$ against $\log_{10} \bar{M}_{w,w}$. If the data for Lucerne galactomannan is removed from this plot, however, the correlation between $\log_{10} s_o$ and $\log_{10} \bar{M}_{w,w}$ becomes comparable to the others. The problem with Lucerne arises from its' much greater value of the ratio $\frac{D_m}{D_A}$ than that of any other galactomannan. This indicates a much larger degree of polydispersity than the other galactomannans, which appears to have resulted in a higher sedimentation coefficient than would be expected for a molecule of the same weight-weight average molecular weight as lucerne galactomannan, but having a similar degree of polydispersity to the remaining galactomannans.

The good correlations between sedimentation coefficient, diffusion coefficient, and molecular weight otherwise obtained would appear to support the idea that galactomannans in solution can be treated as a linear series differing only in molecular weight. The small deviations from linearity obtained could be attributed at least in part to small differences in molecular weight distribution between the different galactomannans (apart from Lucerne), as it seems highly unlikely that material isolated from different sources will have identical distribution.

The relationship between the diffusion coefficient and molecular weight lies almost mid-way between the random coil and rigid rod cases, consequently the conformation of a galactomannan molecule in solution must lie between the random coil and rigid rod conformations, while that between sedimentation coefficient and molecular weight is inconclusive.

Table ($\bar{V}$-5)

Comparison of Sedimentation Coefficients and Diffusion Coefficients with Molecular Weight Averages

Plot of:-	Correlation Coefficient	Equation of Least Squares Line	Power Equation
$\log_{10}s_o/\log_{10}\bar{M}_{s,D}$	0.983	$\log_{10}s_o=0.317\log_{10}\bar{M}_{s,D}-1.104$	$s_o=7.87\times10^{-2}\bar{M}_{s,D}^{0,32}$
$\log_{10}D_A/\log_{10}\bar{M}_{s,D}$	-0.988	$\log_{10}D_A=3.70-0.687\log_{10}\bar{M}_{s,D}$	$D_A=2.00\times10^{-4}\bar{M}_{s,D}^{-0.68}$
$\log_{10}s_o/\log_{10}\bar{M}_{w,w}$	0.915	$\log_{10}s_o=0.307\log_{10}\bar{M}_{w,w}-0.940$	$s_o=1.15\times10^{-1}\bar{M}_{w,w}^{0.31}$
$\log_{10}s_o/\log_{10}\bar{M}_{w,w}$ (excluding Lucerne)	0.978	$\log_{10}s_o=0.289\log_{10}\bar{M}_{w,w}-0.866$	$s_o=1.36\times10^{-1}\bar{M}_{w,w}^{0.29}$
$\log_{10}D_m/\log_{10}\bar{M}_{w,w}$	-0.992	$\log_{10}D_m=3.92-0.719\log_{10}\bar{M}_{w,w}$	$D_m=8,22\times10^{-4}\bar{M}_{w,w}^{-0.72}$

Figure (V–5):– Plots of Sedimentation Coefficient and Apparent Diffusion Coefficient against Sedimentation–Diffusion Average Molecular Weight

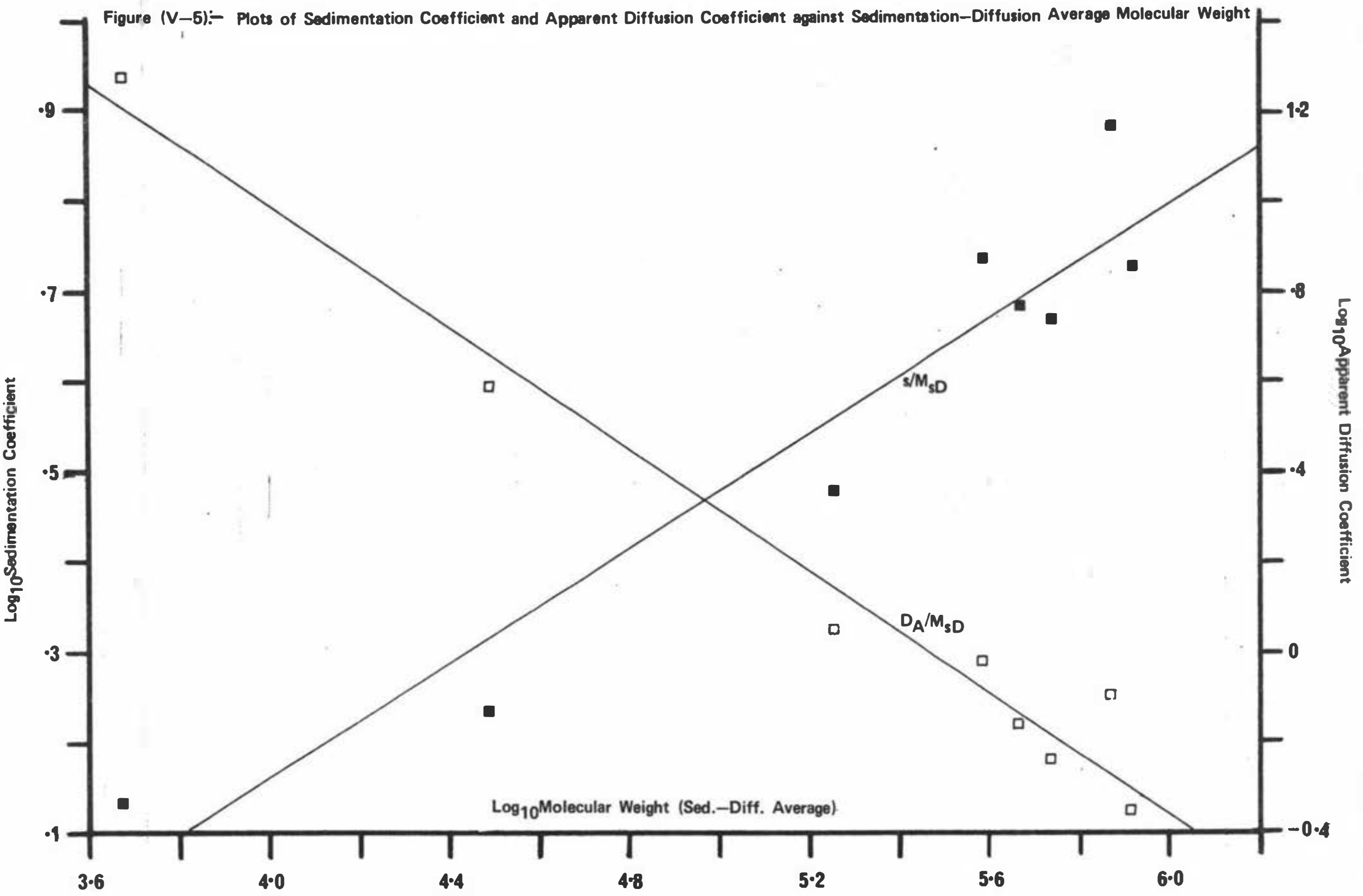
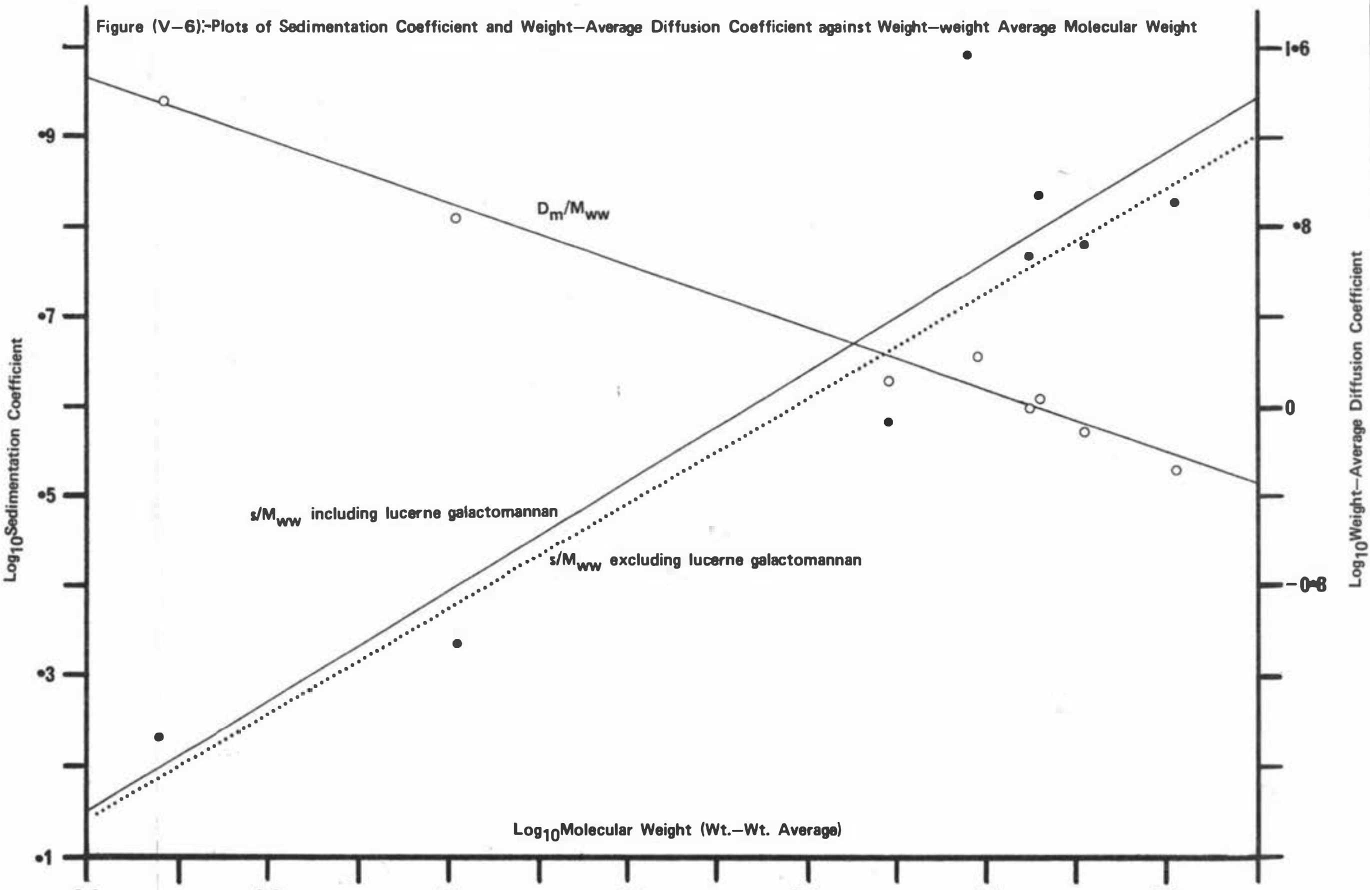


Figure (V–6):-Plots of Sedimentation Coefficient and Weight–Average Diffusion Coefficient against Weight–weight Average Molecular Weight



SECTION VI : MEASUREMENT OF GALACTOMANNAN MOLECULAR WEIGHT DISTRIBUTION

Introduction

Differences in the physical properties of polydisperse but structurally similar or identical molecules may be due in whole or in part to differences in their molecular weight distributions. Such physical properties would include viscosity, which has a known proportionality to molecular weight.

A. Measurement of Sedimentation Coefficient Distribution as an Indication of Molecular Weight Distribution

(1) Introduction

It was shown earlier (page 119) that the relationship

$$s_o = kM^{\alpha} \qquad \text{(IV-1)}$$

(s_o = limiting sedimentation coefficient (weight average)

M = sedimentation / diffusion molecular weight or weight - weight average molecular weight.

k, α = constants)

holds for the galactomannans from Carob, Guar, Lotus pedunculatus, Lucerne, Red Clover, Sophora japonica and Soybean treated as a linear series. No effect of the different mannose : galactose ratios on the values of α and k was evident.

With the known relationship between s_o and M given in equation (VI-1) above, it should therefore be possible to transform a distribution of sedimentation coefficients into a distribution of molecular weights.

The distribution function of sedimentation coefficients (s) in a given system can be defined by g (s). When the system is concentration dependent, as is the case for galactomannans, only s in the extrapolated distribution function g (s) has any physical significance (Fujita (1962)

P 186), i.e. the sedimentation coefficient of each solute at zero concentration of the solution. This can be written as g (s_o), where s_o is the limiting sedimentation coefficient of the solute.

It has already been shown there is a unique relationship between s_o and molecular weight (M) for galactomannans (equation (VI-1) above). In this case it can be shown (Fujita (1962), P 186) that g (s_o) is related to the normalised weight distribution of molecular weight of the sample by:-

$$g(s_o)ds_o = f(M)dM \qquad \text{(VI-2)}$$

$g(s_o)$ and $f(M)$ are now the differential functions of limiting sedimentation coefficient and of molecular weight respectively. It can be further shown that, where s_o corresponds to M ,

$$\int_0^{s_o'} g(s_o)ds_o = \int_0^{M'} f(M)dM \qquad \text{(VI-3)}$$

i.e.

$$G(s_o) = F(M) \qquad \text{(VI-4)}$$

where $G(s_o)$ and $F(M)$ are the integral distribution functions corresponding to $g(s_o)$ and to $f(M)$ respectively (Williams, 1972).

By rearranging equation (VI-2) we obtain

$$f(M) = g(s_o)\,\frac{ds_o}{dM}$$

substituting from

equation (VI-1) gives $f(M) = g(s_o)\cdot\alpha K\left[\frac{s_o}{K}\right]^{(\alpha-\frac{1}{\alpha})}$ (VI-5)

α, K, s_o are all constants, thus a simple transformation of differential sedimentation coefficient distribution into differential molecular weight distribution is available.

Alternatively, the transformation may be made via equations (VI-3) or (VI-4) above.

(2) Measurement of Sedimentation Coefficient Distributions from Sedimentation Velocity Experiments - Outline of Available Methods

It has long been known that under the influence of a centrifugal field, a non-uniform solute will be at least partially fractionated into its components. In an ultracentrifuge, this effect may occur in either a sedimentation equilibrium or a sedimentation velocity experiment.

Both these cases have been well discussed by Fujita (1962) and Williams (1972). As the sedimentation velocity method was used in the present work, that method is the only one which will be discussed.

In the early 1930's Svedburg and his coworkers noted anomalous behaviour of polydisperse solutes in sedimentation velocity experiments. Apparent diffusion coefficients calculated from the refractive index gradient versus distance from the centre of rotation boundary increased markedly with time. They attributed this effect to the polydispersity of the solute.

Gralen (1944) measured the broadening of the boundary (which gives rise to the increasing diffusion coefficient noted by Svedburg) as a function of the concentration of the solution. He plotted the width of the peak at half height against time for a series of experiments at different concentrations, and extrapolated the slope of these plots back to zero concentration. The resultant polydispersity constant (with regard to sedimentation) enabled a distribution coefficient to be calculated for an assumed distribution. Gralen ignored any effects of diffusion. (A similar method was used by Jullander (1945).)

The extrapolation by Gralen to zero concentration was made necessary by the non-ideal nature of the solutions as sedimentation coefficients are usually concentration dependent.

Eriksson (1953) modified Gralen's method by assuming a gaussian distribution of mass over the different sedimentation values in the boundary. He showed that if sedimentation and diffusion of solute occur independently in the solution, the total boundary spreading is the sum of the spreading due to each. Although this will not be true at higher concentrations, it was justified by the extrapolation of the results to zero concentration.

More recent methods are due to Williams and Saunders (1954), Gralen and Langermalm (1952) and Baldwin et. al. (1955). These are all quite similar, and since the method of Gralen and Langermalm was used in the present work, a discussion of the other two is not included. Gralen and Langermalms' method will be discussed in the experimental section.

As is usual, all the above cases involved extrapolation to zero concentration of solute to remove non-ideal, concentration - dependent effects, and thus they provide only a provisional result. The extrapolation is empirical since no theory has been produced to guide it. This difficulty arises because s depends upon the concentrations of all the solutes present, and the complex Johnston - Ogston effects involved produce mathematical problems that have so far remained unsolved.

Williams (1972) describes the methods of Gralen and Langermalm (1952), Williams and Saunders (1954) and Baldwin et. al. (1955), as "somewhat naive", and suggests that a more practical approach is found in the works of Wales and Rehfeld (1962). The calculations required in the latter work however, are complex, and require extensive computer analysis.

As a simple treatment was preferable, and Williams (1972) recommends the methods of Gralen and Langermalm (1952) or Baldwin et. al. (1955) over that of Williams and Saunders (1954), Gralen and Langermalms' method was employed. It should be noted that despite Williams' comments on the "naivete" of this method, it is still widely employed.

(3) Determination of a Sedimentation Coefficient Distribution Using the Method of Gralen and Langermalm (1952)

(a) Outline of the Method:

The method of Gralen and Langermalm differs somewhat from those previously outlined.

Plots of refractive index gradient ($\frac{dn}{dr}$) against distance from the centre of rotation (r) which are normally obtained in sedimentation velocity experiments were transformed into plots of concentration gradient ($\frac{dc}{dr}$) against distance (r) (see fig (VI-1a)). This requires the assumption that the specific refractive index increment of a mixture of molecules of the same type is independent of molecular weight, which is generally accepted as true (Fujita, 1962).

The plots of $\frac{dc}{dr}$ versus r were next transformed into plots of concentration gradient with respect to sedimentation coefficient ($\frac{dc}{ds}$) against sedimentation coefficient (s) (fig (VI-1b)) by using the formula

$$s = \frac{s_A \log_e (\frac{r}{r_o})}{\log_e (\frac{r_A}{r_o})} \qquad \text{(VI-6)}$$

where r_o = distance of the meniscus from the centre of rotation.

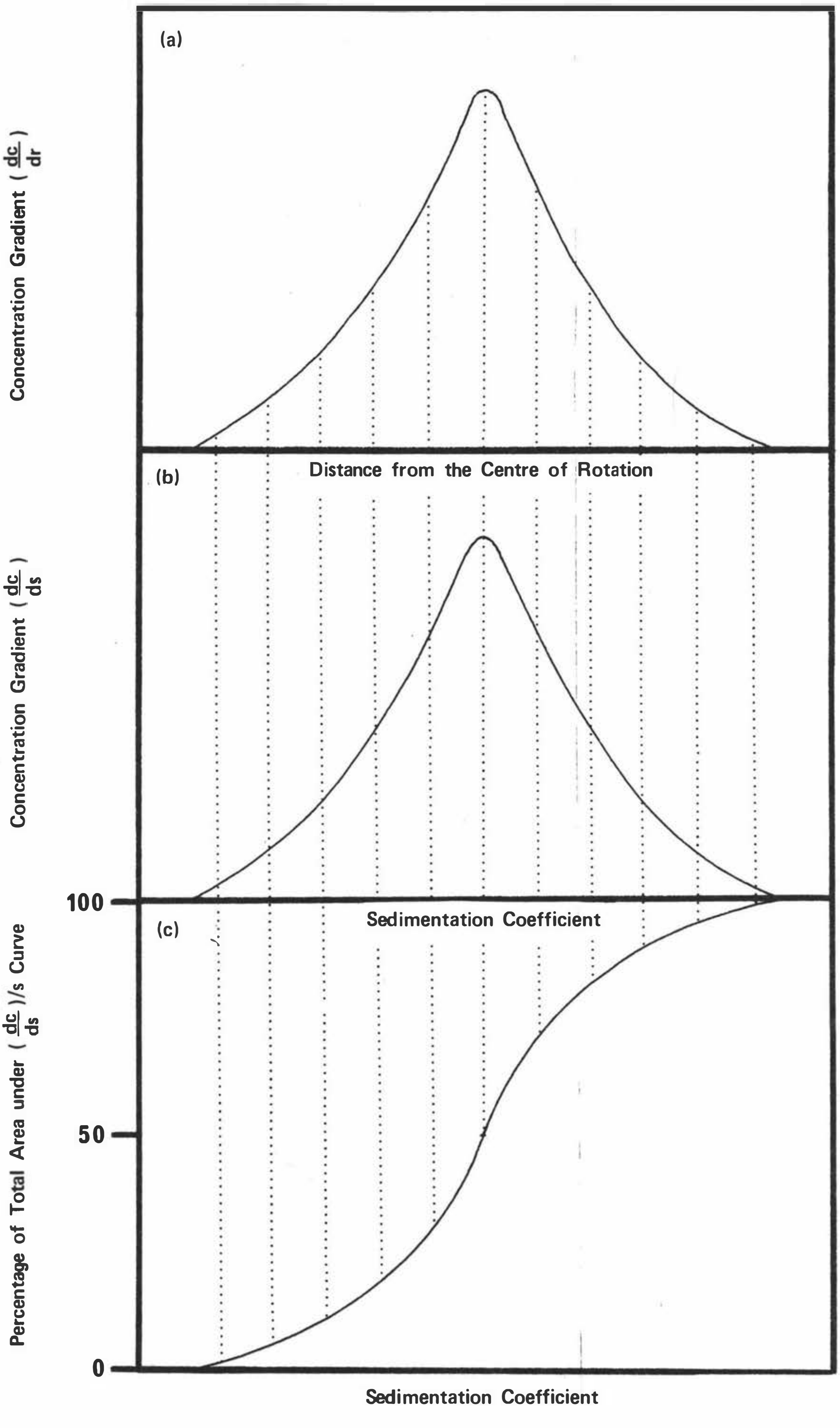
r_A = distance of the apex of the boundary from the centre of rotation (for a gaussian peak).

s = sedimentation coefficient at distance r from the centre of rotation.

s_A = sedimentation coefficient of the apex of the boundary.

These curves were integrated for a number of different concentrations of solute (i.e. different sedimentation velocity experiments) by firstly measuring the area under the curve for specific increments of sedimentation

Figure (VI–1) :-Transformation and Intergration of an Experimental Sedimentation Boundary



coefficient. Secondly, the cumulative percentage of the total area under the curve against sedimentation coefficient was plotted to yield the integrated plot (see fig (VI-1c)).

Values of the sedimentation coefficient at known percentages of total area under the $\frac{dc}{ds}$ versus s curve were obtained from fig (VI-1c) by interpolation. These values (for a series of curves obtained at different solute concentrations) were plotted against solute concentration. Extrapolation to zero concentration yields the integrated distribution curve of limiting sedimentation coefficient (s_o).

Differentiation of this distribution curve, performed graphically in a method analogous to that described for integration, yields the differential form of the distribution curve of limiting sedimentation coefficient.

This method neglects any effects on boundary shape due to diffusion or pressure.

(4) Experimental

The sedimentation velocity experiments and results used were the same as those outlined in Section (V), part (B), page 100.

(a) Calculation of Results

(i) Calculation of Sedimentation Coefficient Distributions:

Calculations were made using the method of Gralen and Langermalm (1952) outlined previously.

One sedimentation curve for each concentration of a series for a particular galactomannan was selected. The selection was made so that it's broadening had reached such a stage that all possible details were at their clearest without interference from the meniscus or the cell bottom. Between three and five concentrations were used for each galactomannan. As no suitable sedimentation curves were available for Soybean or Lotus pedunculatus I galactomannans due to their rapid spreading by diffusion, no calculations of their sedimentation coefficient distributions were carried out.

Gralen and Langermalm neglected diffusion effects in their calculation as they were working with systems containing linear polystyrene molecules which have low diffusion coefficients. Although galactomannans may be regarded as approximately linear since the galactose sidechains are short, an estimate of the effects of diffusion was sought.

Sophora japonica galactomannan, has the highest diffusion coefficient of those used in these calculations. The effect of diffusion on it's sedimentation boundary was accordingly investigated using the method of Eriksson (1953), since the boundary was gaussian.

Eriksson deduced that

$$B^2_{\text{sed total}} = B^2_{\text{diff}} + B^2_{\text{sed}} \qquad \text{(VI-7)}$$

B = peak width at half height

under the conditions stated in the introduction, (i.e. Gaussian boundary, with sedimentation and diffusion occurring independently in the solution.) Using

$$B^2_{\text{diff}} = 4\pi Dt \qquad \text{(VI-8)}$$

(Eriksson, ibid)

D = diffusion coefficient

t = time in seconds from beginning of sedimentation velocity experiment

then

$$B_{\text{sed}} = \sqrt{B^2_{\text{Total}} - 4\pi Dt} \qquad \text{(VI-9)}$$

combining equations (VI-7) and (VI-8).

Substitution into the above gave a maximum error of less than 1.5% if the effects of diffusion on the boundary were neglected.

Diffusion effects have therefore been neglected in all cases in calculating the sedimentation coefficient distributions.

Figure (VI–2):–Transformed and Integrated Curves of Total Area under the Sedimentation Boundary against Sedimentation Coefficient for Sophora japonica Galactomannan.

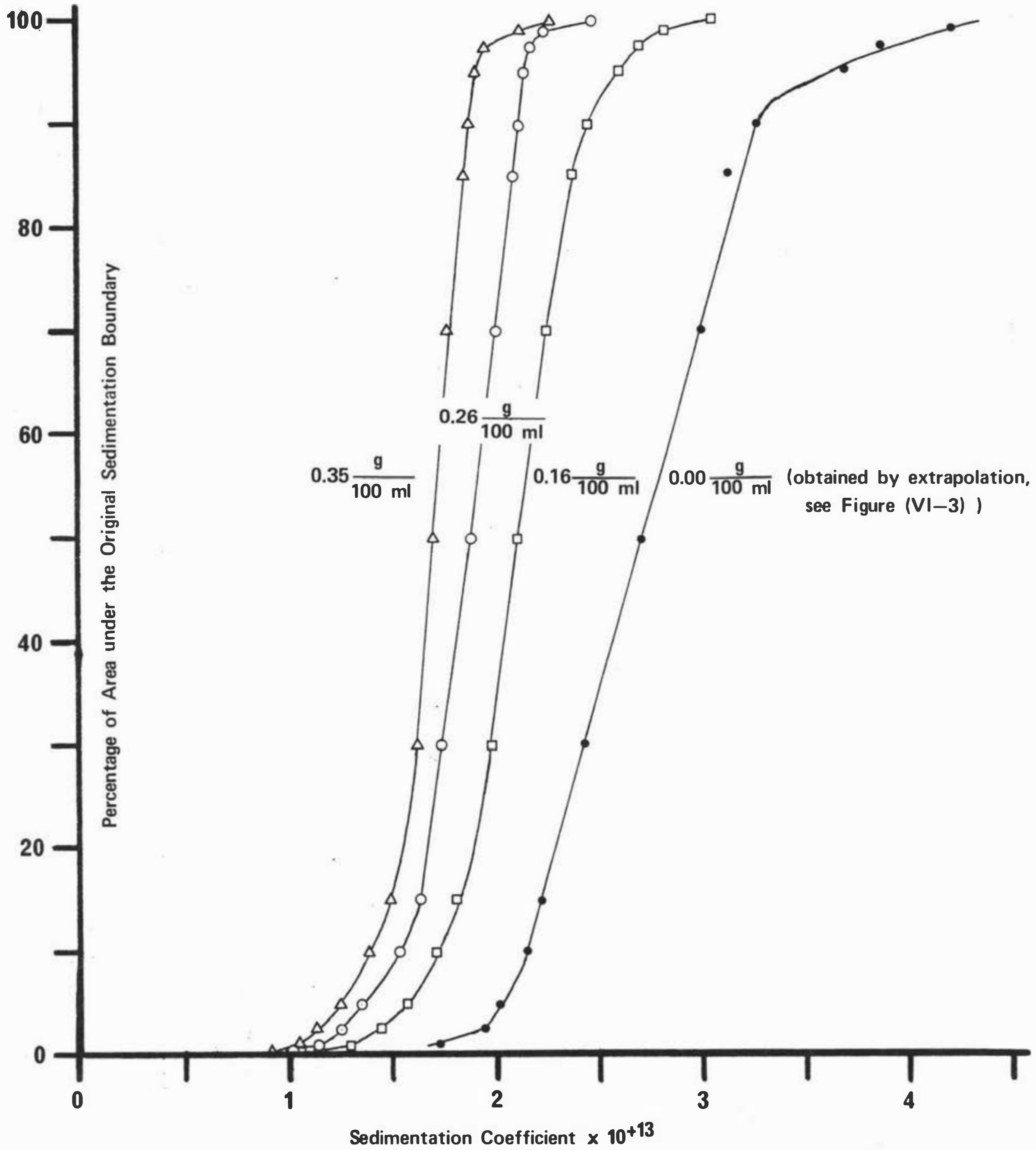
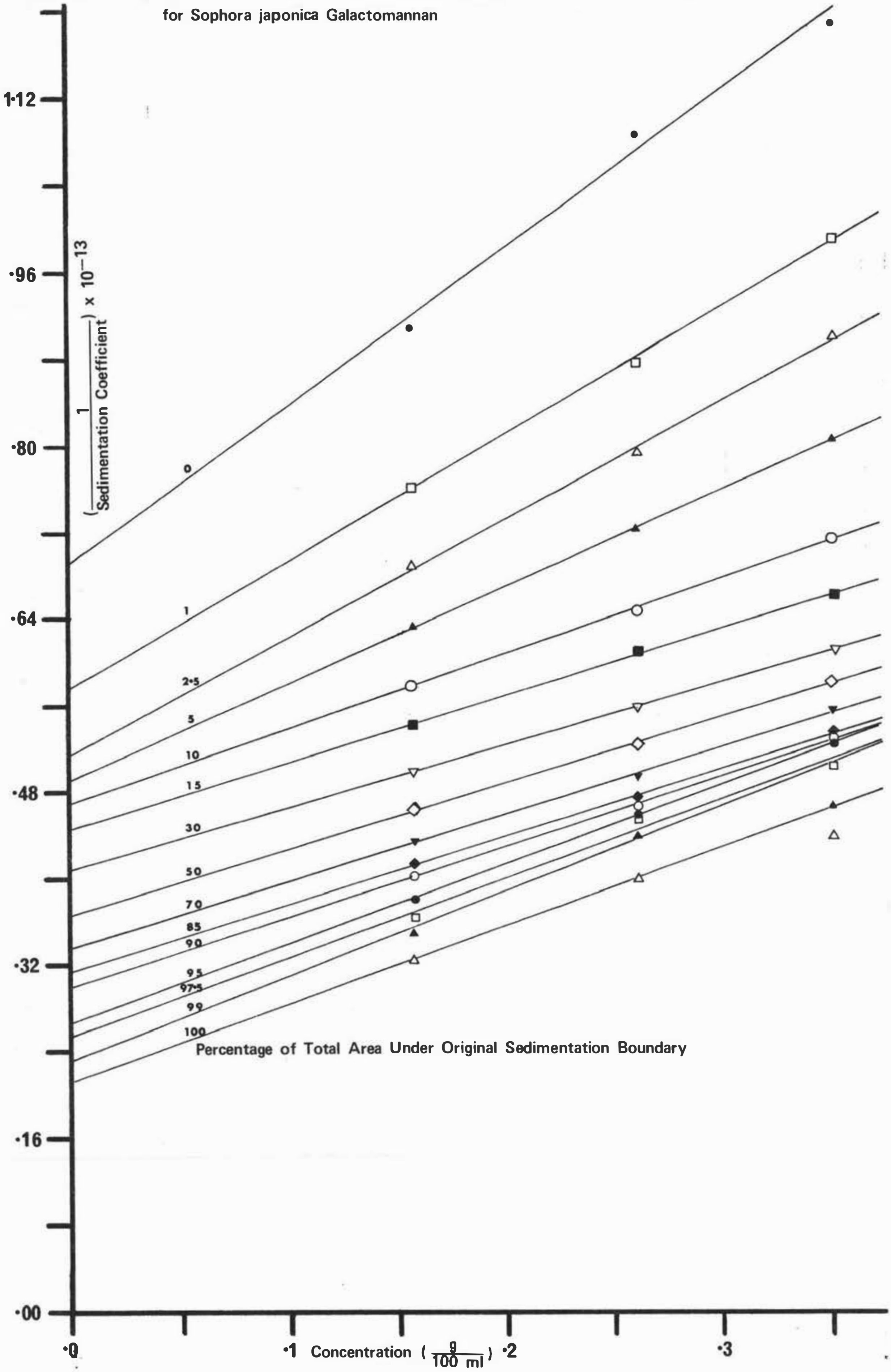


Figure (VI–3):–Extrapolation of Integrated Sedimentation Boundary to Zero Concentration for Sophora japonica Galactomannan



The integrated plot of concentration gradient against sedimentation coefficient was made in the normal way, and values of sedimentation coefficient were read off it at cumulative values of area of 0, 1, 2.5, 5, 10, 15, 30, 50, 70, 85, 90, 95, 97.5, 99 and 100% because of the shape of the plot (see fig (VI-2) for Sophora japonica galactomannan).

In a modification of Gralen and Langermalms' method, the reciprocals of the sedimentation constant values obtained above were plotted against concentration and extrapolated to zero concentration (see fig (VI-3) for Sophora japonica galactomannan). This modification was necessary because unlike the polystyrene used by Gralen and Langermalm, galactomannans do not have a linear dependence of sedimentation constant on concentration, and such plots are difficult to extrapolate. Reciprocal sedimentation coefficient / concentration plots are linear, however (see page 107). The values of reciprocal sedimentation coefficients at zero concentration were converted back to limiting sedimentation coefficients, and the integrated curve of concentration gradient / limiting sedimentation coefficient plotted (see fig (VI-2) for Sophora japonica). This curve was differentiated by substracting the value of the concentration gradient (%) from 100 at regular intervals of limiting sedimentation coefficient, and the resultant limiting sedimentation coefficient distribution plotted.

(b) Comparison of Sedimentation Coefficient Distributions of the Galactomannans

The limiting sedimentation coefficient distributions of the different galactomannans were plotted on a common plot for comparison (see fig (VI-4)). On this plot the following points may be noted.

(i) The plots are reasonably gaussian, apart from those of Lucerne and Lotus pedunculatus II galactomannans, although the deviation in the latter case is not greatly marked.

(ii) While the plot for Lotus pedunculatus II galactomannan tails towards both high and low sedimentation coefficients, the remaining plots tail towards high sedimentation coefficients.

(iii) The extremely broad distribution for Lucerne galactomannan would be expected, since some of the freeze dried sample always remains insoluble. This implies a broad distribution of molecular weight. A broad limiting sedimentation coefficient distribution would also be expected for Red Clover galactomannan for the same reason , but this was not observed. The reason for this is not known, but the possibility exists that Red Clover seeds contain two distinct galactomannans, one water soluble and the other insoluble.

(iv) The broader distribution of limiting sedimentation coefficient of Carob galactomannan compared to that of Guar is predicted by a similar observation of the molecular weight distributions by Hui and Neukom (1964).

(v) The general shape of the limiting sedimentation coefficient distribution of Guar galactomannan is very similar to a molecular weight distribution determined by Koleske and Kurath (1964) for a fraction of the galactomannan triacetate of Guar. They also used the Gralen - Langermalm method to calculate the molecular weight distribution.

(vi) Apart from Lucerne and perhaps Lotus pedunculatus II galactomannans, no major differences in physical properties could be predicted on the basis of these limiting sedimentation coefficient plots. (Inherent in this conclusion is the result that plots of identical shape would arise from conversion of the limiting sedimentation coefficient distribution to a molecular. weight distribution via equation (VI-5).

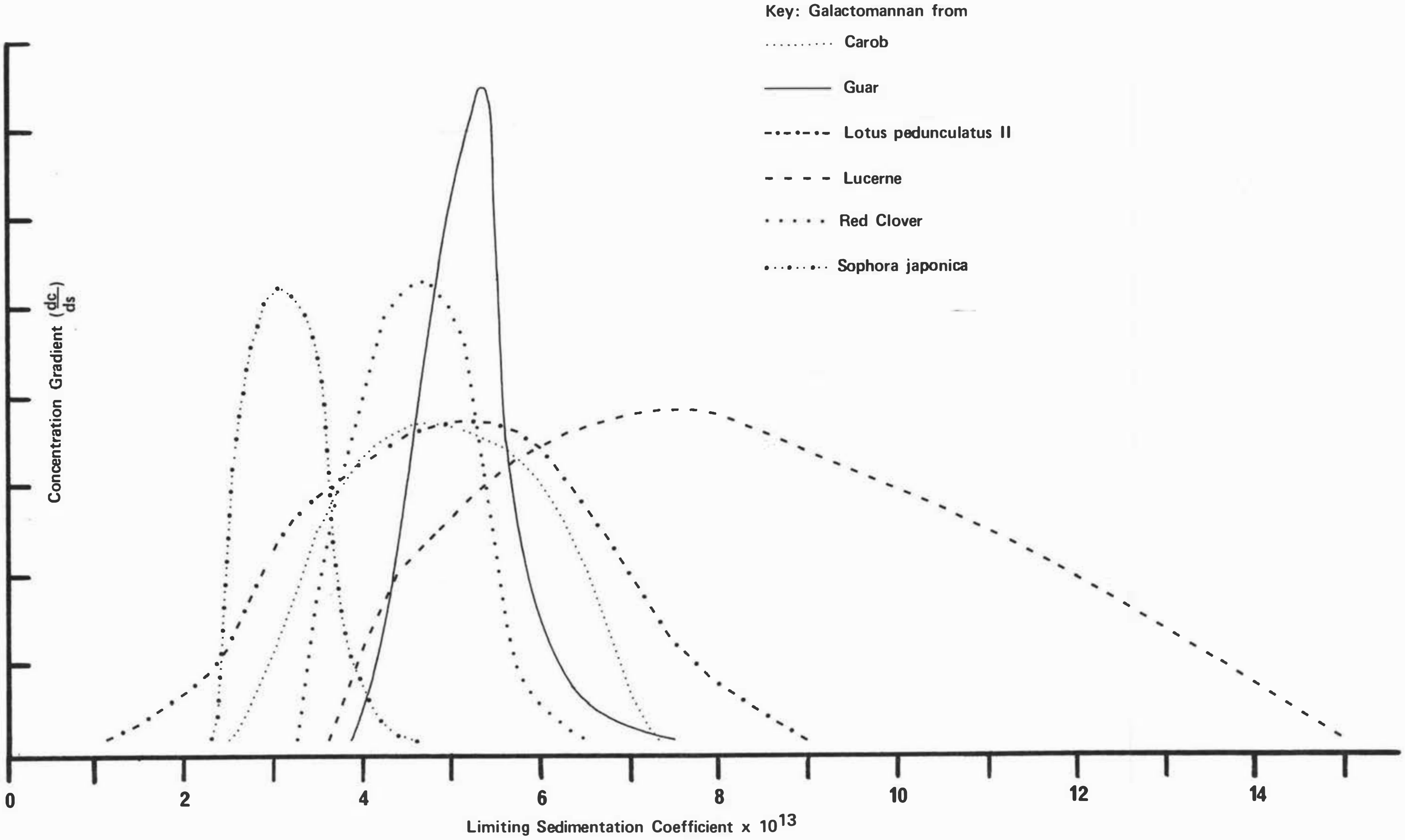


Figure (VI–4) :– Distribution Curves of the Limiting Sedimentation Coefficient Values of the Galactomannans

B. Diffusion Coefficients as on Index of Polydispersity

(1) Introduction

It was shown earlier (Section V, page 97) that for polydisperse systems it is possible to calculate different averages of the diffusion coefficient depending upon the method used. The two average diffusion coefficients calculated were D_A, the apparent diffusion coefficient (lying between the number and weight averages), and D_m, the weight - average diffusion coefficient.

Henley (1962) has suggested the use of the ratio $\frac{D_m}{D_A}$ as a qualitative index of polydispersity. This follows as D_m will be further from the weight - average value the greater the spread of molecular weights.

Although this method is less rigorous than that using the sedimentation coefficient distribution, it was felt that it would be of interest to compare the results from both methods. This method also provides an estimate of the polydispersity of Lotus pedunculatus I and Soybean galactomannans which was not available from the sedimentation coefficient distributions.

(2) Results

Values of D_m / D_A, with diffusion coefficients taken at zero concentration in each case, are given in table (VI-1)

Table VI-1

Values of the Ratio $\frac{D_m}{D_A}$ for Galactomannans

Galactomannan	$\frac{D_m}{D_A}$	Literature Value of $\frac{D_m}{D_A}$
Carob	1.67	1.7 (Kubal and Gralen, 1948)
Guar	1.23	
Lotus pedunculatus I	1.26	
Lotus pedunculatus II	1.15	
Lucerne	2.71	
Red Clover	1.12	
Sophora japonica	1.17	
Soybean	1.80	

(3) Conclusions

The order of increasing polydispersity of the galactomannans as shown by the sedimentation coefficient distributions was: Guar $<$ Sophora japonica $<$ Red Clover $<$ Carob $<$ Lotus pedunculatus II $<$ Lucerne. From the ratio of diffusion coefficients of table (VI-1) it is Red Clover $<$ Lotus pedunculatus II $<$ Sophora japonica $<$ Guar $<$ Lotus pedunculatus I $<$ Carob $<$ Soybean $<$ Lucerne.

Although the absolute order is not the same in each case the orders of magnitude, apart from Lotus pedunculatus II are, i.e. (ignoring Lotus pedunculatus I and Soybean) Red Clover $\approx$ Guar $\gtrsim$ Sophora japonica $<$ Carob $<$ Lucerne.

The anomaly in the case of Lotus pedunculatus II is not understood, but it may be due to the different shape of its sedimentation coefficient (and hence molecular weight) distribution to that of the other galactomannans (see fig VI-4).

On the basis of the agreement between the two methods in the magnitude of the polydispersity of the galactomannans, the indexes obtained for Lotus pedunculatus I and Soybean galactomannans from the ratio of $\frac{D_m}{D_A}$ are of real value.

Thus the increasing order of magnitude of the polydispersity of the galactomannans is Red Clover $\approx$ Sophora japonica $\approx$ Guar $\approx$ Lotus pedunculatus I $<$ Carob $\approx$ Soybean $<$ Lotus pedunculatus II (solely on the basis of the sedimentation coefficient distribution) $<$ Lucerne.

SECTION VII : CONCLUSIONS

A. The Relationship Between Viscosity and Molecular Weight : the Staudinger (or Mark-Houwink) Relationship

From empirical evidence Staudinger (1930) suggested the equation

$$[\eta] = K'M \qquad \text{(VII-1)}$$

as a method of determining the molecular weight of a linear polymer from its intrinsic viscosity. For a series of linear polymers of the same structure differing only in molecular weight, K' would be expected to be a constant.

It was later suggested (Mark (1938) and Houwink (1941)) that Staudinger's equation should be modified to the form

$$[\eta] = K'M^a \qquad \text{(VII-2)}$$

(where "a" is also a constant.)

to obtain a better correlation between the intrinsic viscosity and molecular weight.

According to recent hydrodynamic theories the value of the exponent, a, can be used as an indication of molecular conformation in solution (Tanford, 1961, P.409). This conformation is based on either a random-coil model of the molecule in solution (Flory, 1953, P.404 ff), or a rod model. For $a=0.5$ (the lower limit for the random-coil model) the molecule is tightly coiled and impermeable, with no draining of solvent through the molecular coils. For $a=1.0$ (the upper limit for the random-coil model) the molecule is flexibly coiled, allowing free drainage of solvent. For $a > 1.8$, the molecule approximates to a rod with constant diameter and molecular length proportional to molecular weight.

The equation (VII-2) is strictly applicable only to homogeneous polymers. For heterogenous polymers having a spectrum of molecular weights, a viscosity-average molecular weight $\bar{M}_v$ can be defined (Flory, 1953, P.313) such that

$$\bar{M}_V = \left[\sum W_i M_i^a\right]^{\frac{1}{a}} = \left[\frac{(\sum N_i M_i)^{(1 + a)}}{\sum N_i M_i}\right]^{\frac{1}{a}} \qquad \text{(VII-3)}$$

and $\bar{M}_N < \bar{M}_V < \bar{M}_W$

$W_i = \frac{C_i}{C}$, the weight fraction of species i) in the whole polymer.
N_i = the number of molecules of species i)

If a=1, then $\bar{M}_V = \bar{M}_W$, the weight-average molecular weight.

Thus the Staudinger equation becomes

$$[\eta_i] = K'\bar{M}_V^a \qquad \text{(VII-4)}$$

The viscosity-average molecular weight is not normally very sensitive to the value of "a", and $\bar{M}_V$ lies closer to the weight-average molecular weight than to the number-average.

In practice the values of K' and "a" are obtained by making separate measurements of intrinsic viscosity and molecular weight. The average molecular weight used is seldom the viscosity-average, more normally the weight-average, weight-weight average, or number-average is employed as they are easier to determine. This introduces an error into the relationship between $[\eta_i]$ and molecular weight. If the ratio $\frac{\bar{M}_V}{\text{determined average molecular weight}}$ is about the same for all of the samples within a series, however, the error resides in the value of K', and not in the exponent "a" which is of prime interest. If the ratio varies too widely, no consistent relationship will be obtained between the intrinsic viscosity and the measured average molecular weight (Flory, 1953, P.313).

In the present study, plots have been made of the logarithm of the galactomannan intrinsic viscosities (at mean shear rate $\bar{G} = 1$) against the logarithm of their sedimentation-diffusion average molecular weights, (fig. VII-1a), and against the logarithm of their weight-weight average molecular

Figure (VII–1a) :– The Dependence of Galactomannan Intrinsic Viscosities on the Sedimentation–Diffusion Average Molecular Weight

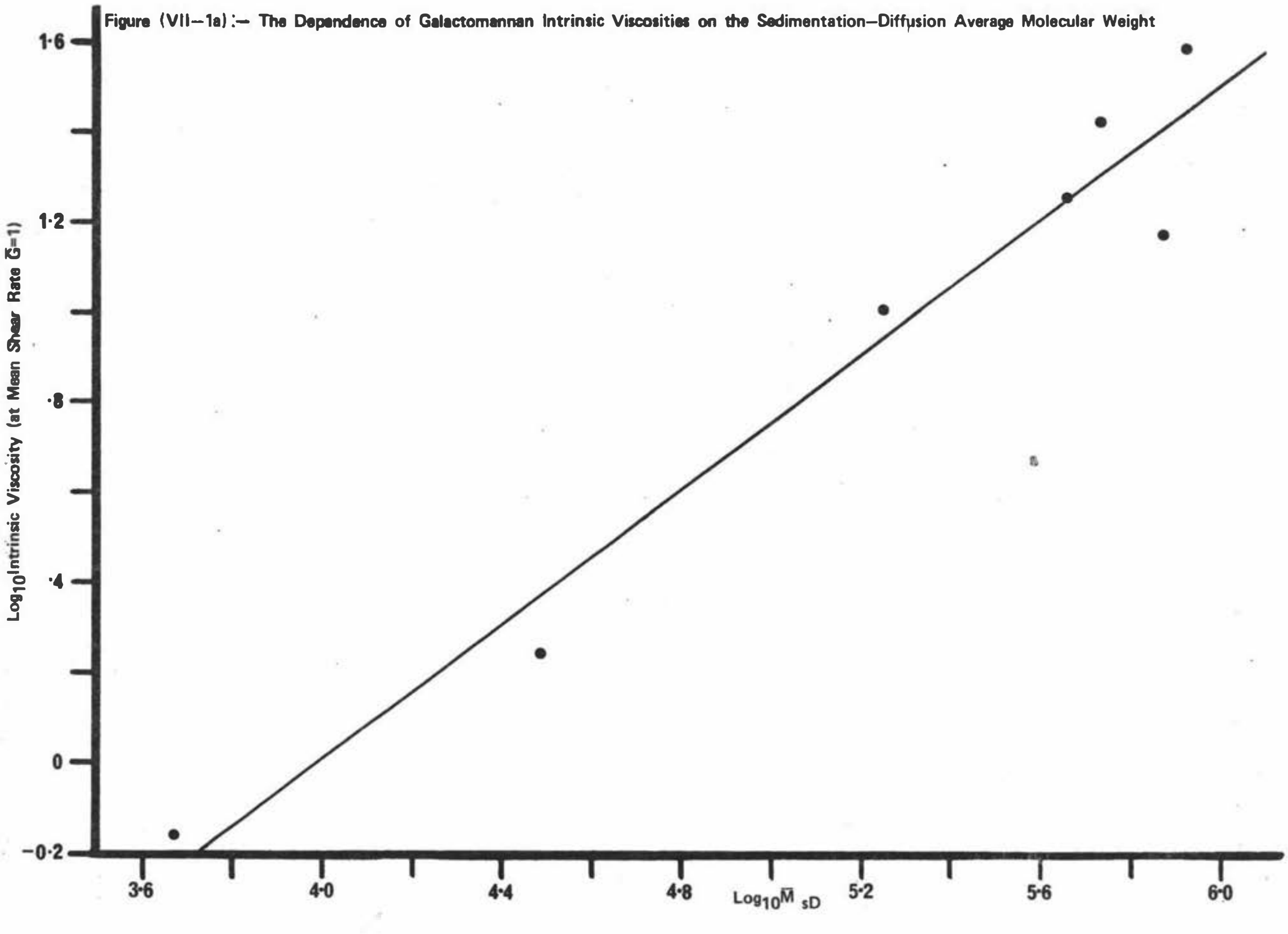
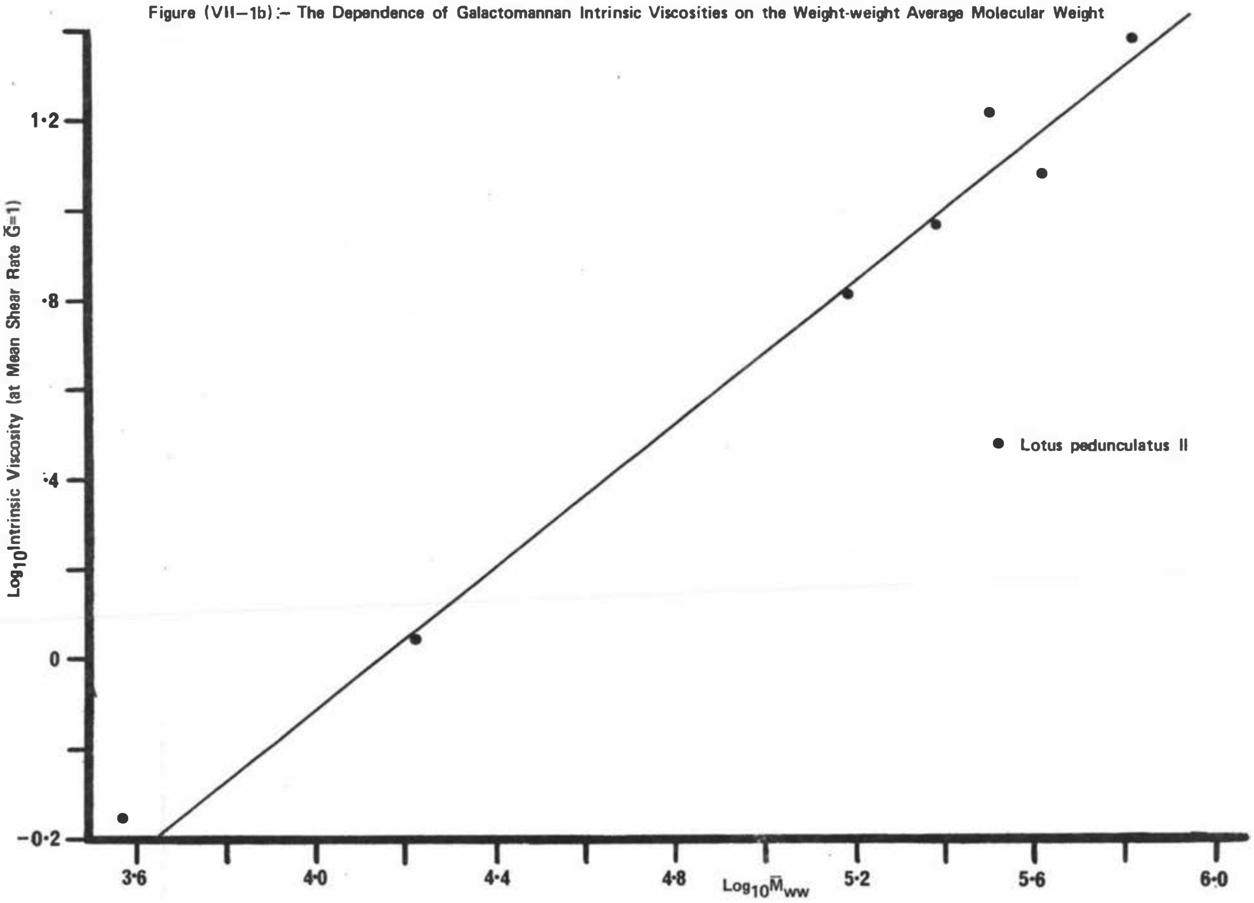


Figure (VII–1b) :– The Dependence of Galactomannan Intrinsic Viscosities on the Weight-weight Average Molecular Weight



weights (fig. VII-1b).

The values of K' and (a) have been determined, and also the correlation coefficients. The molecular weight and intrinsic viscosity of the galactomannan from Lotus pedunculatus II have not been included in the above calculation as the relationship between them does not follow that of the other galactomannans.

Although the molecular weight distribution of Lucerne galactomannan has been shown to be extremely broad (Section VI,P.121), its molecular weight and viscosity data have been included in the calculations above because the relationship between molecular weight and intrinsic viscosity for this galactomannan appears to be the same as the others. Correlation coefficients and the values of K' and (a) appear in table (VII-1).

From the value of (a) it can be deduced that the galactomannan molecule in solution has a conformation between that of a flexibly coiled molecule allowing free drainage of solvent and an imperviously coiled molecule. A similar structure has been found for high molecular weight ($\bar{M}_W > 10^6$) guaran triacetate and some cellulose derivatives in solution; for which values of K' and (a) are presented in table (VII-2) for comparison.

Table (VII-1)

Values of K' and "a" in $[\eta] = K'M^a$ for Galactomannans in Water

Relationship between $\log_{10}[\eta]$ and	Correlation Coefficient	K'	a
$\log_{10}\bar{M}_{s,D}$	0.977	1.04×10^{-3}	0.75
$\log_{10}\bar{M}_{W,W}$	0.987	6.70×10^{-4}	0.81

Table (VII-2)

Values of K' and "a" in $[\eta] = K'\bar{M}^a_{W,W}$ for Guaran Triacetate, Cellulose, and some Cellulose Derivatives in Various Solvents

Polysaccharide	Solvent	Source	K'	a
Hydroxyethyl-cellulose	water	Brown, 1962	1.03×10^{-3}	0.70
Ethylhydroxyethyl-cellulose	water	Manley, 1956	3.7×10^{-4}	0.80
Cellulose	cadoxen	Henley, 1962	-	0.75
Guaran Triacetate ($[\eta] = K'\bar{M}^a_W$)	acetonitrile	Koleske & Kurath, 1964	2.62×10^{-5}	0.87
Galactomannan	water	this work	6.70×10^{-4}	0.81

The difference between the viscosity/molecular weight relationship of Lotus pedunculatus II galactomannan and that of the other galactomannans may be due to the former's different shape in solution. The equations relating the data points of Lotus pedunculatus I and II galactomannans are

$$[\eta] = 7.6 \times 10^{-2}\ \bar{M}^{0.50}_{s,D} \quad \text{(VII-5)}$$

and

$$[\eta] = 0.93\ \bar{M}^{0.48}_{W,W} \quad \text{(VII-6)}$$

The value of the exponent "a" is now that corresponding to a tightly-coiled, impermeable molecule. As Lotus pedunculatus II galactomannan is fully substituted, i.e. one α-1,6 D-galactopyranose unit is linked to every D-mannopyranose unit in the backbone, it seems possible that this may have altered the draining characteristics of the molecule. In this light it is significant that the data points of the highly-substituted Lucerne and Red Clover galactomannans lie below the least-squares line in both plots, whereas the more lightly-substituted Carob, Guar, and Sophora japonica (in one case) galactomannans lie above it. Although the degree of substitution of Lucerne and Red Clover galactomannans is not much less than that of Lotus pedunculatus II galactomannan, it appears that substitution of each mannose of the backbone of a galactomannan molecule by one galactose residue is necessary to observe any drastic changes in solution conformation, and hence hydrodynamic behaviour.

B. Galactomannan Molecular Dimensions from Recent Hydrodynamic Theories

(1) General Basis of the Theories (after Flory, (1953) P.401ff.).

The number of possible conformations of a linear polymer molecule become very large when the number of sub-units (or elements) is greater than about 100. Detailed analysis of the actual molecular conformations becomes impossible and statistical methods must be used. Owing to the large number of statistical elements in a polymer chain statistical formulae may be reduced in nearly all cases to asymptotic expressions which are of the same general form regardless of structure.

The assumption is made that a linear polymer molecule can be represented by a hypothetical chain, consisting of links joined in linear sequence, with successive links randomly oriented to each other. The dimension of such a chain polymer most used to characterize its' spatial conformation is the distance "r" from one end of the chain moleule to the other. This quantity is designated the displacement length of the chain as opposed to the length of the fully extended chain which is the contour length (r_{max}). The statistical average value of r normally used is the root-mean-square end-to-end distance $(\bar{r}^2)^{\frac{1}{2}}$. A further measure of the effective size of the molecule is the average distance of a chain element from the centre of gravity of the molecule. This is normally referred to as the mean radius of gyration of the molecule $(\bar{s}^2)^{\frac{1}{2}}$. For linear chain molecules, $(\bar{s}^2)^{\frac{1}{2}}$ can be obtained from $(\bar{r}^2)^{\frac{1}{2}}$.

Most hydrodynamic theories have the requirement that the value of r, follows Gaussian statistics for the molecules under consideration. This requirement can be written as

$$(\bar{r}^2)^{\frac{1}{2}} = ln^{\frac{1}{2}} \qquad \text{(VII-7)}$$

where l = length of a chain element

n = number of elements in the chain.

In all real polymer chains the direction assumed by a given bond is strongly influenced by its predecessors in the chain, and therefore depends specifically on the structure of the chain unit. The resultant effect is to expand the configuration in comparison with that for a freely jointed chain of the same contour length.

Further problems arise from long range intramolecular interactions. If allowance is made for the finite cross-section of the chain, no two elements remote from each other may occupy the same volume in space (volume exclusion principle), and additionally interference between chain units will also rule out some conformations. This also leads to a more expanded conformation than would be expected from simple statistics.

Solvents would be expected to affect polymer conformation. When the energy of interaction of a polymer element and an adjacent solvent molecule exceeds the mean of the energies of the interaction of the solvent-solvent and polymer-polymer pairs, the molecule will expand to reduce the frequency of contact between pairs of polymer elements. Conversely, where the energy of interaction is unfavourable, more compact conformations in which polymer-polymer contacts occur more frequently will be preferred.

Overall, the problem of determining polymer conformation can be seen to depend on two types of effects. Short-range effects have to do with relationships (bond-length, bond angle) between units of the chain which are sequentially very close. Long-range effects (i.e. thermodynamic interactions, including the interference effect) are in essence dependent on encounters between pairs of polymer elements remote from each other sequentially in the chain. Short-range effects depend only on the molecular parameters, whereas long-range effects depend on both the molecule and its environment. The various hydrodynamic theories arise from attempts to rationalise all the above effects. This is normally achieved by representing the polymer chain used previously as a string of beads (the so-called pearl string model), and considering its frictional properties in solution.

If the solvent is only very slightly perturbed by the molecule as it moves through it, the molecule is described as being "free-draining", and the velocity of the solvent is approximately the same as it would be if the molecule were not present. Experimentally, the above hypothesis was found to be unsound (Flory, 1953, P.605) and was replaced by a consideration of the degree of solvent entrappment by the molecule. Solvent towards the centre of an actual molecule acquires a velocity nearer that of the molecule than that of the surrounding medium. This leads (Flory, ibid) to the concept of an equivalent hydrodynamic sphere, impenetrable to solvent, which displays the same frictional coefficient as the actual molecule.

(2) Outline of Recent Hydrodynamic Theories

(a) Debye, Beuche and Brinkman

Debye and Beuche (1948), and Brinkman (1947 a & b, 1949) used a generalisation of Einstein's theory for impermeable spheres to rationalise linear polymer solution behaviour. They replaced the randomly coiled molecule by an equivalent sphere of uniform resisting point density, where each point is a "bead" of the pearl string model. The flow of liquid through the interior of the sphere was considered as being hindered only to a degree dependent on the average density in space of the polymer molecule in solution. The flow disturbance appears as a volume factor, ϕ_σ or ψ_σ, which are functions of the shielding ratio σ,

$$\sigma = R_s L \qquad \text{(VII-8)}$$

where L = solvent permeability factor, and

R_s = radius of the equivalent sphere.

The amount of shielding in this way determines the exponents in the normal exponential relationships between intrinsic viscosity, diffusion coefficient, sedimentation coefficient, and the molecular weight; i.e. exponents ϵ_1, and ϵ_2 in:

$$[\eta] = KM^{\epsilon_1} \qquad \text{(VII-9)}$$

$$s_o = K_s M^{(1-\epsilon_2)} \qquad \text{(VII-10)}$$

$$D = K_D M^{\epsilon_2} \qquad \text{(VII-11)}$$

s_o = sedimentation coefficient

D = diffusion coefficient

K_s, K_D are constants.

R_s was shown to be related to the root-mean-square end-to-end distance of the molecule $(\bar{r}^2)^{\frac{1}{2}}$ by

$$2R_s = 1.054(\bar{r}^2)^{\frac{1}{2}} \qquad \text{(VII-12)}$$

thus the dimensions of the molecule can be obtained from Debye and Beuches' (1948) equations:

$$[\eta] = \frac{\frac{4\pi}{3} R_s N_A \phi_\sigma}{M} \qquad \text{(VII-13)}$$

$$f = 6\pi \eta_o R_s \psi_\sigma \qquad \text{(VII-14)}$$

f = frictional coefficient of one molecule

η_o = solvent viscosity

hence $F = N_A f$ (VII-15)

N_A = Avagadro's number

F = molar frictional coefficient.

The equations for sedimentation and diffusion coefficients are:

$$s_o = \frac{(1-\bar{v}\rho)M}{F} \qquad \text{(VII-16)}$$

$$D = \frac{RT}{F} \qquad \text{(VII-17)}$$

$\bar{v}$ = partial specific volume of solute (ml/g) p = density of solute (g/ml)

R = Gas constant

T = temperature (in degrees absolute)

Substituting in (VII-16), (VII-17) above from (VII-14) and (VII-15) gives:

$$s_o = \frac{(1-\bar{v}p)M}{6\eta_o R_s N_A \psi_\sigma} \qquad \text{(VII-18)}$$

$$D = \frac{kT}{6\eta_o R_S \psi_\sigma} \qquad \text{(VII-19)}$$

(k = Boltzman's constant)

It was stressed that relationship between $[\eta]$ and molecular weight is indirect, and depends essentially on the type of polymer molecule under consideration. The equations for s_o and D are valid only for a free-draining or near free-draining molecule, with low shielding ratios.

(b) Kuhn and Kuhn

Kuhn and Kuhn (1948, 1950 a & b) considered the polymer molecule in terms of the random coil model. They removed the restriction of the bond direction of a chain element being influenced by its predecessor by replacing the elements with straight-line molecular segments of length A_m and thickness d_h which were randomly oriented. For a chain of the same contour length as the actual molecule the number of bonds will thus be less. A_m becomes a useful measure of chain flexibility, and hence of short-range interactions affecting segment length.

They used large-scale wire models bent in a statistical manner, and, in a series of hydrodynamic experiments, studied their frictional resistance in fluids to translational and rotational motion. In this way a series of equations was derived relating the sedimentation coefficient, the diffusion coefficient, and the intrinsic viscosity to the degree of polymerisation of the molecule (Z):

$$s_o = a_1 + b_1 Z^{\frac{1}{2}} \qquad \text{(VII-20)}$$

$$D = (a_2 + b_2Z^{\frac{1}{2}}) \qquad \text{(VII-21)}$$

$$[\eta] = \frac{Z}{a_3 + b_3Z^{\frac{1}{2}}} \qquad \text{(VII-22)}$$

The constants a_1, a_2, a_3, b_1, b_2, b_3, are independent of Z but depend on the average thickness (d_h) and the length of the statistical chain element (A_m) of the molecule. The original values of the constants derived by Kuhn and Kuhn were revised by Kuhn, Kuhn, and Silberberg (1954) to give better agreement between sedimentation/diffusion and viscosity data.

The segment length of the Kuhn equivalent chain is related to $(\bar{r}^2)^{\frac{1}{2}}$ by:

$$(\bar{r}^2)^{\frac{1}{2}} = A_m\bar{r}_{max} \qquad \text{(VII-23)}$$

where $\bar{r}_{max}$ is the average contour length of the molecule. This latter value can be obtained from:

$$\bar{r}_{max} = b_o\bar{Z} \qquad \text{(VII-24)}$$

where b_o is the length of the monomer unit

$\bar{Z}$ is the average degree of polymerisation of the molecule.

Thus the root-mean-square end-to-end distance of the molecule is:

$$(\bar{r}^2)^{\frac{1}{2}} = A_m b_o\bar{Z} \qquad \text{(VII-25)}$$

(c) Kirkwood and Riseman

Kirkwood and Riseman (1948) studied the polymer molecule in terms of the randomly coiled "pearl string" model, and took into account short-range interactions and the hindered flow of solvent through the molecular coil. They defined b as the bond length of a hypothetical, freely-jointed chain having the same number of bonds as the polymer chain, and hence a much greater contour length. The parameter b thus provides a measure of the stiffness of the molecule (c.f.Kuhn and Kuhn; Debye, Beuche, and Brinkman models).

The flow of solvent through the molecular coils was measured in terms of ς, the frictional coefficient of the monomer unit.

The quantity b can be defined by:

$$(\bar{r^2}) = b^2\bar{Z} \qquad \text{(VII-26)}$$

and Kirkwood and Risemans' theory leads to:

$$b^3 = \frac{3600\ M_o}{N_A(6\pi^3)^{\frac{1}{2}}}\left[\frac{[\eta]}{\bar{Z}^{\frac{1}{2}}}\ \frac{1}{xF(x)}\right] \qquad \text{(VII-27)}$$

$$\text{where } x = \lambda_o\bar{Z}^{\frac{1}{2}},$$

$$\text{and } \lambda_o = \frac{\overset{p}{\zeta}}{(6\pi^3)^{\frac{1}{2}}\eta_o b} \qquad \text{(VII-28)}$$

M_o = monomer weight

By using the fact that xF(x) tends to its maximum value of 1.48 as $\bar{Z}$ tends to infinity, equation (VII-27) can be rewritten in the form (using the revised constants of Kirkwood et. al. (1955)):

$$b^3 = \frac{2435\ M_o}{N_A(6\pi^3)^{\frac{1}{2}}}\left[\lim_{\bar{Z}\to\infty}\left(\frac{[\eta]}{\bar{Z}^{\frac{1}{2}}}\right)\right] \qquad \text{(VII-29)}$$

and the corresponding equations for the sedimentation and diffusion coefficients are:

$$s_o = \frac{M_o(1-\bar{v}\rho)}{N_A\ \zeta}\left[1 + \frac{8}{3}\lambda_o\bar{Z}^{\frac{1}{2}}\right] \qquad \text{(VII-30)}$$

$$D = \frac{kT}{\bar{Z}\zeta}\left[1 + \frac{8}{3}\lambda_o\bar{Z}^{\frac{1}{2}}\right] \qquad \text{(VII-31)}$$

(d) Peterlin

Peterlin (1950, 1952) considered the polymer molecule in solution as a loosely coiled chain having a higher segment density near its centre, and thus a correspondingly higher resistance to solvent flow in this region. In common with the expressions derived by Debye and Beuche (1948), Brinkman (1947, 1949), and Kirkwood and Riseman (1948, 1955), Peterlin's formulae linking intrinsic viscosity and sedimentation coefficient with molecular weight showed that $\frac{M}{[\eta]}$ and s_o were proportional to $M^{\frac{1}{2}}$. From the slopes of plots of these quantities the root-mean-square end-to-end distances can be obtained from: $$(\bar{r}^2)^{\frac{1}{2}} = 1.52 \times 10^{-8} \, (100 \cot \alpha)^{1/3} \, \bar{M}^{\frac{1}{2}} \qquad \text{(VII-32)}$$

where $\tan \alpha =$ slope of $\frac{\bar{M}}{[\eta]} \, / \, \bar{M}^{\frac{1}{2}}$ plot.

(e) Flory and Fox; Mandelkern and Flory

Flory and Fox (1951a & b) also used the random-coil model of the polymer molecule but assumed that the coil permeability to solvent remained constant, and considered the effect of molecular weight on the coil dimensions. From thermodynamic calculations an expansion factor α was defined representing the dependence of the molecular dimensions on long-range effects. If the unperturbed root-mean-square end-to-end distance of the molecule is $(\bar{r}_o^2)^{\frac{1}{2}}$, then the root-mean-square end-to-end distance for the perturbed molecule is:

$$(\bar{r}^2)^{\frac{1}{2}} = (\bar{r}_o^2)^{\frac{1}{2}} \alpha \qquad \text{(VII-33)}$$

Flory and Fox proposed that the intrinsic viscosity of the polymer molecule is proportional to its effective volume in solution, and inversely proportional to its molecular weight. They further suggested that $(\bar{r}^2)^{\frac{1}{2}}$ is also proportional to the effective molecular volume in solution, which leads to:

$$[\eta] = \Phi \left[\frac{\bar{r}_o^2}{\bar{M}} \right]^{3/2} \bar{M}^{\frac{1}{2}} \alpha^3 \qquad \text{(VII-34)}$$

or $$[\eta] = \Phi \, (\bar{r}^2)^{3/2} \, \frac{1}{\bar{M}} \qquad \text{(VII-35)}$$

(by substitution from equation (VII-33))

Φ should be a universal constant, independent of the characteristics of a given chain molecule (apart from the requirement that its solution conformation is that of a random coil).

Mandelkern and Flory (1952) showed that at sufficiently high molecular weights the molecular frictional coefficient (f_o) depends only on the linear dimension of the molecule ($(\bar{r}^2)^{\frac{1}{2}}$ or $(\bar{s}^2)^{\frac{1}{2}}$).

Thus $$\frac{f_o}{\eta_o} = P\,(\bar{r}^2)^{\frac{1}{2}} \qquad \text{(VII-36)}$$

P is also a universal constant, analagous to ϕ, and similarly should be independent of the polymer/solvent system.

Expressing the sedimentation and diffusion coefficients in terms of the molecular frictional coefficients gives (Tanford, 1961, P.349 & P.365)

$$s_o = \frac{\bar{M}(1-\bar{v}p)}{f_o N_A} \qquad \text{(VII-37)}$$

$$D = \frac{kT}{f_o} \qquad \text{(VII-38)}$$

Substituting from equation (VII-36) gives:

$$s_o = \frac{\bar{M}(1-\bar{v}p)}{P\eta_o N_A (\bar{r}^2)^{\frac{1}{2}}} \qquad \text{(VII-39)}$$

$$D = \frac{kT}{P\eta_o\ (\bar{r}^2)^{\frac{1}{2}}} \qquad \text{(VII-40)}$$

Eliminating $(\bar{r}^2)^{\frac{1}{2}}$ between equations (VII-39) and the Flory-Fox equation (VII-35) gives:

$$\frac{s_o\,([\eta]\,\bar{M})^{1/3}}{\bar{M}} = \phi^{1/3}P^{-1}\left[\frac{1-\bar{v}p}{\eta_o N_A}\right] \qquad \text{(VII-41)}$$

For a series of polymers of the same type and in the same solvent, the R.H.S. of equation (VII-41) is constant, thus $\dfrac{s_o([\eta]\,\bar{M})^{1/3}}{\bar{M}}$ would also be expected to be constant.

$\phi^{1/3}P^{-1}$ should be a constant for all polymer systems.

(3) Calculation of Galactomannan Molecular Conformation from Solution Behaviour

(a) General Comments

The hydrodynamic theories previously outlined were, like Staudingers' equation, derived for homogeneous polymer systems. However, provided that the molecular weight distribution of the polymer fractions is very similar, and not too broad, a good approximation of the polymer solution behaviour may still be expected. For this reason the data of Lucerne galactomannan has not been used in combination with that of any of the other galactomannans. Likewise the data of Lotus pedunculatus II has been excluded since this latter appears to be a special case.

The theories of Kuhn and Kuhn, and Peterlin both make use of linear plots of $\frac{\bar{Z}}{[\eta]}$ against $\bar{Z}^{\frac{1}{2}}$, s_o against $\bar{Z}^{\frac{1}{2}}$ and $D\bar{Z}$ against $\bar{Z}^{\frac{1}{2}}$. The Kirkwood and Riseman theory uses the same plots for s_o and D, but replaces the above viscosity plot with the closely related $\frac{[\eta]}{\bar{Z}^{\frac{1}{2}}}$ against $\frac{1}{\bar{Z}^{\frac{1}{2}}}$ plot. These plots have been made (see figs. (VII-2a, b); (VII-3a, b)) using weight-weight average data, as this is better defined mathematically than a sedimentation/diffusion average. The weight-weight average data used appears in table (VII-3).

Table (VII-3)

Galactomannan Molecular Weights, Degrees of Polymerisation, Sedimentation and Diffusion Coefficients, and Intrinsic Viscosities (all at 20°C).

Galactomannan	$\bar{M}_{W,W} \times 10^{-3}$	$\bar{Z}_{W,W}$	$s_o \times 10^{+13}$	$D_m \times 10^{+7}$	$[\eta]$
Lotus pedunculatus I	3.7	23	1.35	24.2	0.69
Soybean	16.6	102	1.72	6.90	1.76
Sophora japonica	152	938	3.02	1.32	10.3
Carob	319	1970	4.65	0.97	26.4
Red Clover	416	2570	4.81	0.77	19.2
Guar	657	4060	5.33	0.54	38.5

(The partial specific volume was taken as 0.63 ml/g, and solvent (water) density as 0.998 g/ml)

The weight-weight average degree of polymerisation ($\bar{Z}_{W,W}$) was obtained from the weight-weight average molecular weight ($\bar{M}_{W,W}$) by dividing it by the molecular weight of an anhydrohexose residue.

Plots of log s_o against log $\bar{M}_{w,w}$, and log D_m against log $\bar{M}_{w,w}$ required by the Debye, Beuche and Brinkman theory have been previously plotted (fig. (V-6). Equations of all plots appear in table (VII-4).

The slopes and intercepts of the experimental plots were equated to those in the theoretical equations in order to obtain the various molecular parameters.

Table (VII-4)

Equations Relating Intrinsic Viscosity, Sedimentation Coefficient and Diffusion Coefficient to Molecular Weight and Degree of Polymerisation

$[\eta] = 6.70 \times 10^{-4}\ \overline{M}_{w,w}^{0.81}$	(VII-42)
$s_o = 1.36 \times 10^{-14}\ \overline{M}_{w,w}^{0.29}$	(VII-43)
$D_m = 8.22 \times 10^{-4}\ \overline{M}_{w,w}^{-0.72}$	(VII-44)
$[\eta] = \dfrac{\overline{Z}_{w,w}}{1.27\ \overline{Z}_{w,w}^{\frac{1}{2}} + 39.3}$ *	(VII-45)
$s_o = (0.069\ \overline{Z}_{w,w} + 1.15) \times 10^{-13}$ *	(VII-46)
$D_m = \dfrac{(0.30\ \overline{Z}_{w,w} + 4.18) \times 10^{-5}}{\overline{Z}_{w,w}}$ *	(VII-47)
* = from figs. (VII-2 and 3a)	

The exponents of equations (VII-42) to (VII-44) show the galactomannan molecule in solution to have a conformation between that of a free-draining molecule and an impermeable coil. The limits of these exponents are (Henley, 1962):

intrinsic viscosity ; impermeable, 0.5 -------------free-draining, 1.0

sedimentation coeff. ; impermeable, 0.5 -------------free-draining, 0.0

diffusion coeff. ; impermeable, 0.5 -------------free-draining, 1.0.

(b) Theory of Debye, Beuche, and Brinkman

The equations used to calculate $(\overline{r}^2)^{\frac{1}{2}}_{w,w}$ were essentially those obtained from Debye and Beuches' theory, i.e. equations (VII 13), (VII-18), and (VII-19). The value of R_s, the radius of the equivalent sphere, was replaced by $(\overline{r}^2)^{\frac{1}{2}}_{w,w}$ using equation (VII-12); i.e.

$$2\ R_s = 1.054\ (\overline{r}^2)^{\frac{1}{2}}_{w,w} \qquad \text{(VII-12)}$$

This gave the following equations:

$$(\overline{r}^2)^{\frac{1}{2}}_{w,w} = \frac{1}{1.054} \left[\frac{6}{N_A \Phi_\sigma \pi}\right]^{1/3} \left[100[\eta]\overline{M}_{w,w}\right]^{1/3} \qquad \text{(VII-48)}$$

$$(\overline{r}^2)^{\frac{1}{2}}_{w,w} = \frac{(1-\overline{v}p)\overline{M}_{w,w}}{6\ N_A \pi 0.527 \eta_o \psi_\sigma\ s_o} \qquad \text{(VII-49)}$$

Figure (VII–2a):— The Dependence of Galactomannan Weight–Average Diffusion Coefficients on the Weight-weight Average Degree of Polymerisation (Kuhn and Kuhn; Peterlin; Kirkwood and Riseman)

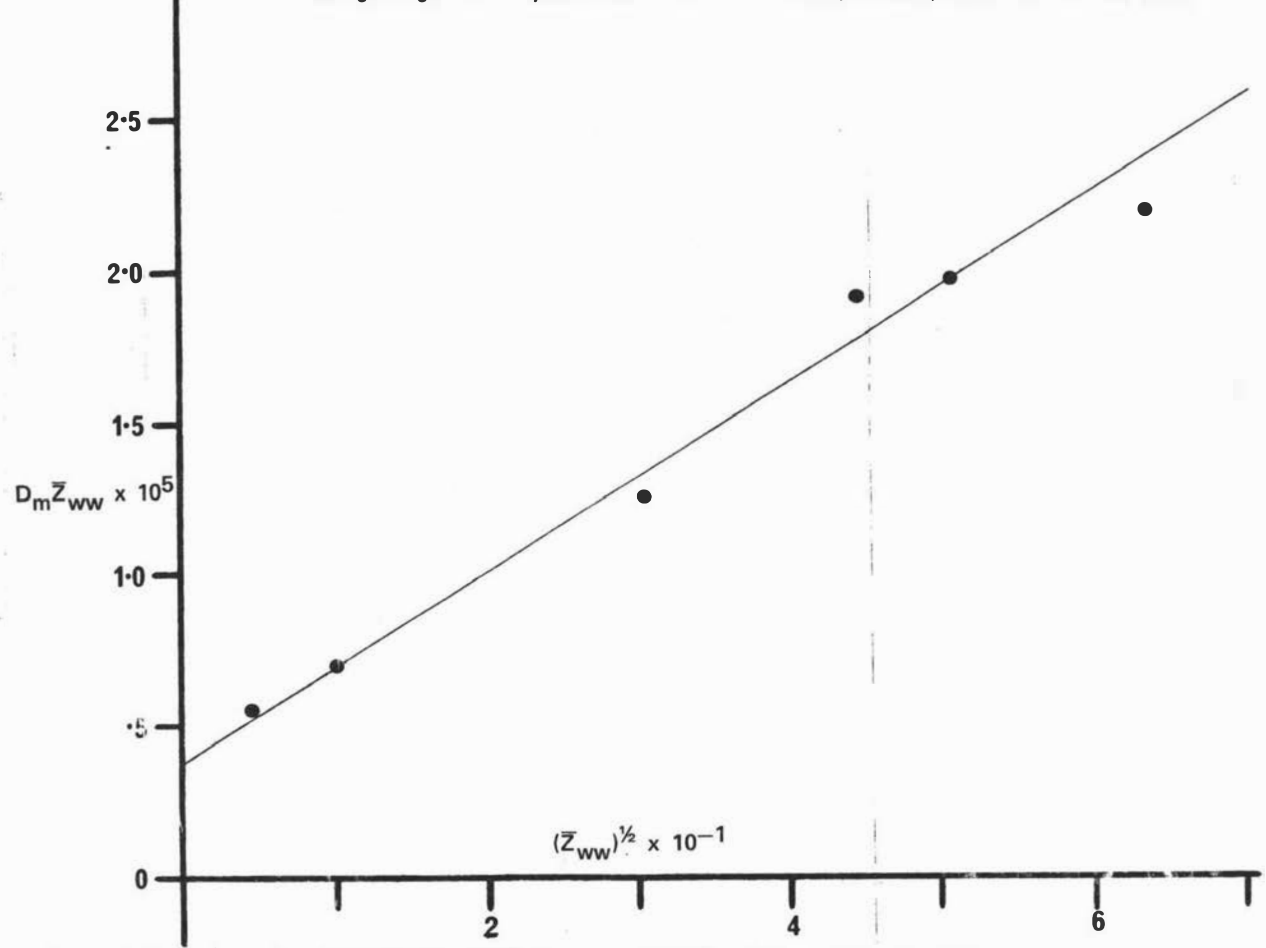


Figure (VII– 2b):— The Dependence of Galactomannan Limiting Sedimentation Coefficients on the Weight-weight Average Degree of Polymerisation (Kuhn and Kuhn; Peterlin; Kirkwood and Riseman).

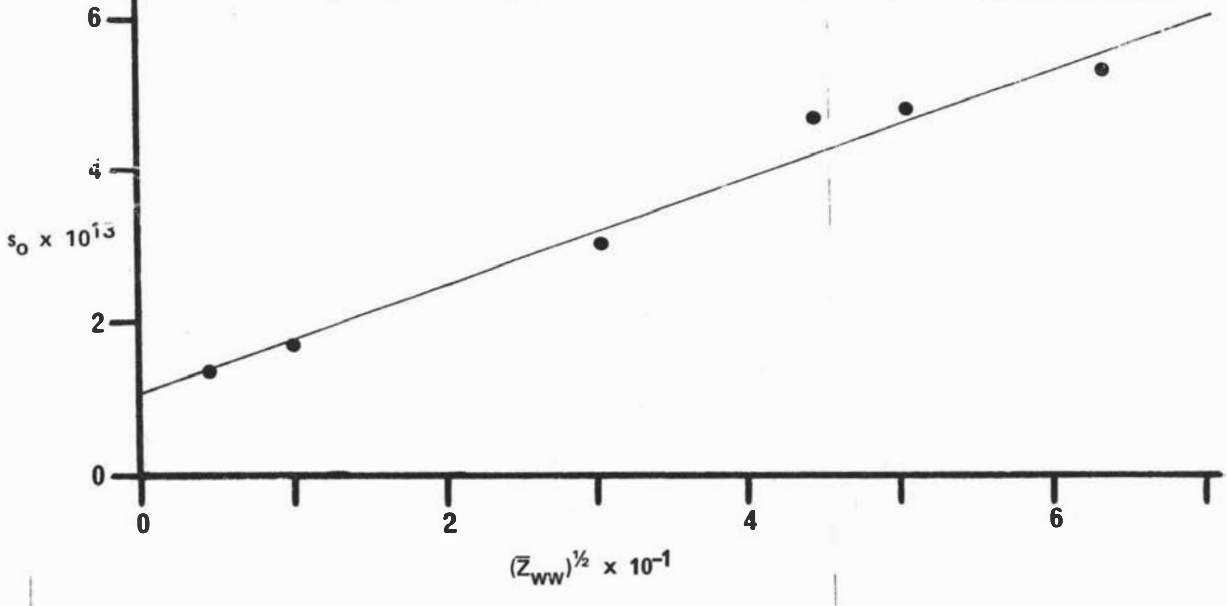


Figure (VII–3a):–The Dependence of Galactomannan Intrinsic Viscosities on the Weight-weight Average Degree of Polymerisation (Kuhn and Kuhn; Peterlin)

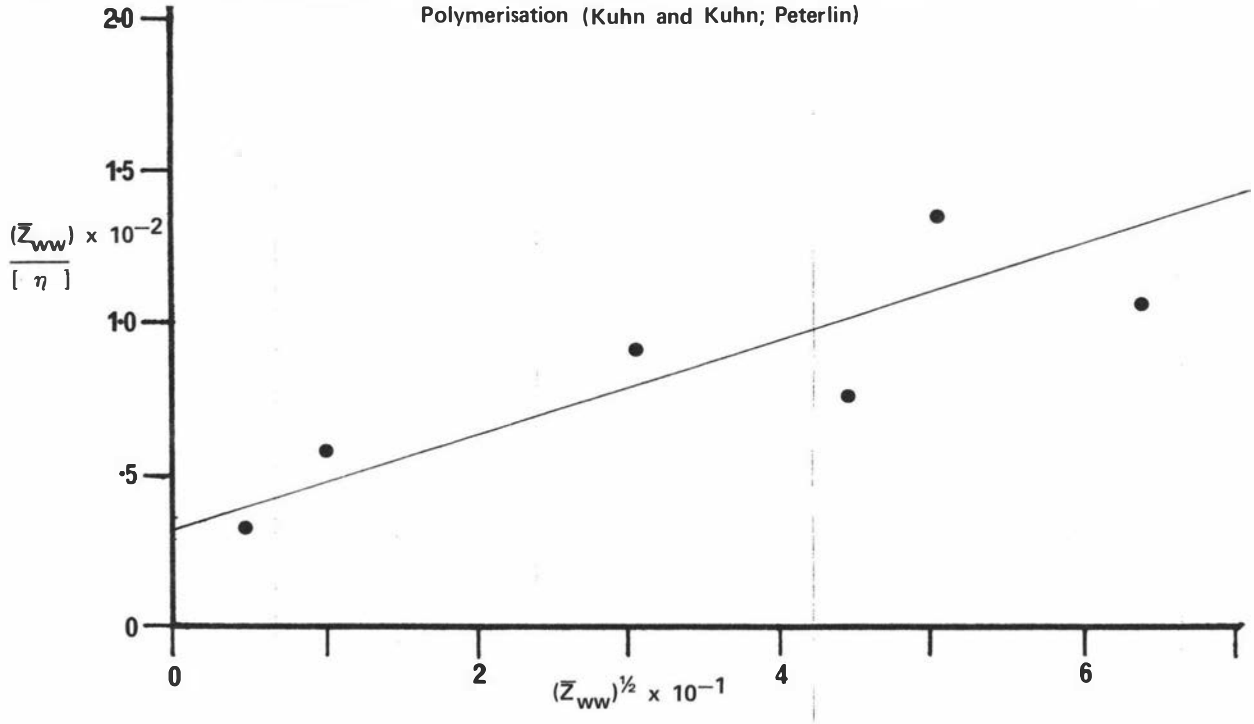
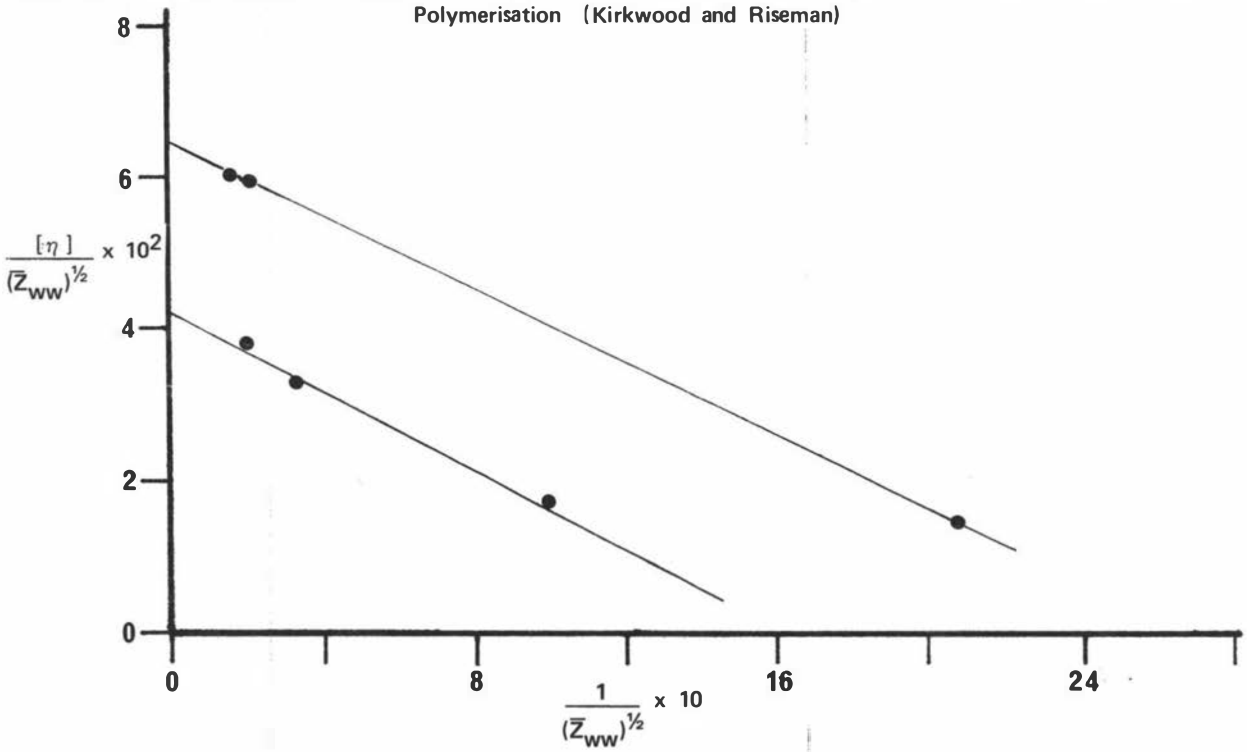


Figure (VII–3b):–The Dependence of Galactomannan Intrinsic Viscosities on the Weight-weight Average Degree of Polymerisation (Kirkwood and Riseman)



$$(\bar{r}^2)^{\frac{1}{2}}_{w,w} = \frac{kT}{6\pi \quad 0.527\eta_o \Psi_\sigma D_m} \tag{VII-50}$$

The values of the sheilding constants (ϕ_σ and Ψ_σ) were obtained from the exponents of equations (VII-42) to (VII-44). The requirement that the exponent of equation (VII-43) + the exponent of equation (VII-44) = 1 is seen to hold (i.e. $(1-\epsilon_2) + \epsilon_2 = 1$; or $0.29 + 0.72 \doteq 1.$)

Values of $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ calculated for each galactomannan appear in tables (VII-8) and (VII-9).

(c) <u>Theory of Kuhn and Kuhn</u>

The equations used in the calculation of A_m, the equivalent chain segment length, and the hydrodynamic radius d_h were (Brown, 1962):

$$[\eta] = \frac{N_A b_o^2 A_m}{100\ M_o} \left[\frac{0.43\ \bar{Z}_{w,w}}{-1.6 + 2.3 \log_{10}\left[\frac{A_m}{d_h}\right] + \left[\frac{\bar{Z}_{w,w} b_o}{A_m}\right]^{\frac{1}{2}}} \right] \tag{VII-51}$$

$$s_o = \frac{M_o(1 - \bar{v}p)}{N_A b_o \eta_o} \left[-0.03 + 0.16 \log_{10}\left[\frac{A_m}{d_h}\right] + 0.136 \left[\frac{\bar{Z}_{w,w}\ b_o}{A_m}\right]^{\frac{1}{2}} \right] \tag{VII-52}$$

$$D_m \bar{Z}_{w,w} = \frac{kT}{b_o \eta_o} \left[-0.03 + 0.16 \log_{10}\left[\frac{A_m}{d_h}\right] + 0.136 \left[\frac{\bar{Z}_{w,w}\ b_o}{A_m}\right]^{\frac{1}{2}} \right] \tag{VII-53}$$

where the modified constants are those of Kuhn et. al. (1954). Substitution of the relevant slopes and intercepts from table (VII-4) into the above equations allowed A_m and d_h to be calculated. The value of b_o, the length of the mannopyranose and galactopyranose residues is 5.15Å as used by Koleske and Kurath (1964). Results are presented in table (VII-5), with those of some water-soluble cellulose derivatives for comparison.

The discrepancy between the values of A_m obtained by sedimentation and diffusion measurements and those obtained by viscometry has been previously noted (Manley, 1956; Brown, 1962; Henley, 1962). Henley

Table (VII-5)

Molecular Parameters from the Kuhn-Kuhn Theory

Sample		Sedimentation	Diffusion	Sedimentation-Diffusion Average	Viscosity
Galactomannan	Am	76 Å	70 Å	73 Å	122 Å
	dh	9.3×10^{-3} Å	2.1×10^{-3} Å	5.7×10^{-3} Å	3.22 Å
Hydroxyethyl Cellulose (Brown, 1962)	Am			51 Å	182 Å
	dh			9.9 Å	1.1 Å
Ethylhydroxyethyl Cellulose (Manley, 1956)	Am			120 Å	216 Å*
	dh			0.1 Å	

* Calculated by Brown, 1962.

interpreted it as a departure from Gaussian statistics (i.e. from $\bar{r}^2 \sim \bar{Z}$), and therefore to partial failure of the hydrodynamic model. Both Henley and Brown found that the value of A_m calculated from light-scattering measurements was nearer that obtained from the intrinsic viscosity than from sedimentation and diffusion measurements. On this basis it would appear that galactomannans in solution are more flexible than the cellulose derivatives from the values presented in table (VII-5).

The diameter of a substituted cellulose chain in water is known to be of the order of 10 Å (Brown, 1962); therefore d_h for galactomannans could be expected to be of a similar magnitude. The d_h value for galactomannans obtained from sedimentation and diffusion measurements is thus totally unrealistic (as opposed to the results of Brown (1962) and Henley (1962)), while that from viscometry measurements is considerably better, but still too small.

The root-mean-square end-to-end distance of each galactomannan was calculated from equation (VII-23) in the form:

$$(\bar{r}^2_{w,w})^{\frac{1}{2}} = (A_m \bar{r}_{max})^{\frac{1}{2}} = (A_m b_o \bar{Z}_{w,w})^{\frac{1}{2}} \qquad \text{(VII-54)}$$

and the results are given in tables (VII-8) and (VII-9).

(d) <u>Theory of Kirkwood and Riseman</u>

The equations used in the calculation of b, the bond length, and ξ, the monomer frictional coefficient were;

$$b^3 = \frac{2435\ M_o}{N_A(6\pi^3)^{\frac{1}{2}}} \left[\underset{\bar{Z}_{w,w} \to \infty}{\text{limit}} \left[\frac{[\eta]}{\bar{Z}_{w,w}^{\frac{1}{2}}} \right] \right] \qquad \text{(VII-55)}$$

$$s_o = \frac{M_o(1-\bar{v}\rho)}{N_A \xi} \left[1 + \frac{8}{3} \lambda_o \bar{Z}_{w,w}^{\frac{1}{2}} \right] \qquad \text{(VII-56)}$$

$$D_m = \frac{kT}{\bar{Z}_{w,w}\ \xi} \left[1 + \frac{8}{3} \lambda_o \bar{Z}_{w,w}^{\frac{1}{2}} \right] \qquad \text{(VII-57)}$$

Substitution of the relevant slopes and intercepts from table (VII-4) allowed b and ξ to be calculated. The value of $\lim_{\bar{Z}_{w,w} \to \infty} \frac{[\eta]}{\bar{Z}_{w,w}^{\frac{1}{2}}}$ was obtained graphically from the intercept of a plot of $\frac{[\eta]}{\bar{Z}_{w,w}^{\frac{1}{2}}}$ against $\bar{Z}_{w,w}^{\frac{1}{2}}$. The graph, fig. (VII-3b) is far from linear, and two possible intercepts were used in separate calculations of b. Results are presented in table (VII-6), again with those from water-soluble cellulose derivatives for comparison.

(b is calculated from sedimentation and diffusion coefficients according to (Kirkwood and Riseman, 1948)):

$$b = \frac{\xi}{\lambda_o} \frac{1}{(6\pi^3)^{\frac{1}{2}} \eta_o} \qquad \text{(VII-28)}$$

Using both light-scattering and viscosity measurements Koleske and Kurath (1964) obtained Kirkwood-Riseman effective bond lengths of 25.4 Å (highest molecular weight) and 23.3 Å respectively for the galactomannan triacetate of guar in acetonitrile. The sedimentation-diffusion values obtained for galactomannans in table (VII-6) are in good agreement with these values. The difference in the intrinsic viscosity values may be attributable in part to the different solvents.

In comparison with the water-soluble cellulose derivatives of table (VII-6) the galactomannans have a similar rigidity in solution on the basis of the Kirkwood-Riseman theory.

Values of $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ were calculated from equation (VII-26) in the form:

$$(\bar{r}^2)^{\frac{1}{2}}_{w,w} = b^2\bar{Z}_{w,w} \qquad \text{(VII-26)}$$

and appear in tables (VII-8) and (VII-9).

(e) <u>Theory of Peterlin</u>

Values of the root-mean-square end-to-end distance of the galactomannan

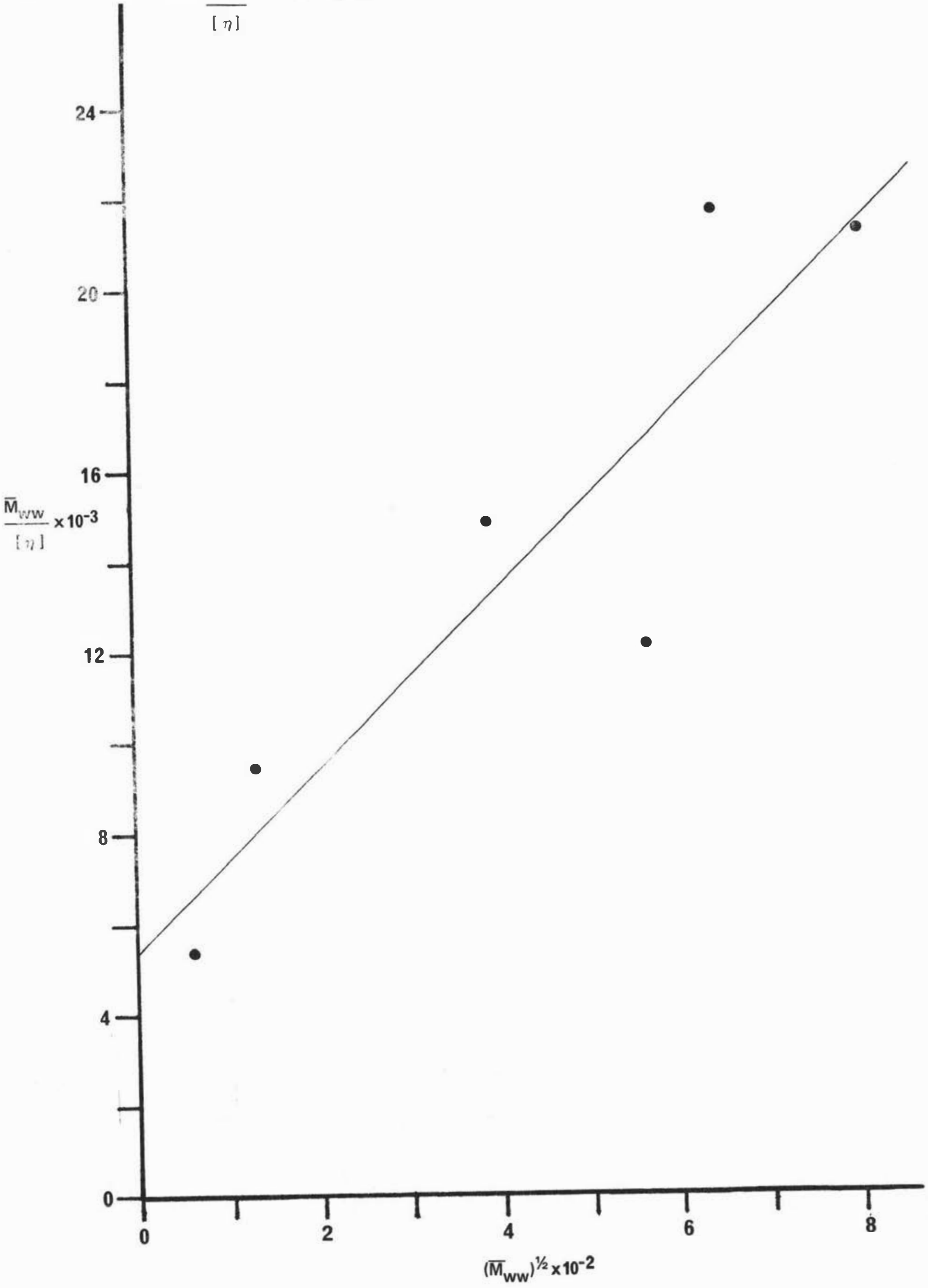
Table (VII-6)

Molecular Parameters from the Kirkwood-Riseman Theory

Sample		Sedimentation	Diffusion	Sedimentation-Diffusion Average	Viscosity
Galactomannan	b	27.1 Å	26.6 Å	26.9 Å	36.4 Å or 41.8 Å
	ζ	8.66×10^{-10}	9.69×10^{-10}	9.18×10^{-10}	
	λ_0	2.23×10^{-2}	2.67×10^{-2}	2.45×10^{-2}	
Ethylhydroxyethyl cellulose (Manley, 1956)	b	37 Å	38 Å*	37.5 Å*	27 Å*
	ζ	11.2×10^{-10}	10.3×10^{-10}*	10.8×10^{-10}*	
	λ_0	2.29×10^{-2}	1.97×10^{-2}*	2.13×10^{-2}*	
Hydroxyethyl cellulose (Brown, 1962)	b			23.3 Å	27.2 Å
	ζ			55×10^{-10}	
	λ_0			19.5×10^{2}	

* Calculated from Manley's data

Figure (VII–4) :– Plot of $\frac{\bar{M}_{ww}}{[\eta]}$ against $(\bar{M}_{ww})^{1/2}$ for Galactomannans (after Peterlin, 1950, 1952)



molecules were obtained from the slope ($\tan\alpha$) of a plot of $\frac{\bar{M}_{w,w}}{[\eta]}$ against $\bar{M}^{\frac{1}{2}}_{w,w}$ (fig. VII-4). Tan α was substituted into equation (VII-32) in the form:

$$(\bar{r}^2)^{\frac{1}{2}}_{w,w} = 1.52 \times 10^{-8} \left(\frac{100}{\tan\alpha}\right)^{1/3} \bar{M}^{\frac{1}{2}}_{w,w} \qquad \text{(VII-58)}$$

Values of $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ are given in table (VII-9).

(f) Theory of Flory, Fox, and Mandelkern.

The values of $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ were calculated from the equations:

$$[\eta] = \Phi \, (\bar{r}^2)^{\frac{3}{2}}_{w,w} \, \bar{M}^{-1}_{w,w} \qquad \text{(VII-59)}$$

$$s_o = \frac{\bar{M}_{w,w}(1-\bar{v}p)}{P\eta_o N_A (\bar{r}^2)^{\frac{1}{2}}_{w,w}} \qquad \text{(VII-60)}$$

$$D_m = \frac{kT}{P\eta_o \; (\bar{r}^2)^{\frac{1}{2}}_{w,w}} \qquad \text{(VII-61)}$$

(where Φ and P are universal constants with value 2.1×10^{21} and 5.1 respectively.)

Results appear in tables (VII-8) and (VII-9).

The value of the quantity $\frac{s_o([\eta]\,\bar{M}_{w,w})^{1/3}}{\bar{M}_{w,w}}$ was calculated for each galactomannan (and appears in table (VII-7)) as according to Mandelkern and Flory (1952) it should be constant for a series of polymer homologs. The value of $\Phi^{1/3}P^{-1}$ also was calculated from:

$$\Phi^{1/3}P^{-1} = \frac{\eta_o N_A}{(1 - \bar{v}p)} \; \frac{s_o([\eta]\,\bar{M}_{w,w})^{1/3}}{\bar{M}_{w,w}} \qquad \text{(VII-62)}$$

as Mandelkern and Flory have predicted that this quantity should have a constant value of 2.5×10^{6}. These results also appear in table (VII-7).

Table (VII-7)

Molecular Parameters from the Flory-Fox-Mandelkern Theory

Galactomannan	$(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ Å				$\frac{S([\eta]\bar{M}_{w,w})^{\frac{1}{3}}}{\bar{M}_{w,w}}$	$\Phi^{\frac{1}{3}}P^{-1}$
	Sedimentation	Diffusion	s-D Average	Viscosity		
Lotus pedunculatus I	32.7	32.7	32.7	108	4.6×10^{-16}	7.6×10^{6}
Soybean	115	115	115	222	3.2×10^{-16}	5.3×10^{6}
Sophora japonica	560	600	580	905	2.3×10^{-16}	3.8×10^{6}
Carob	818	816	817	1590	2.0×10^{-16}	4.9×10^{6}
Red Clover	1030	1029	1030	1560	2.3×10^{-16}	3.8×10^{6}
Guar	1470	1467	1469	2290	2.4×10^{-16}	3.9×10^{6}
Lucerne	370	370	370	1140	4.9×10^{-16}	7.8×10^{6}
Lotus pedunculatus II	729	727	728	905	1.9×10^{-16}	3.2×10^{6}

There is a large disparity between the $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ values from sedimentation and diffusion and those from viscometry. Comment on this is delayed until the general discussion on the hydrodynamic properties of galactomannans.

The values of $\frac{s_o([\eta]\,\bar{M}_{w,w})^{1/3}}{\bar{M}_{w,w}}$ for Sophora japonica, Red Clover, and Guar galactomannans are very constant. For Lotus pedunculatus I and Soybean galactomannans the molecules are possibly too small to fulfill the random-coil conformational requirements; while the high intrinsic viscosity of Carob galactomannan is responsible for its higher value. On the basis of the values found Sophora japonica, Red Clover, Guar, and possibly Carob galactomannans can be approximated to a homologous series of polymers. No conclusions can be drawn about the inclusion of Lotus pedunculatus I and Soybean galactomannans in this series owing to the small size of the molecules.

Similar comments to the above can be applied to the values of $\Phi^{1/3}P^{-1}$ obtained. The most common value of this quantity in table (VII-7) is 3.8×10^6, quite significantly different from the value of 2.5×10^6 predicted by Mandelkern and Flory (1952). The difference may be due to the highly extended conformation expected for galactomannans in solution and their consequent deviation from the Flory-Fox-Mandelkern model.

Table (VII-8)

$(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ from Sedimentation and Diffusion Data (Å)

Galactomannan	Debye-Beuche-Brinkman	Kuhn-Kuhn	Kirkwood-Riseman	Mandelkern-Flory
Lotus pedunculatus I	34.4	93.2	132	32.7
Soybean	121	196	277	115
Sophora japonica	632	595	838	580
Red Clover	1085	985	1390	1030
Carob	860	862	1220	817
Guar	1540	1240	1750	1470

Table (VII-9)

$(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ from Viscosity Data (Å)

Galactomannan	Debye-Beuche-Brinkman	Kuhn-Kuhn	Kirkwood-Riseman	Flory-Fox	Peterlin
Lotus pedunculatus I	99.7	120	175	108	159
Soybean	206	253	368	222	337
Sophora japonica	838	768	1110	905	1020
Red Clover	1450	1270	1850	1560	1680
Carob	1470	1110	1620	1590	1480
Guar	2120	1600	2320	2290	2120

(4) Comments on the Molecular Dimensions Calculated from Hydrodynamic Theories

Values of the weight-weight average root-mean-square end-to-end distance of the galactomannan molecules calculated from sedimentation/ diffusion data and viscometry data were given in tables (VII-8) and (VII-9). The average of the sedimentation and diffusion values is given, since $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ from these two sources did not vary by more than 2% (as would be expected, since both values essentially depend on the frictional coefficient).

In all cases $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ is considerably larger when calculated from intrinsic viscosity measurements than it is when calculated from sedimentation/diffusion values. Brown (1962) found that $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ from light-scattering measurements was much closer to $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ obtained from viscosity data than sedimentation/diffusion data for hydroxyethylcellulose in water. He attributed this to the departure of the hydroxyethylcellulose molecule in solution from random statistics due to its highly extended conformation. Brown also extended the calculations of Manley (1956) and found a similar effect for ethylhydroxyethylcellulose in aqueous solution.

A similar departure of galactomannan conformation in solution from random statistics might be expected from the above evidence. Some proof of this is provided by the calculated values of $\bar{\phi}^{1/3}P^{-1}$ in table (VII-7). Although $\bar{\phi}^{1/3}P^{-1}$ is constant (for Sophora japonica, Red Clover, Carob and Guar galactomannans), it is not equal to the value obtained from systems of linear macromolecules known to approximate random statistics in solution.

Further proof comes from the high values of effective bond length, b, (Kirkwood and Riseman; 1948) and equivalent segment length, A_m (Kuhn and Kuhn; 1948, 1950 a & b) for the galactomannans which are of the same magnitude as those obtained for ethylhydroxyethylcellulose (Manley, 1956) and hydroxyethylcellulose (Brown, 1962).

The values of $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ obtained from sedimentation/diffusion data depend greatly on the frictional coefficient of the molecule, which in turn is determined by the draining effects of the molecular coil. The low permeability of the molecule is shown by the exponent of the Staudinger equation (VII-42) and by the exponents of equations (VII-43) and (VII-44) relating sedimentation coefficient and diffusion coefficient to molecular weight. This low value could be due in part to the galactose side chains of the molecule, and not tight coiling as would be inferred from the equation exponents. The use of relationships between s_o, D_m and $\bar{Z}_{w,w}$ leads to errors when calculating $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ because the low permeability of the molecule is due to a factor other than tight coiling. Intrinsic viscosity is not affected so much because it depends mainly on the effective volume of the molecule (and therefore on $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$), and only to a lesser degree on the coil permeability. Brown (1962) came to the same conclusion for hydroxyethylcellulose, and used it as an explanation for the agreement of his $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ values from light-scattering and viscosity measurements. On this basis, the values of $(\bar{r}^2)^{\frac{1}{2}}_{w,w}$ calculated for galactomannans from viscosity measurements can be concluded to provide a realistic measure of the molecular dimensions in solution, despite the departure of the galactomannan molecule from random statistics.

C. Summary of the Solution Properties of Galactomannans

On the basis of the experimental evidence presented, the following conclusions can be made about the solution properties of galactomannans:-

(1) Their rheological properties can be followed using the empirical equations of Schutz (1970), on the basis of the excellent fit of the experimental data to these equations.

(2) The effect of ionic and non-ionic additives on galactomannan solution viscosity can be explained in terms of a dehydration mechanism.

(3) Galactomannans in solution can be treated as a linear polymer series, with the number of galactose side-chains per molecule having virtually no effect on the hydrodynamic properties (exceptions, see below). The evidence for this is provided by the linear plots of log $[\eta]$, log s_o, and log D_m against log $\bar{M}_{w,w}$, and also by the constant values of $\dfrac{s_o\,([\eta]\,\bar{M}_{w,w})^{1/3}}{\bar{M}_{w,w}}$ as required by the Flory-Fox-Mandelkern theory for a series of linear polymers.

(4) Galactomannans in solution behave in a very similar manner to water-soluble cellulose ethers, such as hydroxyethyl- and ethylhydroxyethylcellulose. This conclusion follows from the similarity of the hydrodynamic parameters calculated from the preceding theories, and was predicted by the experimental results of Koleske and Kurath (1964), and the calculations of Swenson et. al. (1965).

The above summary is subject to the conditions that the molecular weight distribution of the galactomannans concerned is similar, and that the galactose to mannose ratio of the molecule is no less than 1:1.1 or greater than 1:5. The former condition eliminated Lucerne galactomannan from the hydrodynamic calculations. The galactomannan from Lotus pedunculatus II is excluded by the latter condition as its degree of substitution (one galactose sidechain for every mannose in the molecule) appears to have altered its solution conformation from that of the other galactomannans. Galactomannans with a galactose mannose ratio of greater than 1:5 have not been experimentally examined.

From an industrial point of view, it would appear that the galactomannans could be used interchangeably (assuming very similar molecular weights and molecular weight distributions). There are problems with solubility, however, which depends on the mannose:galactose ratio in the molecule (see the example given for Guar and Carob galactomannans given on P.4 of this thesis). Low yields of galactomannan from the seeds of the legumes used in this investigation would also tend to preclude their industrial useage. Considering the number of legume species whose seeds have been found to contain galactomannans, and the still larger number of legume seeds to be investigated (see Bailey, 1971), it would seem extremely likely that high molecular weight, high yielding (20 - 30%) galactomannans will be found that can be used industrially besides those of Guar and Carob.

APPENDIX I

GALACTOMANNANS FROM PLANT SEEDS

SOURCE	GALACTOSE / MANNOSE	Specific Optical Rotation	REFERENCE
Allysicarpus vaginalus D.C.	1:1.44	+57° (0.7,N.NaOH)	Tookey et al (1962)
*Annona muricata	1:4.46	+3.4° (1, H_2O)	Kooiman (1971)
Anthyllis vulneria	1:1.32	+80°	Soemme (1967)
**Arenga sacchifera	1:2.26	+50° (1, H_2O) +35	Kooiman (1971)
		+35° (1,M.NaOH)	Kooiman (1971)
Astralagus cicer L	1:1.32	+64° (0.66,N.NaOH)	Tookey et al (1962)
A. glycyphyllos L	1:1.22	+72° (0.79,N.NaOH)	Tookey et al (1962)
A. nutallianus D.C.	1:1.38	+60° (0.79,N.NaOH)	Tookey et al (1962)
A. sinicus L	1:1.63	+74° (0.71,N.NaOH)	Tookey et al (1962)
A. tenellus BUNGE	1:1.19	+80° (1.20,N.NaOH)	Tookey et al (1962)
**Borassus flabellifer L	1:2.85	+ 9° (0.5,4%NaOH)	Subrahmanyan (1956) Mukherjee (1961)
Caesalpinia cacalaco (Huizache) HUMB & BONPL	$1:2.46^a$		Anderson (1949)
C. pulcherrima (L) Sw	1:1.94 1:3	+ 6° (0.5,M.NaOH)	Bains (1954) Monimoto et al (1962a) Unrau & Choy (1970)
C. spinosa (Tara)	$1:2.73^a$		Castiglioni (1933) Rogers & Brebe (1941) Wise & Appling (1944)
Cassia absus	1:3(I) (II) (III)		Kapoor & Mukherjee (1969) Kapoor & Mukherjee (1971) Kapoor & Mukherjee (1972)

SOURCE	GALACTOSE/MANNOSE		REFERENCE
C. emarginata	1:2.7	+21° (1.2,N.NaOH)	Tookey et al (1962)
C. fistula	1:3.26 1:4		Morimoto et al (1962) Kelkar & Mukherjee (1971)
C. leptocarpa BENTH	1:3.09[a]		Anderson (1949)
C. marylandica	1:3.76	+27° (0.52,N.NaOH)	Tookey et al (1962)
	1:3.76		Tookey & Clark (1965)
C. nodosa	1:3.5		Rizvi et al (1971)
C. occidentalis	1:3		Tookey & Clark (1965)
Ceretonia siliqua L (Carob)	1:2.05[a] 1:2.65[a])		A. Spada (1939)
	1:4[a]		Smith (1948)
	1:5.25[a]		Hirst & Jones (1948)
	1:4[a]		Anderson (1949)
Cercidium torreyanum SARG (Palo verde)	1:3.31[a]		Anderson (1949)
**Cocos nucifera	1:2	-85° (1,4%NaOH)	Rao et al (1961)
	1:2.57	+27° (1.5,0.1MNaOH)	Kooiman (1971)
IConvulvulus tricolor L	1:1.75	+44° (1.49,H_2O)	Kooiman (1971)
Crotalaria incana L	1:2.70	+23° (0.84,N.NaOH)	Tookey et al (1962)
C. intermedia KOTSCHY	1:2.28[a]		Anderson (1949)
C. lanceolata E. MEY	1:2.57	+22° (0.85,N.NaOH)	(Tookey et al (1962) Tookey et al (1963)
C. mucronata	1:2.86 1:3		Morimoto et al (1962)a,b Unrau & Choy (1970)
C. retusa L	1:2.84	+29° (0.62,N.NaOH)	Tookey et al (1962)
C. spectablis ROTH	1:2.84	+20° (0.68,N.NaOH)	Tookey et al (1962)
Cyamopsis tetragonoloba (L) TAUB (guar)	1:1.75[a]	+53.0 (1,N.NaOH)	Heyne & Whistler (1948)
	1:1.70	+53.0 (0.84,NaOH)	Tookey et al (1962)
	1:1.64		Haug (1953)

SOURCE	GALACTOSE MANNOSE		REFERENCE
Delonix regia (Flame tree)	1:4.2 1:2		Anderson (1949) Kapoor (1972)
Desmanthus illinoensis MAC MILL	1:2.69[a]		Anderson (1949)
Desmodium pulchellum	1:2	+60° (,.5M.NaOH)	Sinha & Tiwari(1970)
Gleditsia amorphoides TAUB TAUB	1:2.26		Rique & Pardo (1954)
	1:2.5	+24° (,H_2O)	Cerezo (1965)
G. Ferox DESF	1:3.8		Leschnizer & Cerezo (1970)
G. triacanthos (Honey locust)	1:2.73[a]		Anderson (1949)
		+25° (0.4,H_2O)	Leschnizer & Cerezo (1970)
Glycine max (L) MERR (Soybean)	1:1.5	+65° (1,H_2O)	Whistler & Saarnio (1957)
G. max var. Lindarin I	1:1.43	+68° (1,H_2O)	Aspinall & Whyte (1964)
G. max var. Lindarin II	1:2.35	+26.5°	Aspinall & Whyte (1964)
Gymnocladus dioica (Kentucky coffee bean)	1:2.71[a]		Anderson (1949)
	1:2.69	+29°	Larson & Smith (1955)
** Hyphaene thebaica (Doun palm)	1:19	-28.8° (90%HCOOH)	Khadem & Sallam (1967)
Indigofera hirsuta HARV	1:3.13[a]		Anderson (1949)
I. spicata	1:1.82		Ford (1969)
I Ipomoea muricata	1:1.8	+54° (,NaOH)	Khanna & Gupta (1967)
Laucaena glauca (Willd) BENTH	1:1.32 1:1.33		Unrau (1961) Morimoto et al (1962 a&b)
Lotus corniculatus L	1:1.25	+87°	Soemme (1966)
L. pedunculatus CAV	1:1.04	+82.5°	Richards et al (1968)
L. scoparius	1:1.13	+79° (0.58,N.NaOH)	Tookey et al (1962)
Medicago Hispida GAERTNER	1:1.22	+76° (0.63,N.NaOH)	Tookey et al (1962)

SOURCE	GALACTOSE / MANNOSE		REFERENCE
M. lupulina L	1:1.1	$+85^{\circ}$ $(0.38, N.NaOH)$	Soemme (1968)
	1:1.17		Reid & Meir (1969)
M. orbicularis (L) BARTAL	1:1.56	$+55^{\circ}$ $(0.87, N.NaOH)$	Tookey et al (1962)
M. sativa L		$+93.7^{\circ}$	May & Schultz (1936)
(Lucerne)	2:1[a]		Hirst et al (1947)
	1:1.25		Andrews et al (1952a)
	1:1.20		Reid & Meier (1969)
Melilotus albus	1:1.13		Reid & Meier (1969)
M. indica	1:1.04	$+89^{\circ}$ $(0.73, N.NaOH)$	Tookey et al (1962)
M. officinalis	1:1.20		Reid & Meier (1969)
M. radiata (formerly Trigonella radiata)	1:1.17		Reid & Meier (1969)
Parkinsonia aculeata L	1:2.70	-10 $(0.70, N.NaOH)$	Tookey et al (1962)
Retana raetam	1:4	$+59^{\circ}$ $(0.2, H_2O)$	Ahmed & Rizk (1963)
Sesbania grandiflora POIR	1:1.5		Rao & Rao (1965)
	1:2	$+50^{\circ}$ $(0.5, H_2O)$	Srivastava et al (1968)
Sophora japonica L	1:5.06[a]		Anderson (1949)
	1:5.26	-9° $(1.33, H_2O)$	Kooiman (1971)
Trifolium alexandrium		$+72^{\circ}$ $(\quad , N.NaOH)$	Krishnaswamy (1966)
T. dubium	1:1.17		Reid & Meier (1969)
T. hirtum L	1:1.04	$+88^{\circ}$ $(0.5, N.NaOH)$	Tookey et al (1962)
T. incarnatum	1:1.17		Reid & Meier (1969)
T. pratense L (red clover)	1:1.3	$+78^{\circ}$ $(0.4, H_2O)$	Andrews et al (1952)
T. resupinatum	1:1.04	$+84^{\circ}$ $(0.37, N.NaOH)$	Tookey et al (1962)
T. repens (white clover)	1:1.3	$+77.8^{\circ}$ $(1, H_2O)$	Horvei & Wickstroem (1964)

SOURCE	$\frac{\text{GALACTOSE}}{\text{MANNOSE}}$		REFERENCE
Trigonella caerulea L.SER	1:1.13		Reid & Meier (1969)
T. calliceras FISCH	1:1.13		Reid & Meier (1969)
T. corniculata L	1:1.17		Reid & Meier (1969)
T. cretica L BOISS	1:1.63		Reid & Meier (1969)
T. foenum-graecum L (fenugreek)	1:1.22	$+70^{\circ}$ $(0.22, H_2O)$	Andrews et al (1952b)
	1:1.13		Reid & Meier (1969)
T. hamosa L	1:1.17		Reid & Meier (1969)
T. monspeliaca L	1:1.08		Reid & Meier (1969)
T. Polycerata BIEB	1:1.13		Reid & Meier (1969)

a sugar ratio determined before use of chromatographic methods

* Annonaceae

** Palmaceae

I Convulvulaceae

APPENDIX II

GALACTOMANNANS FROM YEASTS, MOLDS AND FUNGI

SOURCE	GALACTOSE / MANNOSE	Specific Optical Rotation	REFERENCE
Alternaria kikuchiana			Azuma et al (1971)
A. zinniae			Azuma et al (1971)
Aspergillus niger			Sakaguchi et al (1969)
A. fumigatus			Sakaguchi et al (1967)
Lipomyces starkeyi			Sikl et al (1968)
Mycrosporum quinckeam I	1:21.2	+78° (,H_2O)	Bishop et al (1965)
II	1:2.20	+58° (,H_2O)	Bishop et al (1966)
Penicillium chrysogenum	1:1.5		Miyazaki & Yadomae (1968) Sakaguchi et al (1969)
Trichophyton granulosum (mixture)	1:5.25		Bishop et al (1962)
I	1:12.33	+78° (,H_2O)	Bishop et al (1965)
II	1:2.43	+16° (,H_2O)	Bishop et al (1966)
T. interdigitale I	1:7.33		Blank & Perry (1964)
	1:7.33	+80° (,H_2O)	Bishop et al (1965)
II	1:2.03	6.5° (,H_2O)	Bishop et al (1966)
T. rubrum I	0:100	+80° (,H_2O)	Bishop et al (1965)
II	1:3.29	+26° (,H_2O)	Bishop et al (1966)
(mixture)	1:2.24		Suzuki et al (1967)
T. schonleini I	1:6.69	+64° (, H_2O)	Bishop et al (1965)
II	1:2.96	+23° (, H_2O)	Bishop et al (1966)
Trichosporon fermentans			Gorin & Spencer (1968)

APPENDIX III

LITERATURE VALUES OF GALACTOMANNAN MOLECULAR WEIGHTS AND VISCOSITIES

GALACTOMANNAN FROM:-	MOLECULAR WEIGHT	MOL. WT. AVERAGE TYPE	VISCOSITY	REFERENCE
Annona muricata	8,700	[3]Number		Kooiman (1971)
Arenga sachifera	17,000	Number		Kooiman (1971)
Borassus flabellifer L			[1]i=1.25	Mukherjee et al (1961)
Caesalpinia pulcherrima (= Poinciana p.)			[2]r=2.89 (0.1%, 20°C)	Morimoto et al (1962)
Cassia fistula			r=2.11 (0.1%, 20°C)	Morimoto et al (1962)
Ceretonia siliqua (Carob)	520,000	[5]Sedimentation/ Diffusion	i=5	Kubal & Gralen (1948)
	300,000	[6]Weight/Weight		Kubal & Gralen (1948)
	650,000	Number		Hui & Neukom (1964)
Cocos nucifera	125,000	[4]Weight		Rao et al (1961)
	7,200	Number		Kooiman (1971)
Convulvulus tricolor	11,000	Number		Kooiman (1971)
Crotalaria mucronata			r=1.25 (0.1%, 20°C)	Morimoto et al (1962)
Cyamopsis tetragonoloba (Guar)	200,000			Boggs (quoted by Hui & Neukom (1964))
	1,900,000	Weight		Hui & Neukom (1964)
	250,000	Number		Hui & Neukom (1964)
	1,720,000	Weight		Deb & Mukherjee (1962)

GALACTOMANNAN FROM:-	MOLECULAR WEIGHT	MOL. WT. AVERAGE TYPE	VISCOSITY	REFERENCE
Gleditsia amorphoides	19,000	Number	i = 4.4	Cerezo (1965)
Gleditsia triacanthos	17,000 20,000	Number	i = 4.4	Leschnizer & Cerezo (1970)
Glycone max L (Soybean)	32,000	Number	i = 0.35	Whistler & Saarnio (1957)
Leucaena glauca	24,000	Number	r = 2.52 (0.1%, 20°C)	Unrau (1961)
Lotus pedunculatus	100,000	Weight	i = 4	Richards et al (1968)
Sophora japonica	6,000	Number		Kooiman (1971)

1 r = relative viscosity, $= \frac{\text{viscosity solution}}{\text{viscosity solvent}}$

2. i = intrinsic viscosity, $[\eta] = \lim_{c \to 0} \left(\frac{\eta_r - 1}{c}\right)$

3 Number = Number Average Molecular Weight, $M_n = \frac{\Sigma N_i M_i}{\Sigma N_i}$

4 Weight = Weight Average Molecular Weight, $M_w = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i}$

5 Sedimentation/Diffusion = Sedimentation/Diffusion Average Molecular Weight

$$M_{s,D} = \frac{s\,RT}{D_A(1-v\rho)}$$

6 Weight/Weight = Weight/Weight Average Molecular Weight, $M_{w,w} = \frac{s_w RT}{D_m(1-v\rho)}$

APPENDIX IV

GALACTOMANNAN VISCOSITY DEPENDENCE ON CONCENTRATION AND TEMPERATURE (UBBELOHDE VISCOMETERS)

Galactomannan	Temperature(°C)	Viscosity (cS)				
	*Conc$^{n.}$	0.10	0.22	0.43	0.56	0.86
Carob	20	2.26	6.24	33.9	86.4	443.
	30	1.74	4.63	23.9	58.9	301
	40	1.39	3.55	17.3	41.8	208
	50	1.13	2.86	12.9	30.3	147
	60	0.94	2.23	9.61	22.8	106
	70	0.78	1.75	6.94	15.4	77.6
	Conc$^{n.}$	0.10	0.20	0.48	0.55	0.76
Guar	20	3.45	9.85	166	300	2020
	30	2.66	7.34	117	208	1390
	40	2.11	6.63	85.2	149	970
	50	1.69	4.42	61.3	110	692
	60	1.37	3.50	45.7	79.1	500
	70	1.07	2.76	32.1	57.5	353
	Conc$^{n.}$	0.10	0.25	0.50	0.75	1.00
Lotus pedunculatus I	20	1.03	1.04	1.10	1.14	1.20
	30	0.82	0.83	0.87	0.91	0.95
	40	0.67	0.68	0.72	0.74	0.78
	50	0.56	0.58	0.60	0.63	0.66
	60	0.48	0.50	0.51	0.54	0.56
	70	0.42	0.43	0.45	0.47	0.49
	Conc$^{n.}$	0.11	0.22	0.44	0.64	0.84
Lotus pedunculatus II	20	1.78	3.15	8.13	19.2	41.5
	30	1.39	2.43	6.20	14.4	30.9
	40	1.13	1.95	4.82	11.1	24.1
	50	0.93	1.58	3.84	8.60	18.1
	60	0.78	1.31	3.09	6.66	13.7
	70	0.67	1.10	2.49	5.13	9.93
	Conc$^{n.}$	0.09	0.21	0.29	0.32	0.42
Lucerne	20	2.04	4.79	11.2	8.84	18.8
	30	1.58	3.70	8.45	6.63	14.1
	40	1.26	2.92	6.57	5.13	10.9
	50	1.04	2.36	5.20	4.07	8.48
	60	0.82	1.89	4.15	3.29	6.72
	70	0.73	1.54	2.65	3.30	5.33
	Conc$^{n.}$	0.08	0.28	0.36	0.46	0.55
Red Clover	20	1.45	5.63	9.53	16.5	26.1
	30	1.15	4.30	7.21	12.4	19.2
	40	0.93	3.35	5.61	9.53	14.5
	50	0.77	2.61	4.47	7.37	11.2
	60	0.65	2.08	3.51	5.77	8.65
	70	0.55	1.70	2.71	4.22	6.40

Galactomannan	Temperature(^{o}C)	Viscosity (cS)				
	Conc$^{n.}$	0.087	0.18	0.40	0.55	0.80
Sophora japonica	20	1.71	3.23	16.9	40.9	166
	30	1.34	2.66	12.1	28.5	111
	40	1.09	2.10	8.95	21.4	82.2
	50	0.89	1.69	6.92	15.7	58.2
	60	0.76	1.38	5.37	11.7	39.7
	70	0.67	1.15	4.19	8.85	28.1
	Conc$^{n.}$	0.10	0.25	0.41	0.63	0.83
Soybean	20	1.14	1.39	1.70	2.14	2.58
	30	0.91	1.09	1.34	1.68	2.01
	40	0.75	0.90	1.08	1.35	1.62
	50	0.63	0.75	0.91	1.12	1.31
	60	0.53	0.64	0.75	0.93	1.09
	70	0.46	0.54	0.64	0.78	0.91

* = in $\frac{g}{100\ ml}$

Appendix V

Dependence of Galactomannan Viscosity on Concentration and Mean Shear Rate at 20°C.

Galactomannan From:-	C	$\bar{G}$	η		C	$\bar{G}$	η
Carob	0.126	99.9	4.31		0.196	78.4	8.20
		141	4.00			108	7.84
		200	3.80			142	7.47
		282	3.55			205	6.98
		362	3.47			285	6.66
		495	3.37			387	6.39
		674	3.25			510	6.11
		905	3.16			612	5.95
		1210	3.01			868	5.55
		1520	2.94			1070	5.34
	0.351	31.7	35.9		0.538	7.81	190
		41.1	33.9			11.8	174
		60.0	30.6			18.2	158
		105	26.8			24.8	146
		169	23.2			39.5	126
		249	20.8			59.3	115
		316	19.4			90.0	92.8
		401	18.3			123	81.7
		493	17.2			177	67.4
		617	16.0			227	60.4
	0.631	4.59	371		0.875	2.00	1730
		7.92	327			4.48	1460
		11.7	310			8.98	1070
		13.4	288			11.8	1020
		16.6	281			15.2	922
		22.1	260			19.6	838
		32.2	224			24.7	713
		49.2	188			32.1	645
		71.1	162			43.5	559
		102	139			55.1	477
Guar	0.084	111	3.76		0.201	65.1	9.57
		169	3.47			89.2	8.95
		240	3.28			126	8.33
		324	3.14			193	7.59
		416	3.02			283	7.03
		534	2.94			394	6.59
		711	2.83			519	6.21
		962	2.75			618	5.99
		1150	2.71			812	5.58
		1440	2.64			1020	5.28

C = Concentration($\frac{g}{100\ ml}$)
$\bar{G}$ = Mean Shear Rate (sec^{-1})
η = Viscosity(cP)

Appendix V (cont)

Galactomannan From:-	C	$\bar{G}$	η		C	$\bar{G}$	η
Guar (cont)	0.343	18.7	52.4		0.534	8.51	333
		30.5	46.2			12.3	295
		50.3	39.7			18.4	258
		74.7	34.9			25.7	224
		130	28.6			38.9	177
		195	24.4			57.2	148
		296	20.7			90.5	114
		339	19.5			127	94.6
		503	16.6			162	80.2
		655	14.9			194	72.6
	0.631	2.65	796		0.828	1.07	4800
		4.02	714			2.21	4470
		6.57	590			4.93	2950
		10.5	493			6.51	2410
		16.9	400			9.57	1760
		23.9	355			13.7	1460
		32.1	306			18.8	1210
		43.6	260			25.7	946
		58.6	218			34.5	785
		78.8	185			44.1	658
Lotus pedunculatus I	0.105	245	1.18		0.184	235	1.21
		285	1.14			279	1.17
		326	1.15			339	1.15
		393	1.10			403	1.11
		534	1.07			531	1.08
		651	1.06			640	1.07
		764	1.06			744	1.07
		927	1.05			939	1.06
		1190	1.03			1190	1.05
		1431	1.03			1420	1.04
	0.333	239	1.28		0.525	219	1.27
		273	1.21			257	1.25
		332	1.18			304	1.20
		414	1.13			423	1.17
		516	1.12			538	1.15
		648	1.11			657	1.14
		780	1.09			782	1.13
		958	1.09			943	1.12
		1240	1.07			1240	1.11
		1460	1.07			1450	1.11
	0.659	240	1.30		0.908	213	1.39
		267	1.27			256	1.30
		332	1.24			310	1.29
		433	1.20			412	1.25
		549	1.18			530	1.23
		664	1.17			656	1.22
		788	1.16			761	1.21
		1010	1.15			965	1.20
		1220	1.14			1140	1.19
		1414	1.13			1400	1.17

Appendix V (cont)

Galactomannan From:-	C	$\bar{G}$	η		C	$\bar{G}$	η
Lotus pedunculatus II	0.159	238	1.74		0.314	176	2.75
		267	1.72			212	2.67
		322	1.72			263	2.62
		409	1.67			318	2.60
		509	1.65			400	2.59
		670	1.63			489	2.57
		816	1.62			601	2.53
		1080	1.62			801	2.51
		1260	1.62			997	2.49
		1450	1.61			1200	2.48
	0.498	111	4.66		0.608	115	6.43
		139	4.50			148	6.32
		197	4.42			207	6.20
		239	4.30			270	6.09
		329	4.21			388	5.98
		431	4.12			529	5.89
		582	4.08			625	5.84
		720	4.04			700	5.78
		870	4.02			842	5.71
		998	3.99			1030	5.64
	0.757	80.7	9.69		0.804	65.4	12.1
		99.8	9.50			80.9	11.8
		130	9.23			109	11.5
		150	9.09			165	11.1
		180	9.14			257	10.8
		338	8.16			368	10.6
		447	7.98			529	10.3
		613	8.58			684	9.99
		800	8.44			826	9.77
		1020	8.33			1010	9.56
Lucerne	0.083	211	1.74		0.134	244	2.52
		257	1.81			257	2.52
		329	1.80			290	2.51
		405	1.75			328	2.49
		501	1.74			462	2.40
		588	1.73			526	2.39
		676	1.71			596	2.36
		797	1.69			755	2.31
		887	1.68			889	2.27
		972	1.67			998	2.25
	0.254	106	7.13		0.484	62.0	17.1
		123	7.02			77.6	16.5
		134	6.96			117	15.5
		163	6.79			172	14.5
		197	6.59			227	13.5
		255	6.45			250	13.5
		316	6.25			290	12.9
		365	6.15			350	12.5
		450	6.00			472	11.7
		501	5.93			578	11.2

Appendix V (cont)

Galactomannan From:-	C	$\bar{G}$	η		C	$\bar{G}$	η
Lucerne (cont)	0.596	40.5			0.732	22.5	55.2
		43.0	30.3			28.9	53.3
		56.5	28.3			34.3	51.0
		99.7	25.1			44.4	48.9
		146	23.3			72.6	44.0
		170	22.7			105	40.8
		194	22.0			134	38.1
		255	20.2			166	35.9
		442	17.4			201	34.0
		591	17.3			254	31.7
Red Clover	0.050	197	2.15		0.095	157	3.42
		237	2.10			189	3.32
		295	2.06			225	3.22
		398	2.00			285	3.13
		490	1.95			420	2.75
		600	1.92			540	2.71
		756	1.89			710	2.63
		965	1.86			855	2.57
		1105	1.84			1018	2.53
		1260	1.83			1209	2.46
	0.180	97.3	6.71		0.317	29.6	36.1
		129	6.34			47.0	32.0
		176	6.02			72.2	28.2
		239	5.74			132	23.6
		320	5.48			201	20.1
		437	4.86			280	17.9
		559	4.70			374	16.3
		716	4.46			489	14.9
		842	4.32			583	13.9
		1001	4.20			683	13.1
	0.368	28.4	49.6		0.422	8.96	168
		44.7	44.0			16.3	132
		60.6	40.2			26.0	116
		95.0	33.9			45.6	93.6
		143	29.7			63.0	83.3
		196	26.0			92.8	69.3
		276	22.8			121	60.5
		381	20.1			145	55.4
		443	19.0			188	48.1
		544	17.5			223	44.3
Sophora japonica	0.079	219	1.77		0.142	167	2.95
		258	1.75			211	2.86
		312	1.70			230	2.82
		384	1.67			357	2.70
		463	1.65			479	2.62
		592	1.63			566	2.58
		715	1.61			696	2.53
		977	1.57			915	2.48
		1250	1.54			1120	2.44
		1470	1.52			1470	2.37

Appendix V (cont)

Galactomannan From:-	C	$\bar{G}$	η		C	$\bar{G}$	η
Sophora japonica (cont)	0.331	51.0	13.2		0.500	30.0	44.2
		73.5	12.1			38.6	41.4
		93.2	11.7			65.7	37.0
		140	10.9			96.0	32.8
		197	9.90			124	31.1
		292	9.25			181	26.7
		426	8.46			303	23.0
		574	8.02			403	21.1
		784	7.36			505	19.6
		988	7.01			622	18.3
	0.591	16.2	90.9		0.785	8.09	282
		22.0	84.5			10.8	267
		32.1	80.0			14.9	244
		50.1	67.6			24.4	209
		55.7	63.7			31.8	181
		87.3	55.3			41.4	163
		138	47.8			62.9	141
		212	39.6			84.1	123
		286	36.0			102	111
		342	33.8			139	99.8
Soybean	0.078	230	1.31		0.153	208	1.46
		265	1.27			251	1.40
		314	1.24			281	1.38
		394	1.21			387	1.32
		502	1.18			502	1.29
		615	1.17			639	1.27
		723	1.16			740	1.26
		916	1.15			924	1.25
		1120	1.13			1080	1.24
		1370	1.12			1320	1.23
	0.354	226	1.71		0.507	225	2.12
		284	1.66			260	1.93
		305	1.65			279	1.93
		407	1.61			409	1.88
		505	1.59			496	1.87
		613	1.58			630	1.84
		739	1.56			762	1.82
		956	1.55			930	1.81
		1160	1.54			1070	1.78
		1340	1.53			1190	1.77
	0.626	197	2.22		0.788	204	2.64
		273	2.16			254	2.58
		347	2.12			299	2.55
		469	2.09			383	2.49
		559	2.07			519	2.43
		671	2.05			631	2.40
		760	2.04			756	2.37
		922	2.04			936	2.35
		1030	2.03			1080	2.34
		1240	2.02			1300	2.33

Appendix VI-a

Effect of Added Sucrose on Galactomannan Solution Viscosity

Galactomannan from:	C*	Sucrose (%,w/w)	η*	$\bar{G}$*	Galactomannan from:	C*	Sucrose (%,w/w)	η*	$\bar{G}$*
Carob	0.602	0	279	10.8	Guar	0.651	0	636	7.31
			248	18.9				605	8.35
			232	25.0				532	11.5
			210	29.8				437	18.2
		1	251	10.8			1	676	7.02
			228	16.2				607	9.31
			208	24.3				556	11.6
			188	33.8				474	16.6
		10	255	12.3			10	629	7.54
			232	19.0				566	9.93
			221	24.8				509	12.8
			205	32.7				432	18.5
		20	287	11.4			20	609	7.74
			257	19.1				551	9.99
			239	25.6				493	13.1
			227	30.6				423	18.4
		30	307	11.8			30	626	7.48
			276	18.8				551	10.2
			247	28.4				490	13.4
			234	34.0				421	18.7
		40	378	10.6			40	691	7.34
			323	19.7				615	9.65
			301	25.1				538	13.1
			278	32.2				438	20.2
		50	527	11.4			50	797	7.67
			465	17.8				725	9.53
			417	24.7				644	12.6
			365	35.9				571	16.6

* C = Concentration of Galactomannan ($\frac{g}{100\ ml}$ of original solution)

η = Viscosity (cP)

$\bar{G}$ = Mean Shear Rate (sec^{-1}).

Appendix VI-a (cont)

Galactomannan from:	C*	Sucrose (%,w/w)	η^*	$\bar{G}^*$	Galactomannan from:	C*	Sucrose (%,w/w)	η^*	$\bar{G}^*$
Lotus pedunculatus I	0.758	0	1.25	242	Lotus pedunculatus II	0.261	0	3.59	271
			1.21	364				3.55	403
			1.18	469				3.50	572
			1.15	682				3.47	694
		1	1.29	250			1	3.64	257
			1.21	398				3.57	388
			1.18	718				3.54	504
			1.20	552				3.49	687
		10	1.60	284			10	4.39	274
			1.56	396				4.34	400
			1.53	544				4.29	575
			1.52	667				4.25	736
		20	2.78	243			20	5.99	287
			2.23	417				5.91	440
			2.20	568				5.87	538
			2.19	671				5.82	694
		30	3.63	264			30	8.70	256
			3.57	400				8.61	373
			3.57	560				8.46	599
			3.56	675				8.35	712
		40	7.28	266			40	14.9	256
			6.79	379				14.7	377
			6.77	590				14.5	524
			6.75	742				14.3	674
		50	17.0	267			50	25.1	255
			16.9	406				24.7	355
			16.8	489				24.2	515
			16.8	685				23.9	668

Appendix VI-a (cont)

Galactomannan from:	C*	Sucrose (%,w/w)	η^*	$\bar{G}^*$	Galactomannan from:	C*	Sucrose (%,w/w)	η^*	$\bar{G}^*$
Lucerne	0.685	0	63.1	23.1	Red Clover	0.650	0	168	14.1
			59.1	32.5				150	19.1
			55.7	44.3				131	25.0
			49.5	77.3				107	45.0
		1	57.4	25.7			1	165	14.3
			54.9	34.6				150	18.2
			52.7	45.1				136	23.7
			45.6	76.4				110	40.2
		10	59.2	25.4			10	171	12.4
			56.8	35.3				146	19.3
			54.6	45.1				132	24.7
			49.3	76.4				106	43.2
		20	64.2	26.1			20	169	14.9
			62.9	35.3				150	20.3
			61.5	41.7				136	26.1
			58.5	74.1				110	44.6
		30	79.3	26.2			30	176	14.6
			75.5	34.2				159	19.6
			72.0	42.1				143	25.8
			63.9	78.4				118	42.5
		40	101	27.6			40	202	15.3
			97.0	34.3				186	19.1
			92.6	44.6				162	27.8
			83.2	78.1				139	42.6
		50	162	27.6				285	13.0
			156	35.7				260	17.2
			150	44.4				225	26.7
			133	75.9				197	38.9

Appendix VI-a (cont)

Galactomannan from:	C^*	Sucrose (%,w/w)	η^*	$\bar{G}^*$	Galactomannan from:	C^*	Sucrose (%,w/w)	η^*	$\bar{G}^*$
Sophora japonica	0.516	0	80.9	24.8	Soybean	0.669	0	2.05	286
			77.2	31.5				2.00	409
			72.8	41.9				1.97	514
			68.3	56.1				1.94	723
		1	78.4	25.7			1	2.09	276
			73.8	35.3				2.06	402
			70.7	43.9				2.02	507
			66.1	58.1				1.97	714
		10	78.6	25.7			10	2.60	288
			74.3	35.3				2.59	396
			70.9	44.2				2.53	475
			66.9	57.4				2.50	658
		20	102	24.7			20	3.56	284
			95.3	34.3				3.53	354
			90.4	43.5				3.50	499
			83.5	58.0				3.46	688
		30	116	26.1			30	5.43	319
			109	34.5				5.40	402
			101	47.5				5.34	523
			93.8	64.6				5.32	705
		40	142	24.9			40	9.72	295
			134	32.1				9.65	384
			124	44.1				9.55	499
			113	60.3				9.51	688
		50	208	24.6			50	22.6	269
			189	37.9				22.4	350
			179	45.6				22.3	459
			169	58.0				22.2	686

Appendix VI-b

Effect of Added Sodium Chloride on Galactomannan Solution Viscosity

Galactomannan from:	C*	Sodium Chloride (%,w/w)	η*	$\bar{G}$*	Galactomannan from:	C*	Sodium Chloride (%,w/w)	η*	$\bar{G}$*
Carob	0.554	0	196	13.0	Guar	0.561	0	384	7.15
			182	19.3				344	10.1
			169	26.3				317	12.7
			159	34.8				281	17.7
		5	167	14.4			5	289	7.10
			152	18.7				263	10.1
			149	25.5				240	13.9
			134	36.2				207	21.2
		10	159	14.0			10	277	6.94
			151	17.6				256	9.51
			141	25.4				234	12.8
			133	34.1				206	18.9
		15	167	13.7			15	279	7.10
			160	17.9				255	9.51
			152	24.3				225	13.1
			142	33.4				191	21.1
		20	185	15.3			20	327	7.30
			177	19.6				302	9.57
			167	26.4				271	13.3
			159	34.5				206	18.7
		25	212	14.8			25	390	7.30
			199	20.4				355	9.76
			188	26.1				312	13.9
			176	34.3				265	20.9

* C = Concentration of Galactomannan ($\frac{g}{100\ ml}$ of original solution)

η = Viscosity (cP)

$\bar{G}$ = Mean Shear Rate (sec^{-1}).

Appendix VI-b (cont)

Galactomannan from:	C*	Sodium Chloride (%,w/w)	η^*	$\bar{G}^*$	Galactomannan from:	C*	Sodium Chloride (%,w/w)	η^*	$\bar{G}^*$
Lotus pedunculatus I	0.526	0	1.21	313	Lotus pedunculatus II	0.496	0	4.26	316
			1.18	411				4.22	419
			1.15	560				4.18	565
			1.14	729				4.17	688
		5	1.31	323			5	4.14	302
			1.27	420				4.10	415
			1.25	540				4.06	540
			1.21	717				4.04	683
		10	1.42	311			10	4.35	310
			1.39	418				4.32	413
			1.36	527				4.28	530
			1.34	734				4.27	671
		15	1.61	312			15	4.84	313
			1.56	420				4.80	404
			1.53	508				4.77	534
			1.52	679				4.74	662
		20	1.85	290			20	5.63	303
			1.80	431				5.57	445
			1.77	548				5.54	546
			1.76	703				5.49	662
		25	2.24	290			25	6.86	325
			2.18	416				6.80	455
			2.15	582				7.32	537
			2.14	701				6.93	709

Appendix VI-b (cont)

Galactomannan from:	C*	Sodium Chloride (%,w/w)	η^*	$\bar{G}^*$	Galactomannan from:	C*	Sodium Chloride (%,w/w)	η^*	$\bar{G}^*$
Lucerne	0.556	0	23.2	48.4	Red Clover	0.591	0	31.0	47.6
			22.5	60.4				28.9	59.6
			21.6	76.6				28.1	72.6
			20.1	120				25.6	115
		5	19.8	48.0			5	24.9	49.9
			19.1	62.5				24.3	62.8
			18.4	82.9				22.9	83.7
			17.4	121				23.2	119
		10	19.7	47.5			10	20.3	45.1
			18.9	65.5				24.2	57.8
			18.4	79.4				23.3	75.1
			17.3	126				21.9	107
		15	21.3	50.8			15	26.8	49.7
			20.5	65.1				25.9	62.2
			19.3	89.7				24.9	79.4
			18.7	126				23.4	114
		20	25.3	48.1			20	31.2	47.6
			24.0	68.4				29.7	65.6
			23.3	87.0				28.4	83.6
			21.7	123				26.5	123
		25	30.8	50.1			25	36.5	52.3
			29.7	64.0				35.2	65.2
			28.6	81.4				33.5	84.2
			27.2	111				31.6	114

Appendix VI-b (cont)

Galactomannan from:	C*	Sodium Chloride (%,w/w)	η^*	$\bar{G}^*$	Galactomannan from:	C*	Sodium Chloride (%,w/w)	η^*	$\bar{G}^*$
Sophora japonica	0.338	0	12.7	102	Soybean	0.621	0	2.14	296
			12.3	126				2.10	383
			11.8	167				2.06	538
			11.4	222				2.04	657
		5	12.5	101			5	2.01	324
			12.2	122				1.96	415
			11.7	165				1.95	478
			11.3	212				1.93	711
		10	12.9	106			10	2.14	297
			12.5	132				2.10	422
			12.2	170				2.08	563
			11.7	222				2.06	713
		15	14.1	101			15	2.41	313
			13.6	134				2.37	399
			13.2	178				2.34	530
			12.7	227				2.32	670
		20	15.8	103			20	2.84	306
			15.3	132				2.76	422
			14.8	173				2.70	492
			14.1	247				2.69	682
		25	18.2	103			25	3.32	325
			17.5	133				3.31	417
			17.0	173				3.30	527
			16.1	248				3.22	694

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APPENDIX VIII

Further Comments on Viscometry Methods

(i) Equilibration: After the viscometer was filled with galactomannan solution, freshly withdrawn from the refrigerator, it was equilibrated in the waterbath at 20°C until two successive flow-times agreed within 0.2 seconds.

(ii) Measurements of Viscosity at Differing Temperatures: After viscosity measurements had been carried out at 20°C, the measurements at successive 10°C increments were made without interruption until 70°C was reached. At each temperature the attainment of equilibrium was established by the constancy of successive readings over a time interval of 45 minutes at 20°C, decreasing to 15 minutes at 70°C.

(iii) Hysteresis: These experiments were not carried out, although it would have been desirable to do so. A limiting factor would be the loss of solvent from the galactomannan solutions at temperatures above 50°C, which would eventually lead to a hysteresis effect other than that due to changes in the galactomannan.

APPENDIX VII

Definition of a Gum (Paul and Palmer, (1972), p.204)

The term "gum" has come to apply to all polysaccharides or their derivatives that can be dispersed in water to produce gels or very viscous solutions or dispersions. Under this definition, these dispersions need not necessarily be gummy or tacky; those that are slimy or mucilaginous are also included.

The important characteristics are their hydrophilic nature and ability to form dispersions of high viscosity even at low concentrations.

(Paul, P.C. and Palmer, H.H. (1972)
"Food Theory and Applications", pub. J. Wiley & Sons, Inc., New York.