(i) DEGRADATIVE AND ANALYTICAL STUDIES OF PLANT GUM EXUDATES WITH PARTICULAR REFERENCE TO GUM ARABIC (*ACACIA SENEGAL*)

(ii) THE MECHANISM OF INTERACTION BETWEEN UNLIKE CELLULOSIC ETHERS AND GALACTOMANNANS IN SOLUTION.

by

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PREFACE

The research in this thesis comprises of two sections. The first section of the research (Chapters I-IV) investigates structural degradations and analytical studies of plant gum exudates, carried out at Edinburgh University.

The second section (Chapters V-VII) investigates and develops a mechanism of interaction which exists between various water-soluble cellulose ethers and galactomannan gums in solution. The thesis was sponsored by Courtaulds Fine Chemicals, who produce several commercial cellulose ethers.

DECLARATION

I hereby declare that this thesis was composed by myself and that it is based upon results of original research experiments, carried out by me (unless otherwise stated) within the Chemistry Department, University of Edinburgh, and Courtaulds Fine Chemicals Research Department, Coventry, from October 1988 to September 1991. None of the work included in this thesis has been submitted for any other degree or professional qualification.

Some of the analytical data reported in Chapters III and IV have been published or are in press; e.g.

(a)D.M.W.Anderson, D.M.Brown-Douglas, N.A.Morrison and W.Wang, Food Addit. Contam; 1990, 7, (3), 303.

(b)D.M.W.Anderson and N.A.Morrison, Food Addit. Contam; 1990, 7, (2), 181.

(c)D.M.W.Anderson and N.A.Morrison, Food Addit. Contam; 1990, 7, (2), 175.

(d)D.M.W.Anderson and N.A.Morrison, Food Hydrocolloids; 1989, 3, (1), 57.

The research findings from Chapter VII have been presented at the Cellucon 1993 Conference in Lund, Sweden and a publication is currently in press.

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CHAPTER I GENERAL INTRODUCTION.

The technological (i.e. non-food) use of water-soluble polymers dates back thousands of years. Polysaccharide gums are hydrocolloids, and a wide range of applications in the construction, detergent, mining, oil well drilling, food and pharmaceutical industries has been developed since World War II. The natural gums can be classified into three distinct categories according to raw material source; plant gum exudates, seaweed extracts and seed endosperm gums (1). The polysaccharide gums of interest in this section of the thesis are the plant exudate gums. Exudate gums are of considerable importance (2) commercially and as the products of the specific wound response (3) gummosis, are also of biological interest.

The ability of these exudate gums to dissolve readily in water to give viscous solutions makes them attractive commercially. They are widely used in cosmetics, tablet coatings, adhesives and paints (4). They are commonly used as thickeners, suspending and stabilising agents. Other uses include film forming properties, lubricating agents, and binding agents (5). However their main use is in the food industry as a food additive or as an ingredient in confectionery. Unlike many of the polysaccharides which are used in foods to alter (6) the rheology of the solution by thickening, ie. as a viscosity modifier or

as gelling agents at 1-2% polymer concentrations, some exudate gums are soluble up to 50% solution concentrations and are surface active, that is they can stabilise an oil in water emulsion (7).

The term "gum" designates a wide range of natural products in the form of tears, flakes, or angular fragments, but most commonly as clear amber oval nodules, which are sticky in nature and are exuded by certain tropical trees. This section of the thesis investigates degradative studies of Acacia senegal (gum arabic) and an analytical study of gum exudates, from the Acacia (8), Combretum (9), and Albizia genera (10). No Combretum or Albizia gum exudates are permitted as food additives (11), although they have reportedly been, and still may be, sold in blends as adulterants or as substitutes for gum arabic.

Almost all the worlds output of gum arabic is from the sub-Sahara or Sahel regions of Africa. However other geographical sources of gum exudates for technological uses include; Australia, India, and South America from the stems of sub-tropical shrubs and bushes. Despite much structural elucidation of these gums in recent decades, the precise mechanism of gum biosynthesis is not totally understood (12), exudation usually following mechanical injury or microbiological infestation of the bark. Possible mechanisms of gum formation have been postulated (3) including; enzymatic modification of starches or

cellulosic material, bacterial action or by direct synthesis. It is interesting to note that the formation of a specific gum e.g. gum arabic, by a certain species of tree e.g. Acacia senegal, has been remarkably constant over several decades during which the variation in its analytical parameters has been studied (13). Functionally, the plant gums may act to seal off wounds to prevent further injury and inhibit tissue dehydration in the hot arid climate.

Chemically the plant gum exudates have previously been regarded as complex, partially neutralised salts of acidic hetero-polysaccharides (14). The major neutral sugars which occur commonly in the gums in varying proportions are D-galactose, L-arabinose, L-rhamnose, whilst smaller quantities of D-xylose and D-mannose occur in certain species. The acidity of the gums commonly arises from the presence of D-glucuronic acid and its 4-O-Methyl derivative, but small proportions of D-galacturonic acid occur in certain genera. Although some studies had reported a small protein content in certain gums as early as 1954 (15,16), other earlier investigators ignored, or failed to analyse for, the presence of nitrogen in plant gums (17). Although it was shown to be functionally important and not entirely isolatable, the precise location of the protein in certain gum exudates is still under considerable debate. Certain gums although essentially polysaccharide in nature are now more

correctly referred to as proteoglycans with a protein content reported as high as 53% in Acacia difficilis gum (18,19).

Chapter III of this thesis presents results of degradative studies on gum arabic, which is the gum exuded by Acacia senegal. Good quality samples of gum arabic occur as nodules, which are amber to clear in colour, odourless, tasteless and totally water-soluble. The essential property of a food additive is its total lack of toxicity and, although it had long been assumed to be safe, toxicological evidence was required for the continued use of gum arabic in food formulations (7,20). Ideally a food additive and its metabolites should be non-toxic, non-carcinogenic and non-allergenic, both when ingested in small quantities and on long term exposure to the additive. The gum was re-affirmed as GRAS in the U.S.A in 1974. Following the positive toxicological data obtained in response to the requests for evidence of its safety, gum arabic was awarded the status "ADI not specified" by JEFCA in 1982, providing that the gum conforms to the established regulatory specifications for its identity and purity. The regulatory specifications have been recently revised by JEFCA in 1991, as discussed in Chapter III (21).

The two severe Sahelian droughts of 1973-1974 and 1983-1984, both resulted in heavy losses of Acacia senegal trees, and this has led to

suggestions by gum traders that physiological adaptations may have occurred in the trees that survived. It was further suggested, but later disproved (13), that the properties of the exudate gum may also have changed over this period, involving certain well establised analytical parameters such as the specific rotation. As a separate issue, labour cost and transportation cost escalations as a result of the isolated location of the gum sources, have increased markedly the price of good quality gum arabic, now currently (1993), $3100 U.S. dollars per tonne ex Port Sudan, with the price having been as high as $5000 in October 1987.

This has led to unscrupulous gum traders using other much cheaper exudate gums from other botanical sources, which are not permitted in foodstuffs, as adulterants or substitutes for gum arabic. However during the frequent periods of shortage throughout the droughts, many industries were forced to use modified starches and various alternative hydrocolloids as substitutes for gum arabic. When the supply of gum arabic was resumed many did not wish to return to the usage of expensive gum arabic as their formulations and processing facilities had had to be altered. This led to a situation where gum arabic was only cost-effective in special performance formulations where cheaper alternatives could not be substituted due to the unique functionality of gum arabic such as its

ability to effectively stabilise oil in water emulsions. The variation in the analytical properties of the gum from 1950-1989 from different geographical sources are investigated (13) in chapter IV.

Gum arabics' most valuable property is its ability to stabilise oil in water emulsions (7,22). Suggested mechanisms by which this occurs have been based on gum molecules encapsulating oil droplets and stabilising the emulsion with hydrophobic sugar and amino acid moieties. This study investigates several degradative and fractionation studies on gum arabic's structure and how its performance as an emulsifying agent is affected by these changes. Many studies have reviewed the earlier structures proposed and indeed some have proposed new structures for gum arabic (14,23). It has been long established that the gum's structure is based on a highly branched galactan framework with arabinose side chains (5,14,27,29). It has been shown that the rhamnose residues occur mainly in terminal positions on the periphery of the gum structure as do the acidic sugar residues (24). The relatively hydrophobic rhamnose residues have also been related to the gum's performance as an emulsifier (25).

Fractionation of gum arabic by several groups of workers has suggested that the gum is composed of several molecular weight fractions which differ not only in their overall protein content but in their amino acid composition (24,26,27). It has been

reported that only a small proportion of the total quantity of the gum arabic used in emulsification stability is adsorbed (26). Approximately 98% of the gum is not adsorbed at the oil-water interphase and is not surface active. However it is the high protein, high molecular weight fraction that has been shown to adsorb. Although enriched proteinaceous fractions have been isolated no fraction of gum arabic has been isolated that is completely void of protein (23,30). It is this factor that indicates that certain amino acid residues are covalently linked (28) and not hydrogen bonded (i.e. occurring as an impurity), to certain sugar residues.

Chapter III of this thesis reports studies of the effect of ionising gammma irradiation on the analytical parameters and the emulsification properties of gum arabic. This is investigated because of the increasingly likely effect of processed foods being irradiated to prolong their shelflife (31,32), and because it may have been assumed that the structure and ultimately the functionality of the gum is unaltered by this irradiation. The results presented in this thesis contradict previous publications on this subject and suggest that the molecular weight, intrinsic viscosity and both the emulsification activity and stability of the gum are all reduced by low levels of gamma irradiation.

An attempt to deproteinate (33,34,35) gum arabic on the basis that the various molecular weight fractions of Acacia senegal have differing solubilities in a co-solvent mixture, was carried out and the fractions obtained were analysed. The gum was partially deproteinated and a nitrogen-enriched and a nitrogen-depleted fraction were obtained. It was interesting that the amino acid composition as well as the proteinaceous content was altered on fractionation. The various fractions were characterised for various analytical parameters.

After a good quality gum arabic sample was subjected to a very mild sequential Smith-degradation (36,37), its five sequential degradation products obtained were analysed for their amino acid composition, relative sugar ratios, and their emulsification activity and stability. These degradations were less extensive than in previous sequential degradations carried out by other workers (36), and suggest new evidence on the role of certain peripheral amino acids in the functionality of the polysaccharide.

The last fractionation study was carried out by analysing the gum fraction that is adsorbed by limonene after 24 hours and also the material that became destabilised from the oil-in-water emulsion.

All four degradative techniques tend to give complementary analytical information on the gum's complex macromolecular structure.

The analytical parameters used to characterise various gums and molecular weight fractions of gums are; total ash, nitrogen, amino acid and methoxyl contents; specific optical rotation, intrinsic and Brookfield viscosity, equivalent weight and uronic acid content, and the neutral sugar ratios. The emulsification stability and activity measurement of an oil in water emulsion was also determined. These parameters give a characteristic overall "fingerprint" of the gum being analysed. ^{13}C NMR spectra were also obtained for several of the gums to complement the combination of the above parameters and give unequivocal evidence for the identity of a gum.

The amino acid composition is one of the most sensitive methods of establishing the identity of a botanical species. Indeed much analytical work has been carried out in recent years (21), to obtain amino acid data to support chemotaxonomy of gums previously studied (38). Indeed this has brought to light evidence that in Acacia species some amino acids located on the periphery of the macromolecular structure of the gum are not only structurally important but also contribute to the functionality of the macromolecule (36).

The genus Acacia was subdivided into five sections in 1875 by Bentham (39), and his

classification has largely been supported by chemical analytical data to date (36). In chapter IV of this thesis an analytical study of four proteinaceous Acacia gums from Bentham's series Gummiferae is carried out in terms of their carbohydrate and amino acid compositions. None of these gums are permitted for usage as food additives and none have been characterised previously.

In chapter IV, seven exudate gums from the genus Albizia, which is commonly confused with the genus Acacia, and six gums from the genus Combretum are also characterised in terms of their analytical parameters. These thirteen gums are not included in the American GRAS or any other national list of permitted additives and the availability of their data may help prevent their usage in any food formulation. However the Combretum gums are readily available at low prices in East and West Africa and are commonly offered for sale there as "gum arabic". They are the most common adulterants of Acacia senegal in commercial blends, offered by certain large, aggressive commercial suppliers, at prices below the Sudanese controlled export price for gum arabic.

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CHAPTER II

EXPERIMENTAL METHODS

CHAPTER II EXPERIMENTAL METHODS

II (i). GENERAL METHODS

Weighings. All accurate weighings were made within the range of the graticule scale (range 0-100mg) of a Stanton Unimatic model C.L.1. single-pan balance, having the accuracy of ± 0.1mg.

Moisture contents were determined by heating to a constant weight at 105°C.

Ash contents were determined by heating to a constant weight in a muffle furnace at 550°C.

Dialyses of polysaccharides, to isolate soluble low molecular weight material, were carried out in Visking cellophane tubing (Medicell International Ltd., London) in a 5 litre vessel of distilled water. Removal of low molecular weight material was achieved by dialysis against running tap water for 48-72 hours unless otherwise stated.

Reductions in volume were carried out with a rotary evaporator at temperatures below 37°C to prevent gum degradation, and loss of functionality, unless otherwise stated.

Electrodialyses of polysaccharides were carried out in a three-compartment perspex cell fitted with cellophane membranes. The water in the outer electrode chambers was regularly changed to prevent overheating and kept

below 40°C. Electodialysis (applied voltage= 300 volts) was continued until a current ceased to flow.

Carbon, hydrogen and nitrogen contents were determined with a Perkin Elmer 240 Elemental analyser. Nitrogen contents were also determined by a semi-micro Kjeldahl method.

Cationic contents were determined by Atomic Absorption Spectroscopy on a Pye-Unicam SP9 model, by firstly dissolving the ashed polysaccharide in dilute hydrochloric acid, then filtering, using Whatman No 541 filter paper. The cationic content was analysed using an air/acetylene flame against standard salt solutions (1).

Tannin contents were determined by adding 0.1ml of ferric chloride solution (9g ferric chloride hexahydrate made up to 100ml with water) to a 2% fully hydrated gum solution. Positive tannin contents were quantified by a colorimetric method at 430nm using tannic acid as a reference standard.

Methoxyl contents were determined by a vapour phase infrared method (2,3), a calibration curve was based on known weights of methyl iodide. Infrared spectroscopy was carried out with a Perkin-Elmer 137 spectrophotometer. Vanillin was used as an internal standard to check for the completeness of recovery.

Equivalent weight determinations on exhaustively electrodialysed polysaccharides were carried out by

direct titration with standard sodium hydroxide solution (ca. 0.01N).

Uronic acid contents were calculated from the equivalent weight as (17,600/E.W.), i.e values are expressed as uronic acid anhydride.

Quantitative estimation of sugars. Sugars were separated from hydrolysates by chromatography in various solvents (a-d) on Whatman 3MM papers. After elution from the paper in boiling water, sugars were estimated colorimetrically by the phenol-sulphuric acid method (4), and sugar ratios were determined. The optical density was read on a Unicam SP 1300 spectrophotometer at 430nm. Calibration curves were obtained from known weights of sugars.

Chromatographic separations. Paper chromatography was carried out on Whatman No. 1 papers, unless otherwise stated, with the following solvent systems (v/v).

a) ethyl acetate, acetic acid, formic acid, water (18:3:1:4).

b) benzene, butan-1-ol, pyridine, water (1:5:3:3, upper layer).

c) ethanol, 0.1N hydrochloric acid, butan-1-ol (10:5:1). (5)

d) ethyl acetate, pyridine, water (10:4:3).

Before using solvent system (c), papers were dipped in 0.3M sodium dihydrogen orthophosphate and air dried.

Reducing sugars were detected by spraying chromatograms with a saturated solution of aniline oxalate in ethanol/water (1:1 v/v), then heating at 105°C for 2-3 minutes in an oven.

Emulsification Capacity Determination.

A standard oil-in-water emulsification test for soluble hydrocolloids has been developed. Two aspects were considered; the ability to form an emulsion ("the emulsification activity", E.A) and subsequently to stabilise it ("the emulsification stability", E.S) (6,7).

D-limonene or paraffin oil (0.5ml) was added to the filtered gum solution (2mls of a 5% polymer solution, Whatman No. 42 filter paper) and homogenised with an Ultra-Turrax Model T25 for 60 seconds at 15,000 rev/min. Immediately 0.1 ml of the emulsion was withdrawn, diluted (1:100) with distillied water, and the turbidity absorbance measured at 500nm on a Perkin-Elmer ultraviolet/visible spectrometer against a blank.

Emulsion stability The remaining emulsion was used to fill up to the 1.0ml mark of a 1.0ml syringe, and allowed to stand, clamped vertically, for 30 minutes, after which the lower half of the emulsion (0.5ml) was

dispersed into distilled water (50ml). The absorbance at 500nm of this diluted emulsion was measured against the blank. The emulsion stability (E.S) was calculated as the absorbance of this diluted (1:100) lower half of the emulsion (stored for 30 minutes) expressed relative to the original emulsification stability (E.A) :-

$$\% \text{ E.S} = \frac{\text{Abs.at 500 nm of emulsion stored for 30 min x 100}}{\text{E.A. at 500 nm of freshly prepared emulsion}}$$

Brookfield viscosities were determined on a model RVT viscometer on 25% w/w gum solutions at 25°C, using spindle 2 at 20 r.p.m.

pH values were determined using a PW9420 model pH meter for 25% w/w gum solutions at 25°C.

Acetyl content was determined by dissolving gum (300mg) in sodium hydroxide (5ml, 4M) solution and warming gently. This liberates the acetate groups. This is added to a semi-micro Kjeldahl apparatus with sulphuric acid (5mls 33%). Steam is passed through for 20 minutes to carry the evolved acetic acid into a known volume of sodium hydroxide (0.014M) which is then back-titrated with a standard sulphuric acid solution. Glucose pentacatate was used as a standard reference material (8).

Nuclear Magnetic Resonance Spectroscopy. ^{13}C Fourier-transform NMR spectra were recorded overnight

for 10% gum solutions in D_2O at room temperature, at 50.320 MHz with a Brüker WP200 SY spectrometer. All spectra were recorded under identical instrumental conditions (9).

II (ii). PHYSICAL METHODS.

Specific rotations of aqueous solutions were measured using the sodium D-line with a Perkin-Elmer model 141 polarimeter at 20± 2°C. All solutions were first clarified by passage through filters of average pore size 0.8µm(Millipore Ltd., Bedford, Mass., U.S.A) with a stainless steel filter holder attached to a syringe (20ml). Concentrations of gum were corrected for insoluble material after filtration (10).

Viscosity determinations were carried out in a M-sodium chloride in an Ubbelohde suspended-level dilution viscometer at 25 ± 0.1°C. Polymer and solvent solutions were filtered carefully using a 0.80µm millipore filter, before additions were made to the viscometer. Flow times were measured to within 0.1 second by means of a digital stop watch. The isoionic dilution technique was used; a solution of the gum (6mls, 1-2%) was placed in the viscometer and the flow time measured. Flow times were obtained for successive dilutions with M-sodium chloride solutions (four additions of 2mls each). Since preliminary experiments

had indicated that any loss of gum was negligible, concentration values were estimated from the dry weight of gum dissolved in a known volume.

Assuming the densities of M-sodium chloride and gum solutions to be equal for low concentrations of gum, the intrinsic viscosity number $[\eta]$, is given by;

$$[\eta] = \lim_{c \to o} \frac{\eta sp}{c} = \lim_{c \to o} \frac{t - t_o}{c t_o}$$

where C is the concentration of gum (g/ml) and t_o and t are the flow times (seconds) for solvent and solution respectively. Linear extrapolation of the plot of $\frac{t-t_o}{ct_o}$ against c at t=0 gives the intrinsic viscosity $[\eta]$,(11).

II (iii). CHEMICAL METHODS

Small scale polysaccharides hydrolyses were carried out overnight with sulphuric acid (1N) on a boiling water bath, unless otherwise stated. Hydrolysates were neutralised with barium carbonate, filtered, deionised using Amberlite IR-120(H) resin, and concentrated to a viscous syrup on a rotary evaporator.

Periodate oxidations of polysaccharides were carried out in darkness at room temperature. The formic acid released was estimated titrimetrically (12), with standard sodium hydroxide solution (0.014N) for sequential 1ml portions of the polymer solution. Methyl

red was used as indicator.

Amino acid hydrolysis. A sufficient amount of gum sample to give 2mg of nitrogen (12.5mg of crude protein) was weighed and transferred quantitatively into a 100ml two-necked round-bottomed flask. Anti-bumping granules and 80mls 6N hydrochloric acid were added to the flask which was fitted with a 800mm air condenser. The apparatus was purged with oxygen-free nitrogen at 5lbs/in^2. The contents were heated under reflux at approximately 160°C for 20 hours under a continuous stream of nitrogen. The resultant hydrolysate was filtered through Whatman No 42 filter paper and evaporated to dryness at 42°C. The residue was dissolved in 20ml 0.1N citrate buffer, filtered through a 0.22µm Millipore filter and stored at -20°C in glass vials pending analysis.

Analysis was effected on a Rank Hilger Chromaspek amino acid analyser as follows:
A suitable aliquot (normally 50µm) is applied to a 350 X 3mm stainless steel column of cationic exchange resin (6µm beads from Rank Hilger) and the constituent amino acids separated at high pressure (ca. 2,000 lbs/in^2) by elution with lithium citrate buffers of increasing ionic strength and pH. The eluted amino acids are detected by reaction with ninhydrin in a continuous flow analytical system, and quantified by references to standard solutions at 570nm (440nm for proline and hydroxyproline).

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CHAPTER III

DEGRADATIVE STUDIES OF GUM ARABIC

CHAPTER III INTRODUCTION

Gum Arabic is defined as "a dried gummy exudate obtained from the stem or branches of *Acacia senegal* (L.) Willd. or of related species of *Acacia*" (1) and as such is a permitted food additive (E414) within the EEC. It has been assessed as toxicologically safe, the "not specified" category of Acceptable Daily Intake (A.D.I.) being assigned in 1982 (2). The existing specifications for gum arabic were recently revised by JEFCA in 1990; " to reflect more closely the gum that had previously been toxicologically evaluated". The Revised Specification differed from that superseded, in that the nitrogen content and the specific rotation values of the gum arabic samples, now had to be specified, and had to fall in a specified range, and the botanical origin of gum arabic was modified to "*Acacia senegal* and its closely related species" (3).

The composition of commercial gum arabic is variable however, depending on its country of origin, climatic conditions and method of processing (4). It is important however for manufacturers to receive a constant supply of uncontaminated good quality gum arabic (5). It is therefore necessary to have precise specifications for the purity and identity of gum arabic for trade and enforcement purposes to prevent other exudate gums being sold as substitutes or adulterants in blends with gum arabic (6).

Good quality gum arabic (microbiologically "clean", very soluble, odourless and colourless) is used in the pharmaceuticals, cosmetics but predominantly in the food industry as an effective oil-in-water emulsifier (7,8). It is also used to a lesser extent to influence the body, texture and viscosity of foods; for example to inhibit ice crystal formation, or preventing sugar crystalisation in confectionary, or as a glazing agent. Inferior grades (darker and less readily soluble) are used in lithography, paints, inks and ceramics (9).

Gum arabic is a highly branched, complex, hetero-polysaccharide (10). It exists in nature as the partially neutralised salt of an acidic polysaccharide, arabic acid, containing various proportions (2-4% ash) of the cations; calcium, magnesium, sodium, potassium and iron as well as minor proportions of zinc, manganese and cobalt.

The polysaccharide gum is composed of five carbohydrate moieties: complete mild acid hydrolysis of the gum yields D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and small proportions of 4-O-methyl glucuronic acid. Various structural studies (11,12,13), have suggested that the gum consists of a highly branched β-1,3 linked galactopyranosyl backbone, with side-branches of galactopyranose linked β-1,6 containing arabinopyranose, arabinofuranose and rhamnopyranose (14). Glucuronic acid and

4-O-methylglucuronic acid residues are located on the periphery of the gum's structure.

It has been shown by another study that all the rhamnose residues are attached to glucuronic acid, and that some galactose units, to which some glucuronic acid groups (not in peripheral positions) are attached, can also carry small numbers of arabinofuranose units (15).

Although the earliest studies of Acacia exudates ignored the possibility of nitrogenous components in the gums (16), the Gum Research Group at Edinburgh University, led by D.M.W Anderson, reported that the all the Acacia species studied since 1959 contained nitrogen (17,18). Other investigators neglected this presence of protein until 1983 (19). Since then there has been widespread recognition that the protein and more precisely the amino acid composition of the gum plays an integral structural role, and contributes to the gum's unique functionality as an emulsifier (20). A small proportion (1-3%) of proteinacous material is present in most exudate gums, although several species contain higher levels for example 53% in Acacia difficilis gum (21). There is recent evidence to suggest that covalent chemical bonding exists between protein and polysaccharide, and the gums are more correctly termed as proteoglycans (22,23,24,25).

Arabinogalactan-proteins are regarded as complex polymeric structures and are widely recognised from a variety of phytochemical sources (26,27). However there is little detailed stuctural imformation on the precise linkage between amino acid residues and carbohydrate moieties in these proteoglycans. One study has proposed that hydroxyproline is linked in an alkali stable β-D-galactopyranosyl bond (28).

Various studies on the fractionation, for example by affinity chromatography (29), of gum arabic have suggested that the structure of the gum is not homogeneous but consists of three fractions. One of these fractions which consists of about 88% of the total mass of the gum is an arabinogalactan which is deficient in protein. Fraction 2 which represents approximately 10% of the gum is an arabinogalactan protein complex which has a higher molecular mass than the arabinogalactan fraction. The study has suggested that this fraction contains approximately 50% of the total protein in the gum. The smallest fraction which is only 1-2% of the total weight of the gum but contains around 50% of the total protein of the gum has been shown to consist of one or more glycoproteins (24).

Despite its widespread use in the stabilisation of citrus oils and other beverage flavour emulsions, there is no precise understanding of its mode of action (30,31,32). This chapter investigates

structural property relationships of gum arabic with respect to oil-in-water emulsion stabilisation, through a series of structural degradations and fractionations of the gum.

CHAPTER III.I. THE EFFECT OF GAMMA IRRADIATION ON THE MOLECULAR STRUCTURE AND FUNCTIONALITY OF GUM ARABIC.

III.I (i) INTRODUCTION

Gum arabic is a complex proteinaceous polysaccharide obtained as the exudate gum of Acacia senegal and is used widely in food and soft drink formulations (30). In the food industry there is continued interest in the possible use of irradiation by ionising gamma rays to extend the shelflife of foods.

The food industry has used a variety of methods over the years to preserve or extend the shelflife of food. These have included; smoking, packaging, dehydration, freezing and using chemical preservatives. Recently there has been a consumer campaign against chemical additives (33), however processed foods rely on additives not only for preservation effects but for flavour, colour, emulsifying and stabilising properties and rheological control. The idea of irradiating foods is however not new. The treatment was tested on strawberries in Sweden in 1916. The Soviet Union used it to prevent sprouting of potatoes in 1958, and disinfection of grain in 1959.

At present the United Kingdom, along with W.Germany and Scandinavia, does not permit irradiation of foods for public consumption. However

proposed laws to permit food irradiation are currently being discussed in Parliament.

Gamma irradiation, and radiation from other sources, for example ultraviolet radiation, have been employed as a means of preservation of some kinds of plant organs used as food material by inhibition of sprouting in storage organs, or delayed ripening of fruits or for the control of growth of microorganisms on such organs. If a safe dosage of irradiation could be used for gum arabic for eradication of microorganisms without affecting the gum's physiochemical properties, the gum could be used in the food industry without fear of growth of microorganisms. Microbiological tests have shown that following a radiation dose of 1MRad on foodstuffs, the contaminating microorganisms were effectively inactivated (34).

Since it is the emulsification stabilising properties of gum arabic that is its commercially important property, it is vital that any procedure aimed at reducing the microbiological level does not degrade the gum and so lower its ability to stabilise emulsions (35).

Food irradiation employs an energy form termed ionising radiation. The process of food irradiation requires the use of gamma rays, which may be generated by the decay of Cobalt-60 or Caesium-137. This results in either an indirect effect whereby the chemically reactive products formed from water

(hydroxide and superoxide radicals), are themselves chemically reactive, and result in a cascade of further reactions, or direct effects resulting in chemical changes induced in molecules, such as polysaccharides. Gamma irradiation is a form of high energy electomagnetic radiation, consisting of self-propagating electric and magnetic disturbances. The energy of the electomagnetic radiation is related to its frequency. The frequency of gamma rays is approximately 10^{20} Hz (36).

Through physical effects gamma rays interact with the molecules that make up the food and also those of food contaminants such as bacteria, moulds, yeasts and parasites, causing chemical and biological consequences which can be utilised. From the standpoint of food irradiation, the most important change in a polysaccharide's structure caused by irradiation is the breaking of some glycosidic bonds. This may result in the formation of low molecular weight material (34). In gum arabic the situation may be more complicated as tests have indicated that protection of the carbohydrate structure may occur as exemplified by the presence of amino acids and protein (37).

Irradiation can denature native proteins, principally through the breaking of hydrogen bonds and other linkages involved in the secondary and tertiary structures. The kind of change caused as a result of gamma irradiation is dose-dependant. Low to

moderate doses may affect the secondary or tertiary structure of the protein whereas higher dosages have yielded detectable changes in the primary structure (34).

Several studies have examined the effect of gamma irradiation on the molecular structure of gum arabic (37,38). One of these studies concluded that sterilising doses of gamma irradiation up to 3 MRads does not have a detrimental effect on gum arabic's ability to stabilise emulsions (38). In that study the bacteriological measurements of the starting material (300-500 micro-organisms/g) did not represent typical commercial gum arabic samples. (A representative bacterial count is more typically 14,000 micro-organisms/g). The study looked at the effect of gamma irradiation on raw, kibbled and spray-dried gum arabic samples. The study found that the intrinsic viscosity of the raw gum remained virtually unaltered whereas the kibbled and spray-dried gum showed a reduction in viscosity after approximately 1.0 and 0.5 MRad doses respectively.

Another study by Dickinson (39) and his co-workers has shown that the emulsification stabilising properties of different samples of gum arabic are directly proportional to the gums' molecular weight or intrinsic viscosity. The findings in this thesis agree with Dickinsons' findings.

The method used in the study by Blake and his co-workers (38), related the emulsification

ability of the irradiated gum to the absorbance of the emulsion as it was formed. This measurement has been termed the "emulsification activity" in this thesis. However the reduction of this value as a function of time, i.e, "the emulsification stability", taken in conjunction with the emulsification activity, which may both occur by different mechanisms, have been found to give more meaningful results (41,42). The emulsion stability can be determined at several time intervals, for example 30 minutes and 300 minutes, to compare trends and reproducibility of results.

III.I (ii) MATERIALS AND METHODS

Gamma Irradiation of gums. Gums were irradiated at the Scottish Universities Research and Reactor Centre in East Kilbride. The gums were exposed to ^{60}Co gamma irradiation for various lengths of time at a dose rate of 0.360 Gy/s, using an industrial irradiator by lowering the samples in a sealed vessel into the radiation source. The exposure dosage was calculated quantitatively by a dosimeter.

Microbiological testing. Gum arabic was subjected to standard microbiological assays. The samples were also subjected to after-pasturisation tests to detect spore-forming bacteria. No spore forming bacteria were found in any gum sample after pasturisation. These

tests were carried out in the Microbiology Department of the Edinburgh University School of Agriculture.

Origin of gum samples. Good quality Sudanese gum arabic (1988) was used throughout Chapter III, to allow direct comparison between results from different degradation and fractionation techniques.

Molecular weights M_w were determined from the Mark-Houwink relationship (43,44);

$$[\eta] = K M_w^a \qquad [1]$$

where K= 0.013 and a= 0.54.

Gum samples . Raw gum nodules were ground and powdered and passed through 150µm and 60µm mesh sieves. The fraction retained by the 60µm filter was put into polythene bags and sent for irradiation.

III.I (iii) RESULTS AND DISCUSSION.

Table III.1 shows the analytical data for the control sample of gum arabic and for the gum sample exposed to a 1 MRad (10 KGys) dose of gamma irradiation. Initially it appears that little change in the gum's composition as a result of the irradiation. Indeed the protein content and the sugar ratio remain unchanged. However the optical rotation of the gum has decreased slightly and the intrinsic viscosity is significantly reduced.

TABLE III.1 Analytical data for control gum arabic and 1 MRad irradiated gum arabic.

Analytical Parameter	Control gum arabic	1 MRad irradiated gum
Moisture, %	9.8	9.7
Ash, % [a]	3.2	3.2
Nitrogen, % [a]	0.34	0.34
Nitrogen conversion factor (N.C.F)[b]	6.59	6.57
Specific rotation in water (degrees)[a]	-28°	-29°
Intrinsic viscosity, mlg^{-1} [a]	15	11
Equivalent weight [a]	1030	1030
Hence % uronic anhydride	17	17
<u>Sugar composition after hydrolysis, %</u> [b]		
Glucuronic acid	17	17
Galactose	48	49
Arabinose	25	24
Rhamnose	10	10

<u>Notes</u>:

[a] Corrected for moisture content.

[b] From tables III.3 and 4.

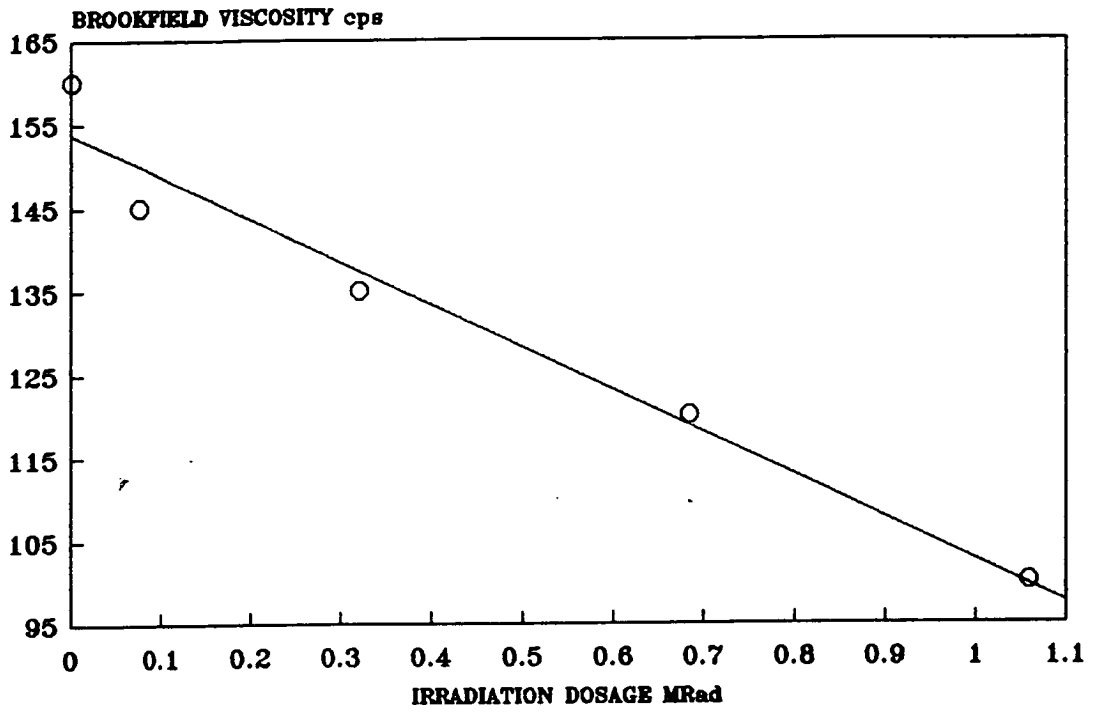
[c] Corrected for protein content.

[d] Including 4-O-methylglucuronic acid.

Table III.2 reveals the extent of the structural degradation that has occurred as a result of gamma irradiation. The intrinsic viscosity of the gum has decreased, as a result of increased irradiation dosage. Therefore the molecular weight of the gum calculated by the Mark-Houwink relationship [1], has correspondingly decreased. It must be added that the microbiological count is also greatly reduced as a result of irradiation, but it appears that the functionality of the gum is also reduced in the irradiation process. This reduction in functionality is indicated initially by the lowering of emulsification activity in a limonene oil-in-water emulsion at 500nm.

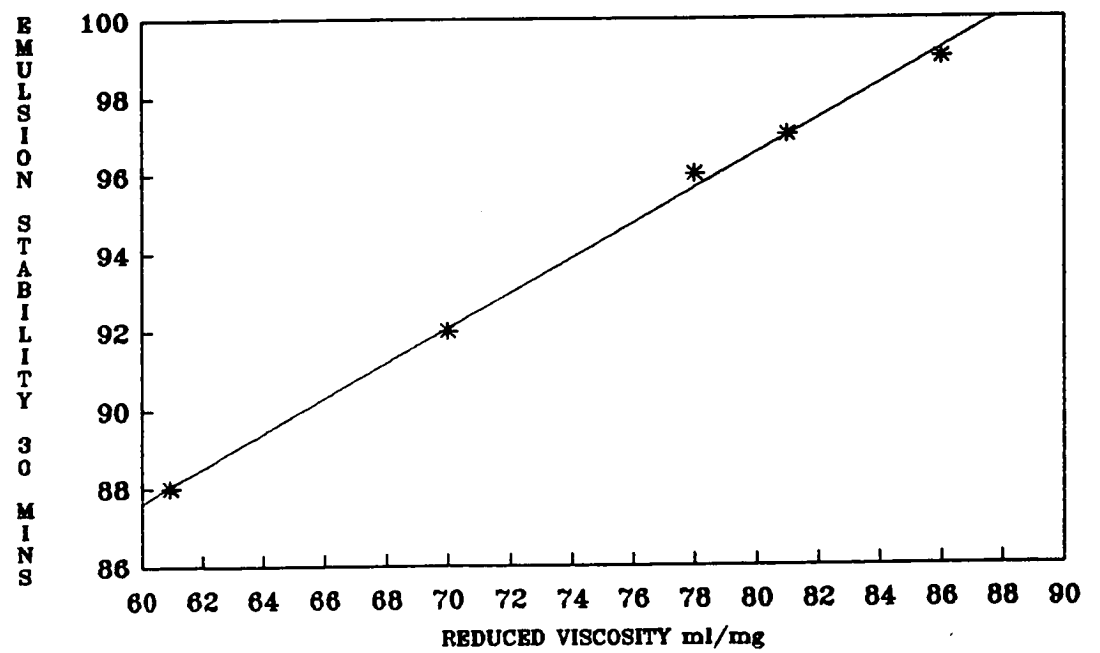
Although the precise mechanism of oil-in-water emulsion formation by gum arabic has not been totally resolved, the high molecular weight, highly proteinaceuos fraction of the gum has been shown to adsorb at the oil-water interface (35). It has been suggested that the hydrophobic amino acids located on the periphery of the gums' structure are adsorbed at the interface and the gum molecules encapsulate oil droplets (18,42). The relatively more hydrophilic sugar moieties presumably lie in the aqueous side of the interface. The molecular weight and hence the viscosity of the gum may suspend the oil droplets and prevent the emulsion from separating into two layers. Therefore if structural degradation involving the periphery of the gum or molecular weight reduction has occurred by

RELATIONSHIP BETWEEN IRRADIATED DOSAGE
AND BROOKFIELD VISCOSITY IN GUM ARABIC



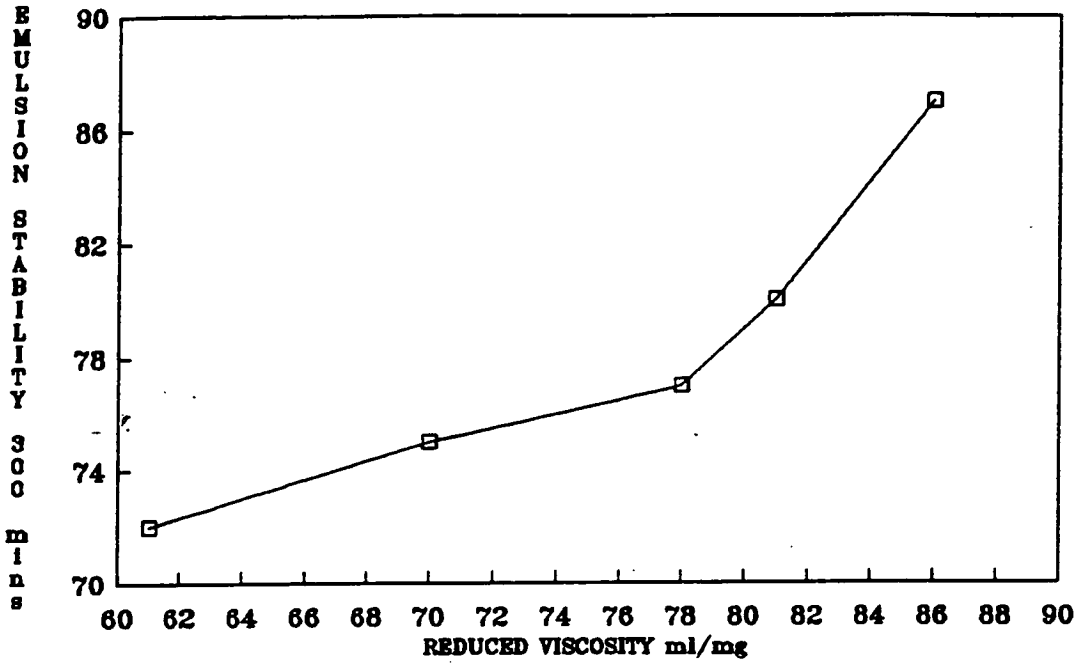
GRAPH III.1
20 r.p.m

RELATIONSHIP BETWEEN EMULSIFICATION
STABILITY AND REDUCED VISCOSITY IN
IRRADIATED GUM ARABIC SAMPLES.



GRAPH III.2
D-LIMONENE OIL-IN-WATER EMULSION

RELATIONSHIP BETWEEN EMULSIFICATION STABILITY AND REDUCED VISCOSITY IN IRRADIATED GUM ARABIC SAMPLES



GRAPH III.3

TABLE III.2 Microbiological total plate counts, intrinsic viscosity, hence molecular weight and the emulsification activity of irradiated samples of Acacia senegal.

Irradiation Dosage (MRad)	Total counts per gram	Intrinsic viscosity (ml/mg)	Hence Mw	E.A (500nm)
0.0	14,200	15.6	502,000	1.682
0.083	1,800	15.2	480,000	1.651
0.320	800	13.1	380,000	1.642
0.684	400	12.5	334,000	1.631
1.060	200	11.6	291,000	1.549

cleavage of vulnerable glycosidic bonds as a result of irradiation, the functionality of the gum will be reduced (22,32).

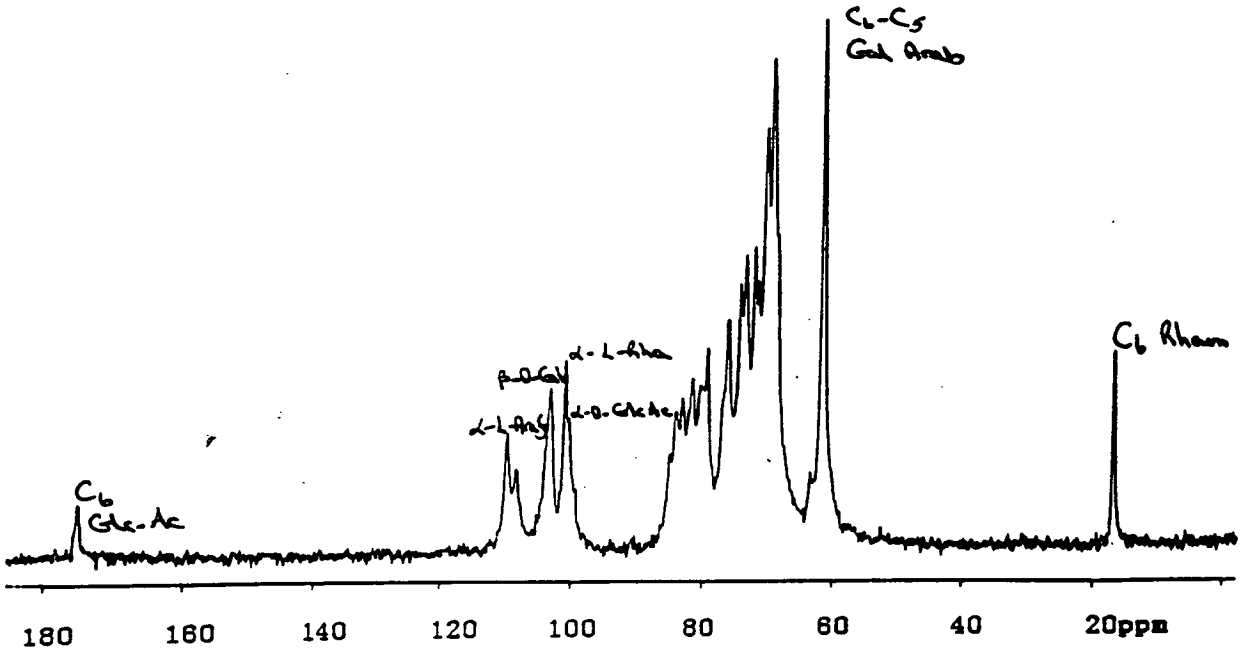
The Brookfield viscosity of the gum samples has also decreased from 160cps for the control gum to 100cps for the 1 MRad irradiated gum sample, as shown in graph III.1. This agrees with the intrinsic viscosity figures in Table III.1, as do the reduced viscosity results shown in graph III.2. This graph displays the relationship between reduced viscosity of the irradiated gums against the emulsification stability of a limonene oil-in-water emulsion, 30 minutes after the emulsion was formed. It is obvious that as well as the emulsification activity being reduced as a result of irradiation of gum arabic, the emulsification stabilities (calculated as a percentage of the original activities) are also significantly reduced as a result of irradiation. A similar trend in emulsification stabilities is maintained when the stabilities are measured 300 minutes after the emulsion was formed in an independent experiment, as shown in graph III.3.

Further structural elucidation was carried out to investigate possible causes of this reduction in the functionality of the gum when gamma-irradiated. The amino acid compositions of the control gum arabic and the 1MRad irradiated gum sample were compared. Initially it appears that there is no

change in the amino acid composition of the two samples, within the limits of experimental error. It appears that no amino acids have been converted into for example the simplest amino acid structure glycine, by a free radical mechanism, or hydroxyproline being converted to proline. The ^{13}C Nuclear Magnetic Resonance (NMR) spectra III.1 and III.2 and the sugar ratios in table III.1 both show that no major changes are apparent. Spectrum III.1 shows a well-resolved ^{13}C-n.m.r spectrum for the parent sample of Acacia senegal which agrees with a previous publications (3,46). The two signals at extreme fields may be assigned unambigiously to C-6 of L-rhamnopyranose, and D-glucuronic acid at 17.7 and 175.6 p.p.m respectively. Approximately seven signals can be resolved from the anomeric carbon resonances (90-110 p.p.m) as shown in spectrum III.1, based on literature values (46).

In the following experiment, 10g of the control gum arabic and 10g of the 1 MRad irradiated gum were dissolved in 100mls of distilled water then dialysed exhaustively against 5 litres of distilled water. Low molecular weight fractions of both gums were isolated from the diffusate. Both the high molecular weight fractions and the diffusate were freeze dried and subsequently analysed. Approximately 1.4g of low molecular weight material was removed from the control gum sample and 2.1g from the irradiated gum which indicates that degradation of the parent gum has

Spectrum III. 1: 13C NMR spectra of control Gum Arabic.



Spectrum III 2: 13C NMR spectra of 1MRad irradiated.
Gum Arabic

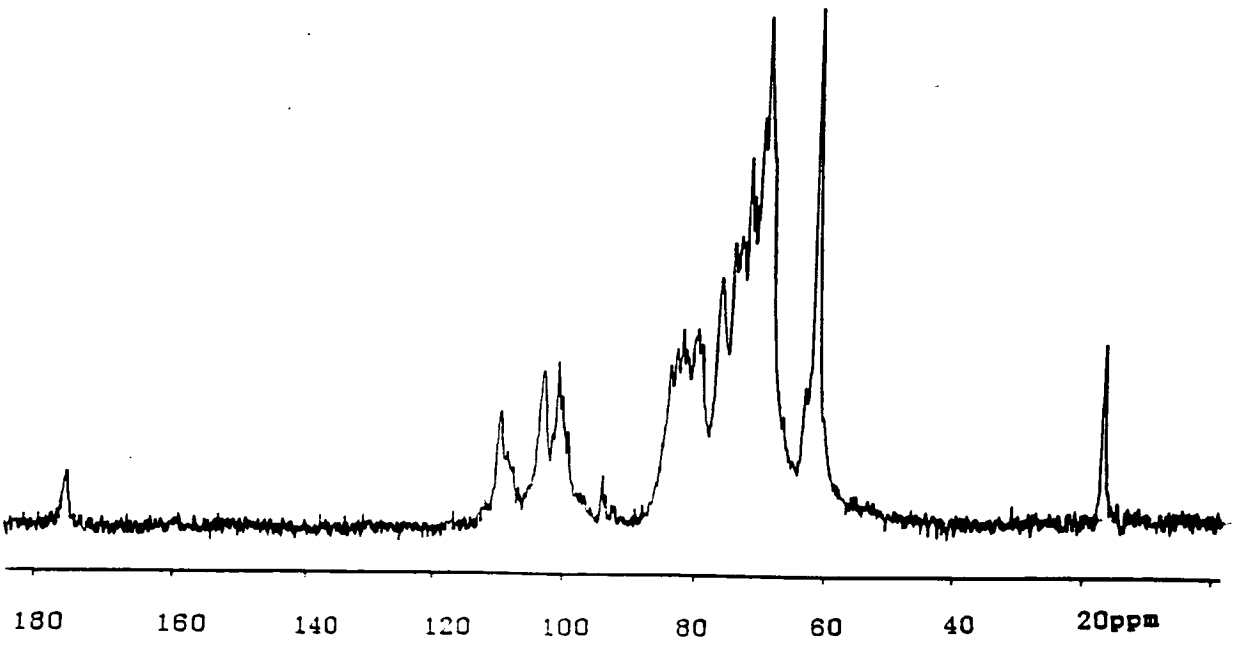


TABLE III.3 Amino acid composition of gum arabic, irradiated gum arabic and dialysed high molecular weight parent gum.

	Control Parent Gum Arabic.	1 MRad Irrad Gum Arabic	High Mol wgt fraction Parent gum
% Nitrogen	0.33	0.33	0.33
Alanine	27	30	24
Arginine	10	11	10
Aspartic acid	55	57	49
Cystine	0	0	1
Glutamic acid	42	44	36
Glycine	54	54	51
Histidine	49	48	51
Hydroxyproline	292	291	315
Isoleucine	12	12	11
Leucine	75	71	71
Lysine	27	28	31
Methionine	1	1	1
Phenylalanine	39	33	35
Proline	63	66	67
Serine	131	130	132
Threonine	74	72	73
Tyrosine	11	12	11
Valine	38	40	31
Nitrogen Conversion factor	6.59	6.57	6.58

TABLE III.4 Amino acid composition of dialysed irradiated and unirradiated gum arabic.

	Low Mol wgt fraction Parent	High Mol wgt 1 MRad Irrad	Low Mol wgt 1 MRad Irrad
% Nitrogen	0.33	0.30	0.53
Alanine	25	14	55
Arginine	6	6	34
Aspartic acid	55	46	38
Cystine	0	2	0
Glutamic acid	33	36	48
Glycine	61	53	49
Histidine	46	52	22
Hydroxyproline	312	361	141
Isoleucine	12	7	29
Leucine	72	65	102
Lysine	36	25	73
Methionine	1	1	1
Phenylalanine	36	22	75
Proline	49	68	64
Serine	129	149	100
Threonine	78	85	68
Tyrosine	12	5	32
Valine	37	3	69
Nitrogen Conversion factor	6.58	6.62	6.46

occurred as a result of irradiation.

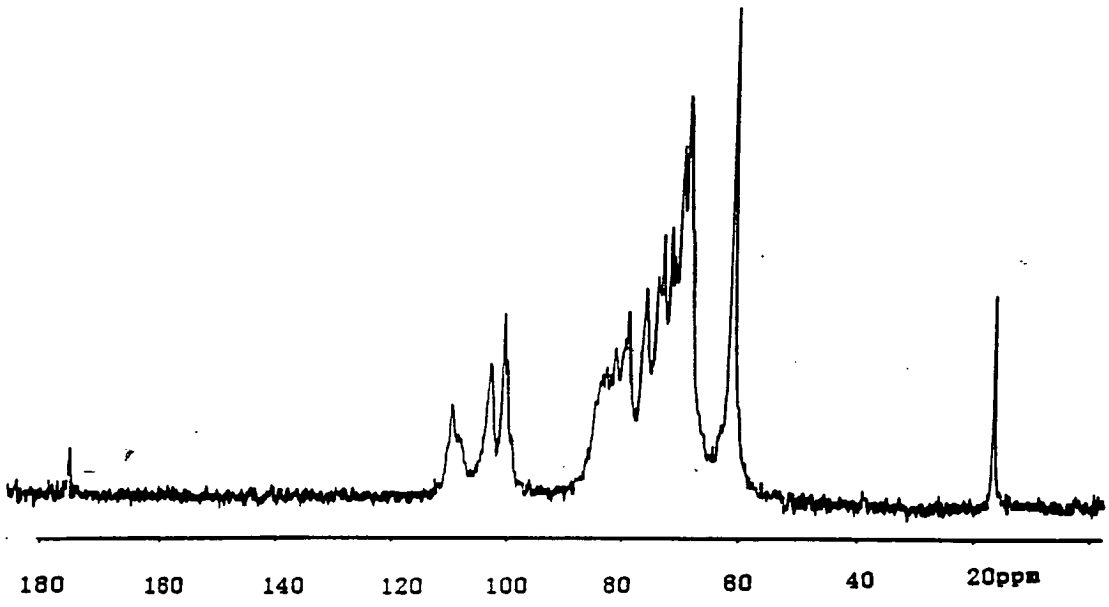
The amino acid compositions of the low and high molecular weight components of the control gum are very similar (Table III.3 and 4), but not identical as comparison of, for example, the proline values indicate. However the amino acid compositions of the low and high molecular weight components of the irradiated gum shows differences. There are major increases in the proportions of hydroxyproline, serine, threonine in the high molecular weight component. These amino acids have been previously identified to be associated with the core of the gum structure (22,47), and appear to have increased in relative terms through the possible loss of some of the peripherally located amino acids as a result of irradiation. Indeed, several relatively hydrophobic amino acids such as phenylalanine, isoleucine, alanine and valine are significantly higher in proportion in the low molecular weight component of the irradiated gum. These amino acids have been reported (22,47) to be located at the periphery of the gum's structure hence are likely to be involved in emulsification processes. Therefore their elimination, at least in part, from the irradiated gum may partially explain the reduction in its emulsification functionality.

When 13C NMR spectra of the low and high molecular weight components of the control gum and the 1MRad irradiated gum samples are compared, similar

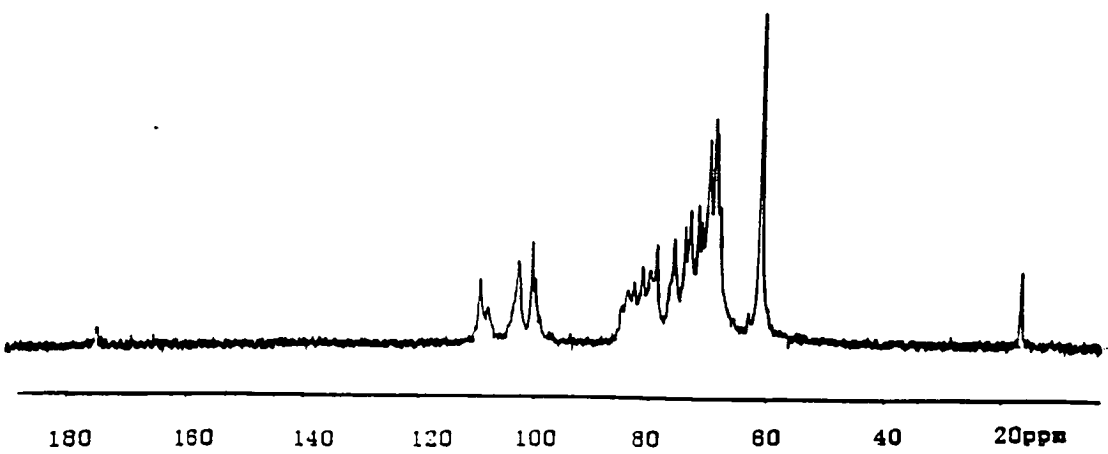
conclusions may be drawn. It appears that the spectra of the low and high molecular weight components of the unirradiated gum are similar, although some small differences are noticable. The spectra of the low and high molecular weight components of the irradiated gum, however, indicate that some structural degradation of the gum has occurred as a result of gamma irradiation; the spectrum of the high molecular weight irradiated gum shown in spectrum III.5 shows differences in the spectrum at around 95-105 p.p.m, a new peak is apparent at 95 p.p.m which is not present in the parent gum. The C-6 rhamnopyranosyl peak appears to have a slight shoulder in spectrum III.5 and not in spectrum III.1 the spectrum of the parent gum, where it appears as a singlet. The spectum of the low molecular weight irradiated material, spectrum III.6 suggests major structural changes from that of the parent gum (29).

These results do not agree with the findings of Blake et al (38) who concluded that "measurements showed that no adverse effects of gamma irradiation, even up to doses of 30KGys (3MRads), on the ability of gum arabic to stabilise emulsions". This study by Blake, irradiated spray dried gum at a dose rate of 0.011Gy/s. Therefore for a dosage of 10KGys, it would require approximately 252 hours in the radiation chamber, a time that could not be justified commercially. In the present study a dose rate of approximately 0.360Gy/s was delivered and the 1MRad

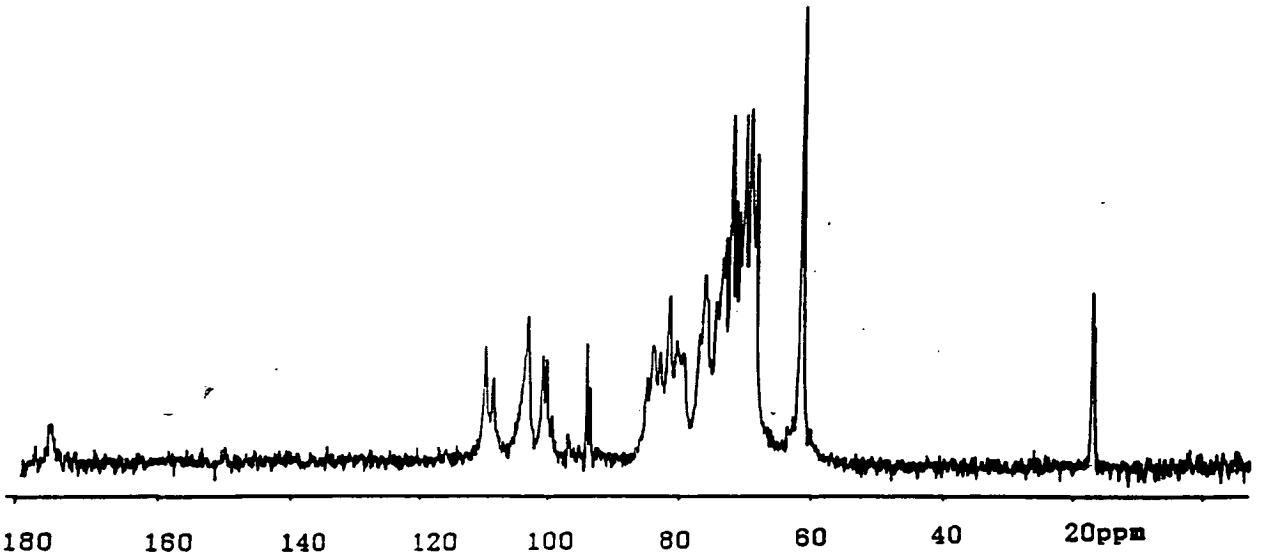
Spectrum III 3: 13C NMR spectra of dialysed high molecular weight component of control gum.



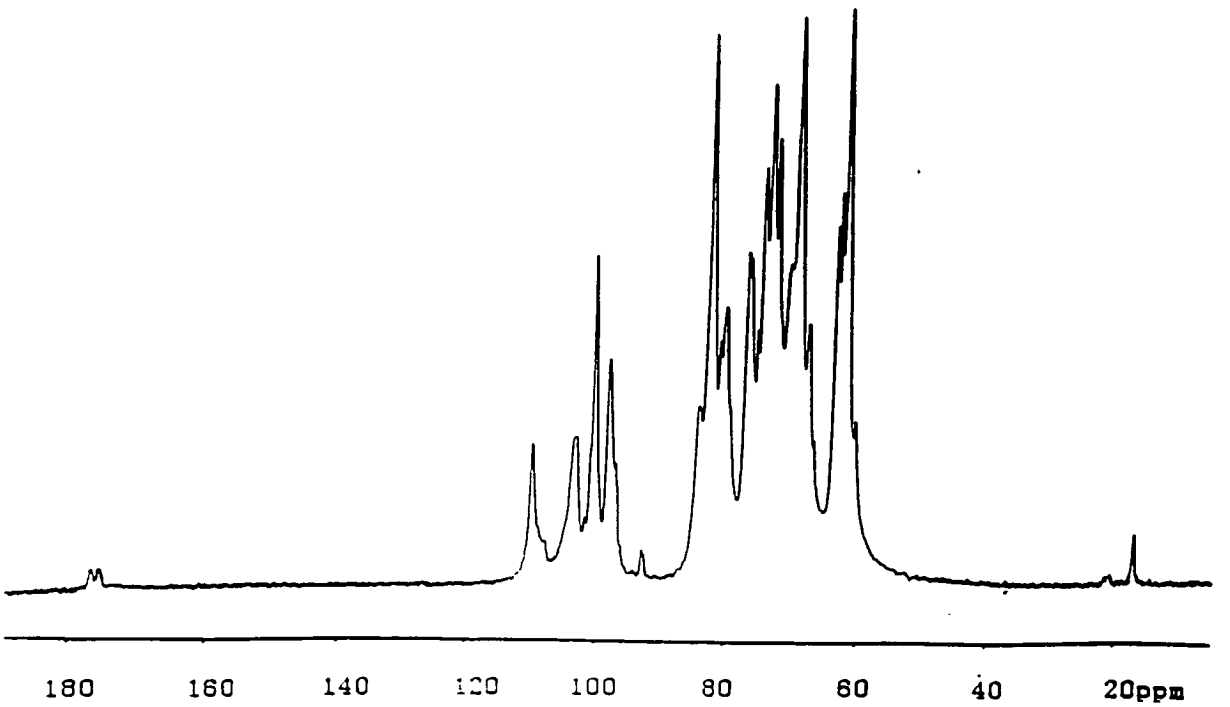
Spectrum III 4: 13C NMR spectra of dialysed low molecular weight component of control gum.



Spectrum III 5: 13C NMR spectra of dialysed high molecular weight component of irradiated gum.



Spectrum III 6: 13C NMR spectra of dialysed low molecular weight component of irradiated gum.



sample was irradiated for up to 6 hours. The study by Blake and co-workers also used a ^{60}Co radiation source for the raw and kibbled gum arabic samples, and a ^{137}Cs source for their spray dried gum which was irradiated at a different location.

CONCLUSION

The experimental evidence presented in this study suggests that gamma irradiation at doses up to 1 MRad (the proposed maximum permitted dose for food use), structurally degrades gum arabic, and reduces the gum's functionality in respect of its ability to form and stabilise emulsions.

CHAPTER III.2. MILD SEQUENTIAL SMITH DEGRADATIONS OF GUM ARABIC.

III.2 (i) INTRODUCTION

Smith and his co-workers first applied the technique of periodate oxidation followed by borohydride reduction then mild controlled acid hydrolysis in 1959 (48). Sequential Smith degradations were subsequently carried out by Anderson, Hirst and Stoddart on gum arabic in 1967 (12). The study carried out by Anderson and McDougal (47), which carried out four sequential Smith degradations on gum arabic extended previous studies by investigating the fate of periodate labile amino acids and sugars; it was established that the overall nitrogen content increased from 0.34% in the original gum to 0.85% in the fourth degradation product, and that the amino acid composition of this degradation product was very different from that of the original gum. Peripherally located rhamnose and most of the glucuronic acid residues were eliminated after the first Smith degradation, and the fourth degradation product contained only galactose, as had been established in the earlier study (12).

The present study attempts to carry out much milder sequential Smith degradations than used previously, where the very objective was for each stage

to be complete. Thus the gum will be periodate oxidised for a much shorter time than in previous studies. Previous studies have oxidised the gum for 48 hours with periodate in darkness for each Smith-degradation stage. This study will oxidise the gum for 30, 120, 360, 1200, and 2000 minutes respectively in five sequential stages, and investigate the fate of periodate vulnerable sugars and amino acids thus the total degradation after these five consecutive very short oxidations could be expected to be much less than resulted from even the first of these stages in either of the previous studies (12,47). The study determines various analytical parameters for each mildly degraded product and also how the functionality of the gum, with respect to emulsion formation and stability, are affected. This will attempt to establish the precise structural role of certain peripheral amino acid moieties in the unique functional properties of the gum.

Following a separate degradative study of gum arabic by Anderson and McDougal (22), it was concluded that some relatively hydrophobic amino acids are involved in peripheral chain-terminating positions in the branched gum macromolecules, whilst other relatively hydrophilic amino acids are located more extensively within the branched galactan core of the gum's highly branched heteropolysaccharide framework.

Each Smith degradation initially

involves a periodate oxidation stage. This oxidation will cleave sugar residues which have two hydroxyls in adjacent positions in the sugar ring. Therefore, galactose residues linked (1-6) are susceptible to oxidation, but residues which are linked (1-3) remain intact. A reduction stage with borohydride follows so that any cleaved sugar residues, aldehyde functional groups are reduced. The resulting polyalcohol is subsequently hydrolysed, at room temperature under mildly acidic conditions to cleave only the periodate-opened sugar residues. This exposes further hydroxyls on the remaining gum and further degradation can then be achieved.

Various workers (15,19) have used results from Smith degradations, with information from other hydrolytic techniques, to revise previously proposed structures for gum arabic and other Acacia gum exudates. Data has been reported for the fate of proteinaceous material in Acacia polyancantha (49), Acacia robusta (50), Acacia tortilis (51), and Acacia seyal (52). Although all these polysaccharides belong to the Acacia genus, they belong to different Bentham's subsections, either Gummiferae or Vulgares and all are structurally different. It has been suggested, from results of sequential Smith degradations (whether their protein composition is enriched or depleted), that gums from these subsections differ further in terms of the location of proteinaceous material within their

macromolecular structures.

Earlier studies by Anderson and Stoddart had indicated that Acacia senegal was inexplicably sensitive to autohydrolysis, and that a proteinaceous brown flocculant precipitate formed (53). The identity of the amino acids involved in each fraction was not investigated in this study. However the isolation, by fractional precipitation of a high molecular weight, high nitrogen fraction (1.0%), and a subsequent low molecular weight fraction almost deficient in nitrogen (0.02%), indicated that a fraction of gum arabic contained regular polysaccharide subunits interlinked with polypeptide chains, and a predominantly lower molecular weight arabinogalactan fraction which possibly consists of individual subunits.

Although further knowledge has been gained on the location of the structurally significant proteinaceous material in Acacia senegal, less has been reported on the role of this material in the functionality of the gum (47).

III.2 (ii) EXPERIMENTAL METHODS

Powdered natural gum arabic from Acacia senegal, (40g) was dissolved in distilled water (500ml) in a 1 litre volumetric flask and 0.25M sodium periodate solution (500ml) was added. The oxidation was followed titrimetrically by measuring the release of

formic acid with time. After time intervals of 30, 120, 360, 1200 and 2000 minutes, a 200ml portion of the gum solution (theoretically 8g assuming no degradation occurs), was withdrawn and added to ethylene glycol (10mls), to stop the reaction: the solution was then dialysed against tap water for 2 days. Sodium borohydride (2g) was then added and the mixture was maintained at room temperature for 30 hours, then dialysed for a further 48 hours. The resulting polyalcohol was hydroylsed with 0.5M sulphuric acid at room temperature for 48 hours after which the solution was neutralised with solid barium carbonate, filtered, deionised (Amberlite resin IR-120 (H)), reduced in volume to ca. 150mls, and dialysed against distilled water (10 litres). After further dialyses against running tap water for 2 days, each fraction was isolated as the freeze dried product and analysed.

III.2 (iii) RESULTS AND DISCUSSION

The yields for each sequential fraction SD0, SD1, SD2, SD3 and SD4, which were oxidised for time intervals of 30, 120, 360, 1200 and 2000 minutes respectively, were 82%, 67%, 55%, 39% and 29%. The analytical parameters determined for each degradation product and the original gum are shown in Table

TABLE III.5 (i) Analytical data for control gum arabic and sequential Smith degradation products.

Analytical Parameter	Parent gum	SD0	SD1	SD2	SD3	SD4
Moisture, %	9.8	3.2	3.1	3.2	3.3	3.1
Ash, % [a]	3.2	n.d	n.d	n.d	n.d	n.d
Nitrogen, % [a]	0.33	0.35	0.38	0.41	0.48	0.54
Nitrogen conversion factor (N.C.F)[b]	6.59	6.62	6.77	6.82	6.84	6.94
Hence protein, % (N.C.F X %N)	2.17	2.32	2.57	2.80	3.28	3.58
Methoxyl, % [b]	0.31	0.26	0.20	0.09	Tr	-
Specific rotation in water (degrees)[a]	-29°	-26°	-24°	-19°	-17°	-11°
Intrinsic viscosity, mlg^{-1} [a]	16	15	13	11	10	9
Equivalent weight [a]	1030	1354	4400	4400	5867	-
Uronic anhydride, %	17	13	4	4	3	-
Sugar composition after hydrolysis, % [c]						
Glucuronic acid [d]	17	13	4	4	3	0
Galactose	47	51	62	67	73	82
Arabinose	25	27	27	23	22	18
Rhamnose	11	9	7	6	2	0

Notes:

[a] Corrected for moisture content.

[b] From tables III.6 and 7.

[c] Corrected for protein content.

[d] Including 4-O-methylglucuronic acid.

III.5(i). Table III.5 (ii) shows how the protein content of the gum is enriched, following successive degradations. It can be initially noticed that there is enrichment of the overall protein content of the gum from a 33/1 polysaccharide/protein molar ratio in the original gum, to a 20/1 ratio in the fifth degradation product. The viscosity of the gum reduces as a result of the Smith degradation from 16ml/g in the original gum to 10ml/g in the fifth degradation product. The peripherally located rhamnose and the terminal glucuronic acids groups are sequentially reduced in each successive degradation product and are totally eliminated in SD4. The polysaccharide remaining after SD4 has an enriched protein content and a branched galactan backbone to which only galactose and arabinose residues are attached. All methoxyl groups are also eliminated in the fourth degradative product.

Table III.6 and 7 show how the amino acid contents per 1000 amino acid residues varies for each sequential degradation product as the proteinaceous component increases from 2.17% in the parent to 3.58% in the fifth degradative product. As reported in a previous study (47), the differences between the amino acid composition of the original gum and SD1 (subjected to a periodate oxidation for 48 hours) was the elimination of many peripherally located amino acids and the relative enrichment of other amino acids located in the core of the branched

macromolecule, associated with the galactan backbone.

This study agrees with these findings. Minor quantities of some amino acids e.g. methionine, isoleucine, tyrosine, arginine and alanine are almost totally eliminated by SD4, and others such as valine, phenylalanine, aspartic acid, and glycine are significantly reduced. These amino acids have been reported to be located at the periphery of the gum structure (22,47) and as they are relatively hydrophobic moieties, they are probably involved in the gum's unique functionality. Hydroxyproline remains the major amino acid in all the fractions, comprising 41% of the total protein in SD4 compared to 27% in the original gum. The relative proportions of hydroxyproline, threonine and serine all increase with successive degradations. All these amino acids have been reported to be located in the core of the branched macromolecule (22), and may be involved in covalent bonding between amino acid and sugar residues in arabinogalactan glycoproteins.

The emulsification ability of the original (parent) gum compared to that of each successive Smith degradation product shows interesting findings. Graph III.4 indicates that the emulsification activity (absorption in U.V at 500nm) of a limonene oil-in water emulsion is greatly diminished by the small changes to the gum structure as a result of sequential periodate oxidations. The emulsion

TABLE III.5 (ii) Relative proportions of sugar and amino acids in <u>Acacia senegal</u> and five Smith degradation products.

Gum fraction	Yield %	Hence wgt	Nitrogen % and factor (a)	Hence composition of products(b) polysac m/moles	protein m/moles	Hence ratio polysac/ protei mm/mm
Whole gum	100%	8.00g	0.33% 6.59	46.2	1.38	33/1
SD 0	82%	6.56g	0.35% 6.62	38.0	1.21	31/1
SD 1	67%	5.36g	0.38% 6.77	31.1	1.11	28/1
SD 2	55%	4.40g	0.41% 6.82	25.3	0.99	25/1
SD 3	39%	3.12g	0.48% 6.84	17.9	0.82	22/1
SD 4	29%	2.32g	0.54% 6.94	13.3	0.66	20/1

<u>Notes</u>

[a] Factors for converting % N to % protein in Table III.6 and 7.

[b] From sugar ratios Table III.5 (i) and amino acid compositions Table III.6 and 7.

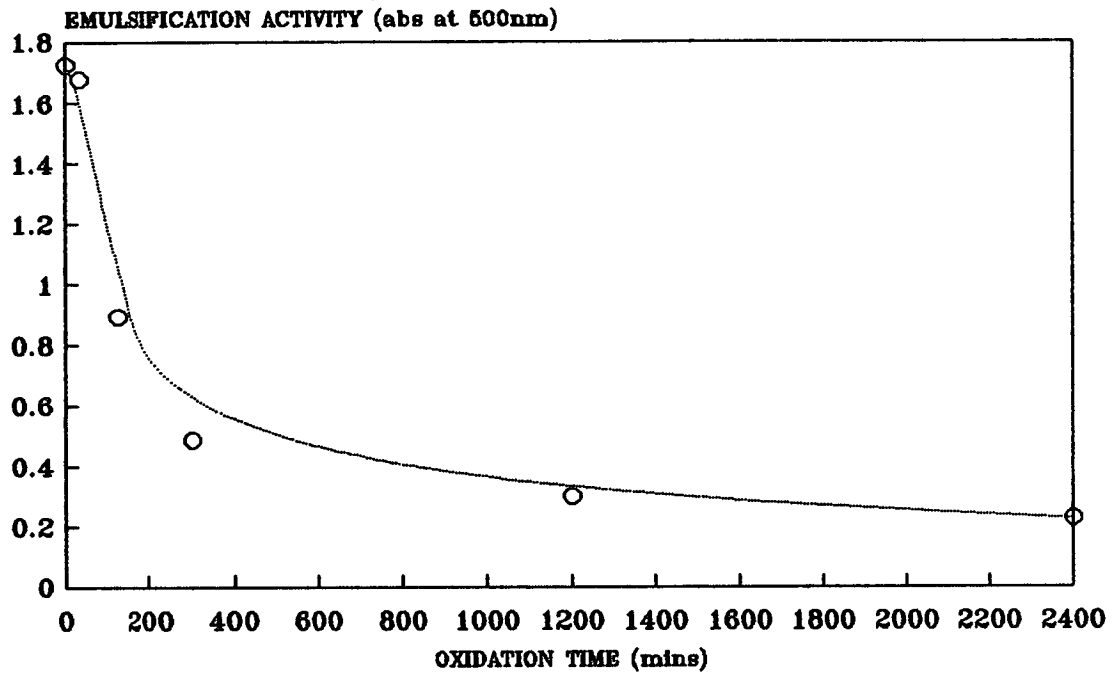
TABLE III.6 Amino acid composition of sequential periodate oxidation products of gum arabic.

	Control Parent Gum Arabic.	SD0 30 mins oxidation.	SD1 120 mins oxidation.
% Nitrogen	0.33	0.35	0.38
Alanine	27	26	19
Arginine	10	8	5
Aspartic acid	55	56	54
Cystine	0	0	0
Glutamic acid	42	46	40
Glycine	54	57	57
Histidine	47	48	40
Hydroxyproline	271	293	314
Isoleucine	13	12	10
Leucine	75	75	73
Lysine	26	19	17
Methionine	3	2	1
Phenylalanine	39	37	37
Proline	74	69	78
Serine	141	141	142
Threonine	74	77	76
Tyrosine	10	7	6
Valine	39	37	31
Nitrogen Conversion factor	6.58	6.62	6.77

TABLE III.7 Amino acid composition of sequential periodate oxidation products of gum arabic.

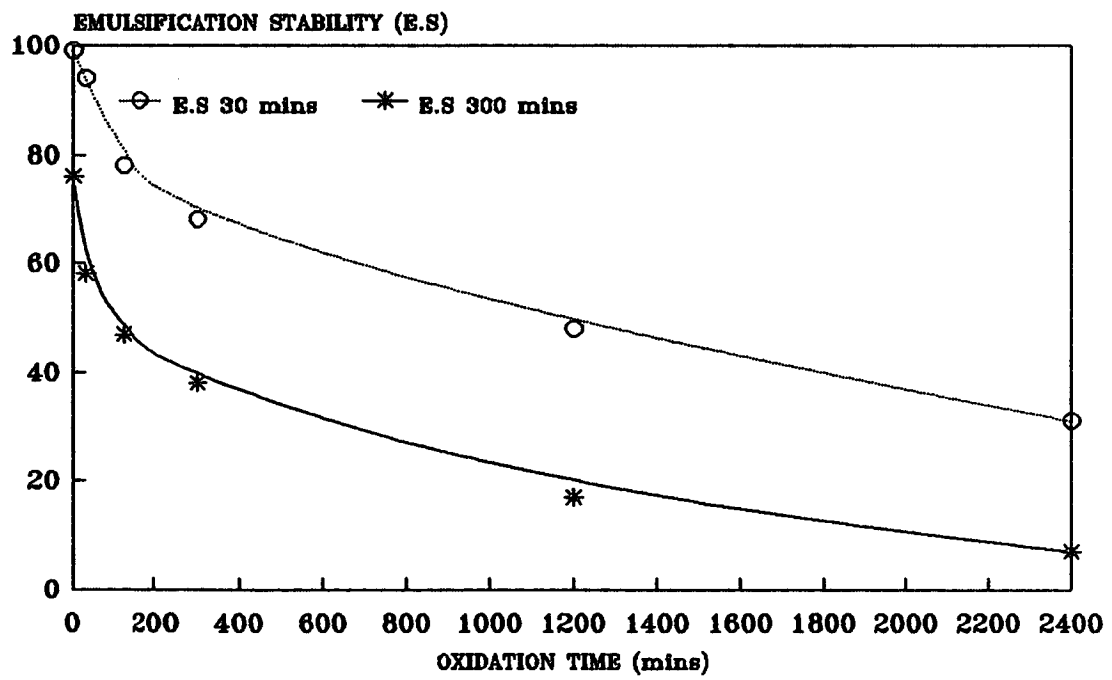
	SD2 300 mins oxidation	SD3 1200 mins oxidation.	SD4 2000 mins oxidation.
% Nitrogen	0.41	0.48	0.54
Alanine	17	15	7
Argininé	5	5	2
Aspartic acid	56	57	30
Cystine	0	0	0
Glutamic acid	35	32	29
Glycine	56	49	37
Histidine	35	33	34
Hydroxyproline	334	354	413
Isoleucine	7	6	2
Leucine	77	75	77
Lysine	15	13	5
Methionine	0	0	0
Phenylalanine	32	24	8
Proline	77	71	77
Serine	147	160	164
Threonine	80	90	106
Tyrosine	5	2	1
Valine	22	14	8
Nitrogen Conversion factor	6.82	6.84	6.94

RELATIONSHIP BETWEEN EMULSIFICATION ACTIVITY AND PERIODATE OXIDATION REACTION TIME.



GRAPH III.4
D-LIMONENE-WATER EMULSION.

RELATIONSHIP BETWEEN EMULSIFICATION STABILITY AND PERIODATE OXIDATION REACTION TIME.



GRAPH III.5
LIMONENE-WATER EMULSION.

stability, which is the turbidity reading at a certain time interval expressed relative to the original emulsification activity, is also reduced as a result of structural degradation (graph III.5). The pattern of reduction is similar in an independant experiment at 300 minutes compared to 30 minutes. Therefore these results suggest that small changes in structure can lead to dramatic changes in the gums functionality and performance as an effective emulsifier.

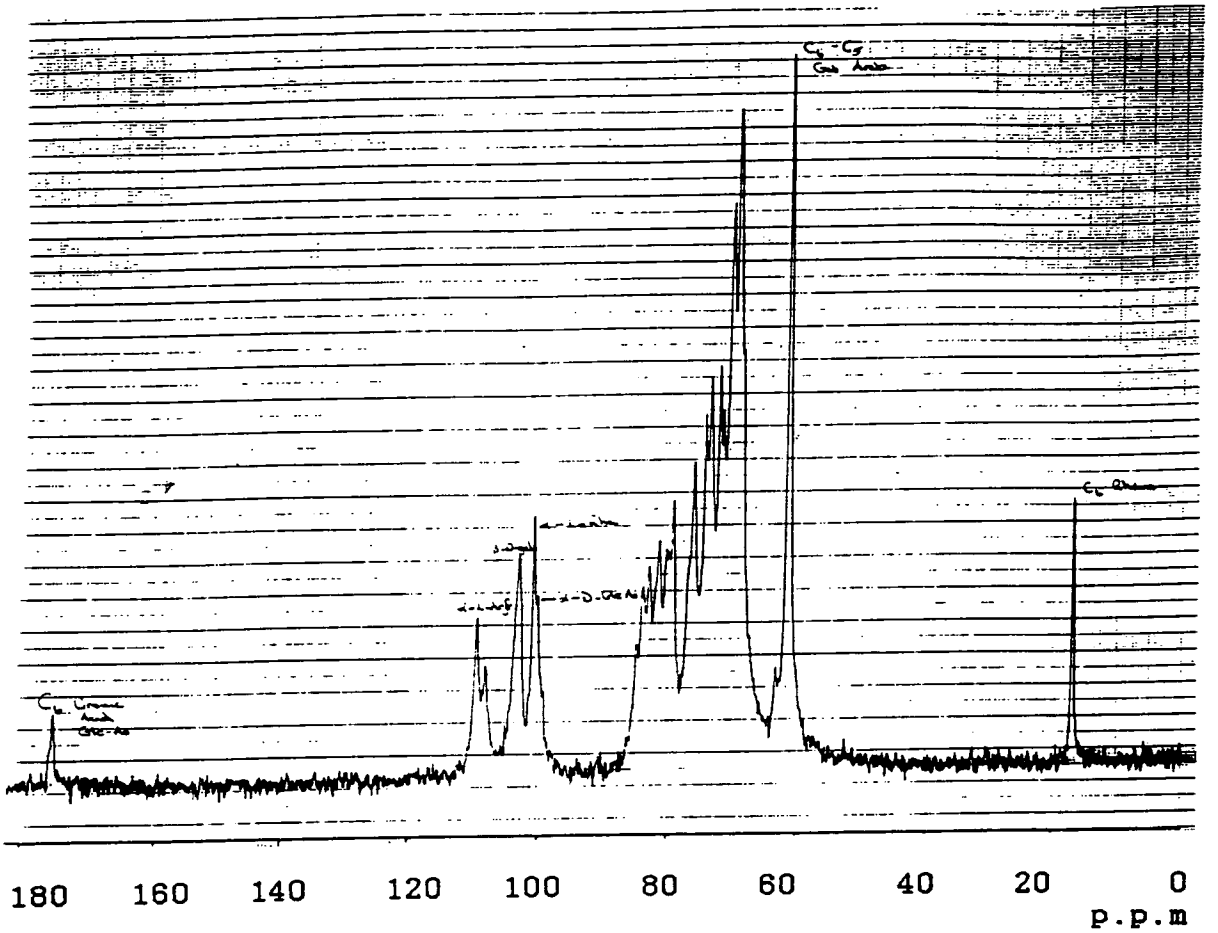
Previous studies (32,39,40), have linked a gums' molecular weight to the gums ability to form and stabilise emulsions. Another study (34) has shown that only a very small amount of high molecular weight, highly proteinaceous material is adsorbed at the oil-water interphase. However the viscosity of the non-adsorbed gum molecules may stabilise the oil droplets in an emulsion (which may be encapsulated by the adsorbed high molecular weight fraction of the gum molecules) and thus prevent phase separation. This study (40) indicated that the viscosity and hence molecular weight of the gum is necessary for gum stability, but perhaps more critical is the role of hydrophobic amino acid and sugar residues in forming the initial emulsion by adsorbing at the oil-water interphase. Although the degraded products SD1 to SD4 have enriched protein contents their ability to form emulsions is greatly diminished.

The gum polysaccharide from Acacia

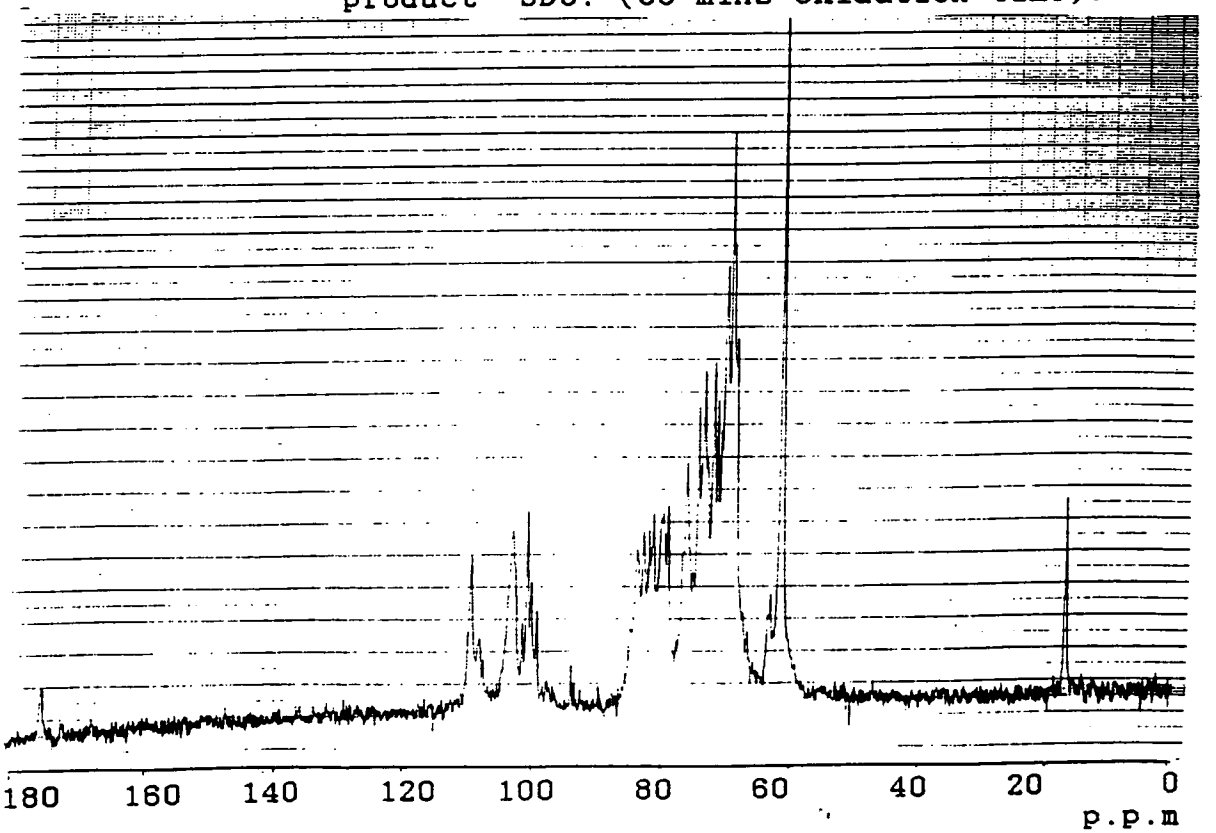
senegal (L.) Willd, in aqueous solution, gave a well resolved ^{13}C-nmr spectrum (46),(spectrum III.7). The two signals at extreme field can be assigned unambigiously to C-6 of L-rhamnopyranose (17.6 p.p.m) and D-glucuronic acid (175.6 p.p.m). In the range for the anomeric carbon resonances (90-110 p.p.m), four more signals can be resolved; α-D-Arabinofuranose C-1 at 109.6 p.p.m, β-D-galactopyranosyl at 104.5 p.p.m, α-L-rhamnopyranosyl at 101.6 p.p.m and α-D-glucuronopyranosyl at 100.8 p.p.m, based on literature values (46). This assumes that L-rhamnose, D-galactose, L-Arabinose and D-glucuronic acid are the only constituents in the gum and does not consider the 2.1% of proteinaceous material. Complete assignment of the non-anomeric ^{13}C resonances in the 60-90 p.p.m region of the spectra proves difficult due to overlapping of signals. The major peak at 62.2 can be assigned to unresolved C-6 galactopyranosyl plus C-5 arabinofuranosyl.

The spectrum, (spectrum III.8) of the polysaccharide obtained after the initial 30 minute periodate oxidation indicates that the the signals previously assigned in the original gum arabic to α-L-rhanopyranosyl (17.6 p.p.m) and D-glucuronic acid (175.6p.p.m) are significantly reduced. This comfirms previous structural studies that these two sugars are located on the peripheral, chain terminal positions of the branched macromolecule. The pattern is similar for

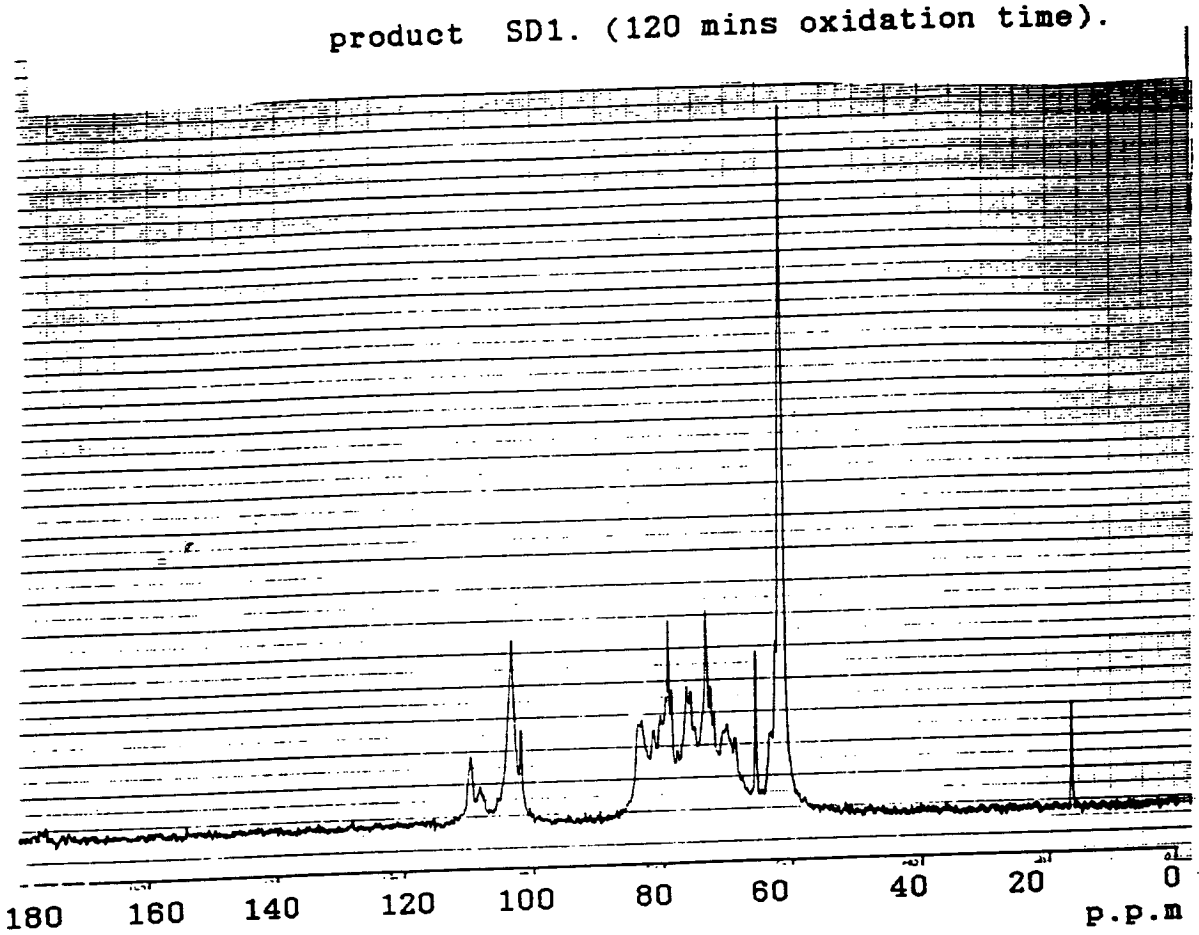
Spectrum III 7: 13C NMR spectra of control untreated gum arabic.



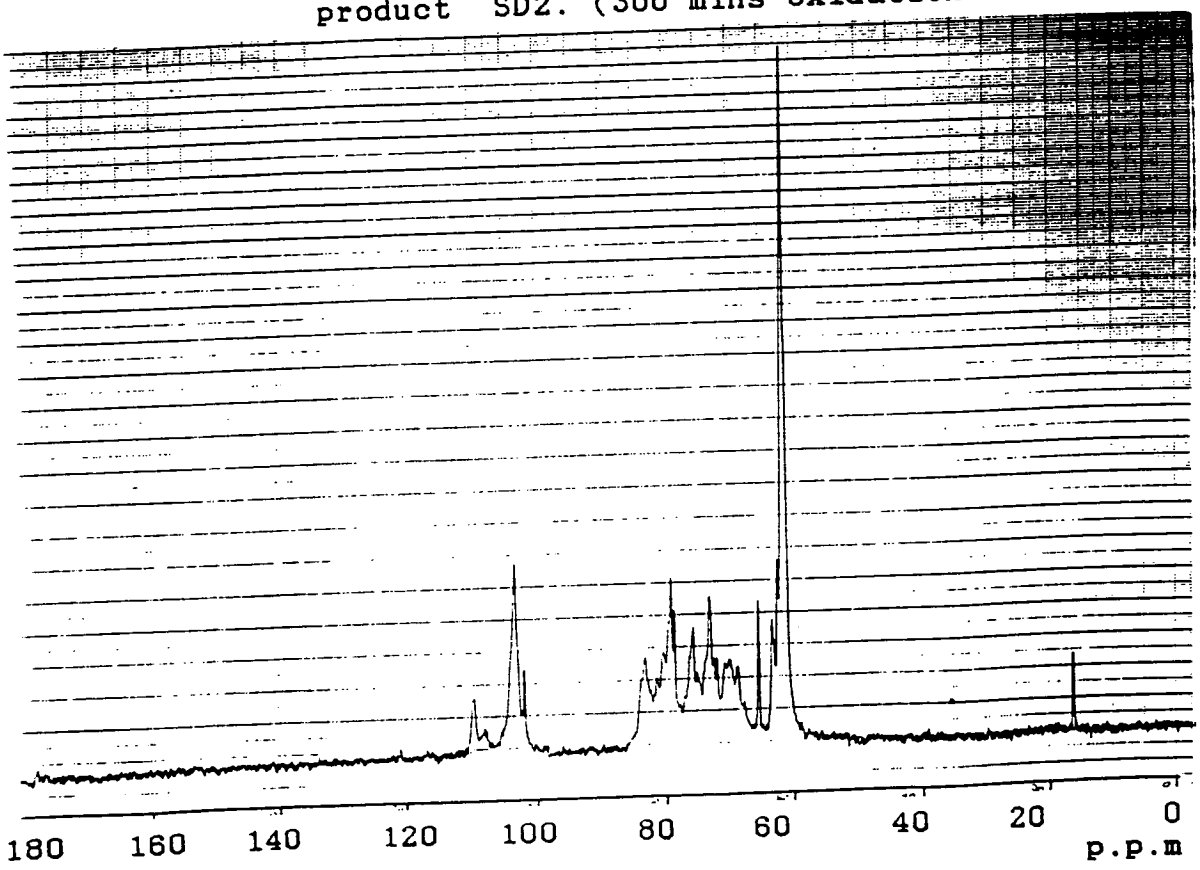
Spectrum III 8: 13C NMR spectra of periodate oxidation product SDO. (30 mins oxidation time).



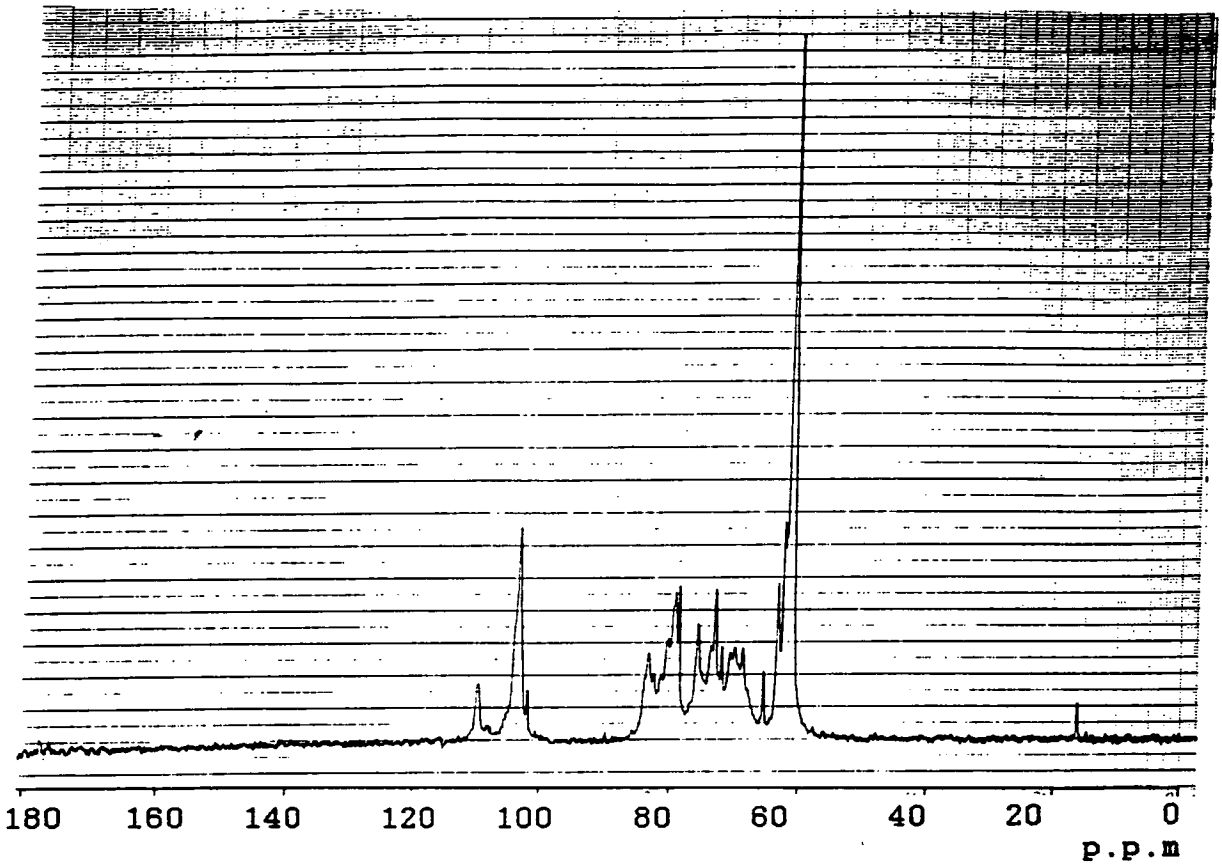
Spectrum III 9: 13C NMR spectra of periodate oxidation product SD1. (120 mins oxidation time).



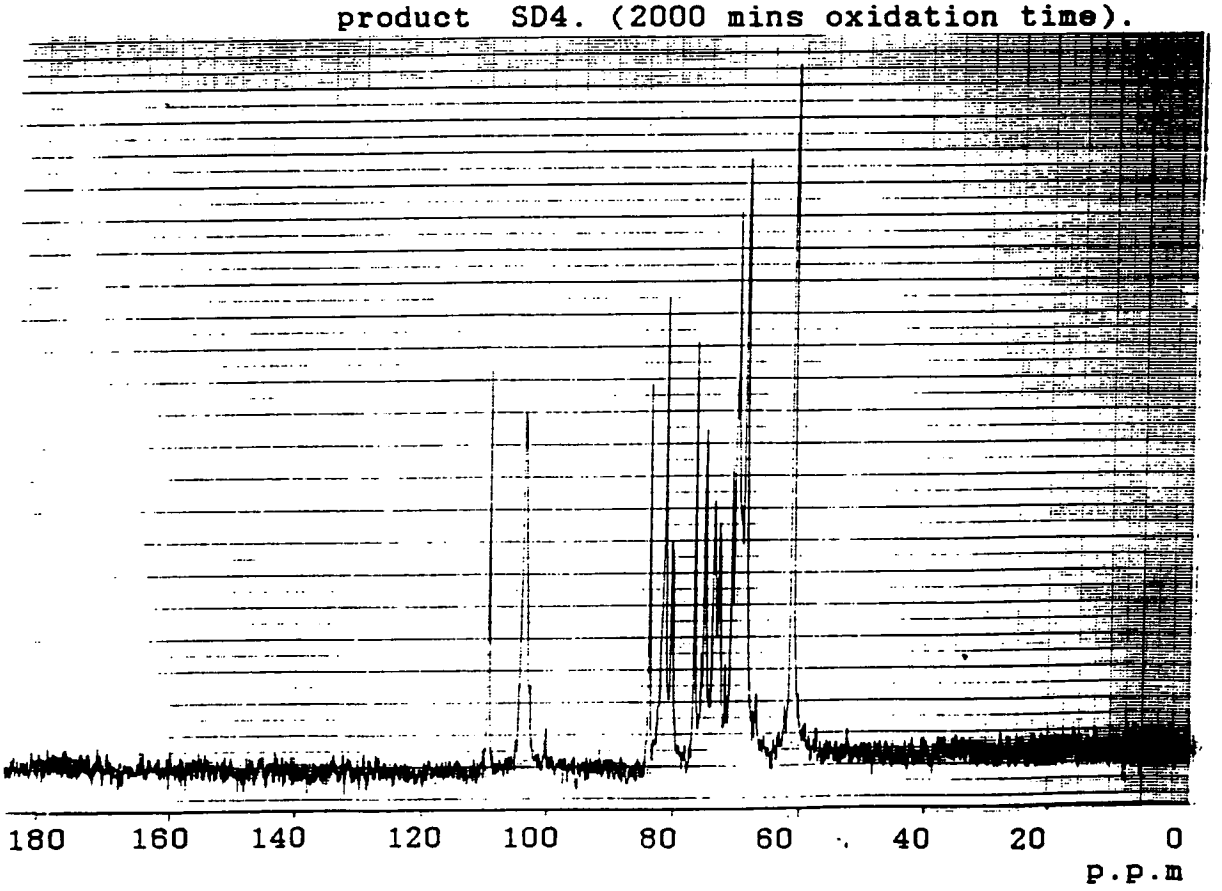
Spectrum III 10: 13C NMR spectra of periodate oxidation product SD2. (300 mins oxidation time).



Spectrum III 11: 13C NMR spectra of periodate oxidation product SD3. (1200 mins oxidation time).



Spectrum III 12: 13C NMR spectra of periodate oxidation product SD4. (2000 mins oxidation time).



SD1 (spectrum III.9), and in spectrum III.10 for SD2, little rhamnose or glucuronic acid residues remain attached to the gum structure. In spectrum III.12 where the polysaccharide SD4 had been oxidised for 2000 minutes, no rhamnose or glucuronic acid residues are present on the remaining protein-enriched galactan core of the branched macromolecule. In this spectrum, the presence of a single C-1 signal for L-arabinose (compare with the double signal at 109.6 p.p.m given by the original (parent) gum arabic, and diminished double peaks in subsequent spectra), suggests that the (1-3) linked α-L-arabinofuranosyl side chains contained no more than two arabinose units in the original gum (earlier studies (12) had proposed that no side chains were longer than three arabinose units).

These results provide more structural evidence on the fate of certain sugars during sequential periodate oxidations, and confirm and extend previous studies (22,46,47) of the structure of gum arabic. The results also confirm the sugar ratios derived from chromatographic techniques in Table III.5(i) for the various polysaccharide degradative products.

CONCLUSION

The results from the extremely mild successive Smith degradations of Acacia senegal, suggest that although only 25% of the total protein

content in the gum structure is surface active (34), the peripherally located (2), chain-terminal hydrophobic amino acid residues play a critical role in the functionality of the gum for effectively forming and stabilising oil-in-water emulsions.

This study has considered the complex macromolecules of Acacia senegal gum, as a whole in five mild sequential Smith degradations. A more complete study could consider the sequential Smith degradation products of various molecular weight fractions of gum arabic.

CHAPTER III.3 DEPROTEINATION AND FRACTIONATION OF ACACIA SENEGAL (GUM ARABIC).

III.3 (i) INTRODUCTION

Gum arabic is a highly branched heteropolymolecular (14) polysaccharide, i.e. it consists of molecules that show a natural variation in their sugar and amino acid composition as well as in mode of linkage and molecular mass. Various fractionations, mild acid hydrolysis, autohydrolysis, sequential periodate oxidations and methylation studies on the whole gum (12,13,22 and 47) and fractions of the gum have indicated that gum arabic consists of a β,1-3 linked galactopyranose core with branches of galactopyranose linked β,1-6; arabinopyranose, arabinofuranose and rhamnopyranose, glucuronic acid and 4-O-methyl glucuronic acid exist as chain-terminating groups and a smaller proportion of glucuronic acid residues also may be linked to the core of the gum through a galactopyranosyl bond (14,15,54).

Another study (25) has shown that the structure posseses a high degree of regularity and proposed that the gum molecule consisted of 64 sub-units each of which had a molecular mass of 8000, which were arranged linearly or may be randomly disposed. Although earlier studies had reported a

protein component in the gum (53,55), and the gum had been fractionated into varying nitrogen contents, most structural studies until recently had concentrated on the carbohydrate component and their modes of linkage in the gum (47).

Various attempts have been made to purify and fractionate gum arabic into several components (53,56). An early study in 1958 (57), on the purification of gum arabic by sequential precipitation by acetone observed that various fractions separated and that the gum was heterogeneous in nature, as each fraction had a different intrinsic viscosity and hence molecular weight. Another study by Heidelberger and co-workers in 1956 (58), using chemical and immunological procedures concluded that the gum was not homogenous and that no precise structural formula could be given. Anderson and Stoddart in 1966 (53) fractionated gum arabic using saturated sodium sulphate solution, into various molecular weight fractions which also differed markedly in protein content, but without yielding a fraction completely devoid of protein. A recent publication by Allain and co-workers (7), separated Acacia senegal gum into various molecular weight fractions by inducing coacervation of the highest molecular weight fractions with successively more concentrated propan-1-ol solutions. However this study did not consider the proteinaceous component of each fraction.

A study by Vandevelde and Fenyo (23) concluded that the gum consisted of two distinct fractions, one lower molecular weight component which was predominately carbohydrate in nature but not completely void of protein, and a higher molecular weight fraction which although only comprising of 30% of the total weight of gum, was more correctly termed an arabinogalactan-protein complex due to its relatively high nitrogen content.

An attempt to deproteinate gum arabic by a protease enyzme (59), concluded that the gum structure consisted of regular carbohydrate blocks of molecular mass approximately $2X10^5$, which are covalently linked to a core polypeptide chain. This has been termed the "Wattle Blossom model" as proposed by Fincher and co-workers (27,28). However this representation does not explain why high molecular weight material is associated with a high protein content, and also why some structurally and functionally important amino acid moieties have been linked to the periphery of the gum structure.

The present study attempts to deproteinate gum arabic by separating various fractions using butan-1-ol. Various attempts (60,61) have been made to fractionate and deproteinate starch, which consists of two major fractions, a straight chain amylose fraction and a branched amylopectin fraction, using similar techniques. Schoch in 1942 (63),

separated starch by selective precipitation using butan-1-ol, the butan-1-ol soluble fraction (amylose) was suggested to be the component in potato and corn starch responsible for gelation and retrogradation characteristics of the parent. Schoch in 1950 (64), separated starch into a linear and a branched fraction using an aliphatic alcohol-water mixture at elevated temperatures.

Gum arabic has been shown to be heterogeneous in nature, and also that only a small amount of a proteinaceous component, about 2% of the total mass of the gum is adsorbed at the oil-in-water interphase in emulsion stabilisation and is responsible for the gum's unique functionality (38). It is possible therefore that a more hydrophobic fraction of the gum may be selectively fractionated by a butanol-sodium chloride solution slurry. This study therefore goes on to treat a protein-depleted fraction of gum arabic with butan-1-ol in an attempt to remove further quantities of proteinaceous material.

The study then compares the fractions obtained from deproteination of Acacia senegal, with comparable fractions from Acacia seyal (refer to Chapter IV.3 for analyses of four Acacia gums not permitted for use as food additives). Acacia seyal (characterised in Bentham's Gummiferae subsection [65]) differs taxonomically from Acacia senegal (Bentham's Vulgares subsection). In clear distinction from Acacia

senegal, Acacia seyal has a high positive specific rotation, a low content of L-rhamnose and of nitrogen, lower intrinsic viscosity and inferior emulsion functionality (52). It is also not permitted on any International list for use as a food additive, but has commonly being blended as an adulterant, and sold as "true" gum arabic, as this is commercially attractive for unscrupulous gum dealers (1).

Published studies on sequential periodate degradations, amd β-elimination treatments by Anderson and co-workers have suggested that the constituent amino acids in Acacia seyal appear to differ quantitatively from those in gum arabic. The paper concluded that the protein component in Acacia seyal (52) was, in contrast, present as a core protein/peptide, as opposed to the three different molecular weight moieties in Acacia senegal. The present study investigates the effect of these structural differences on the behavior of these two gums with respect to attempted deproteinations by butan-1-ol.

III.3 (ii) MATERIALS AND METHODS

Good quality Sudanese gum arabic from Acacia senegal, (5g) was dissolved in distilled water overnight (20mls), to give a 20% solution, which was filtered through fine muslin cloth to remove insoluble

impurities. Sodium chloride solution (5M, 25mls) was added. This gives a 45ml solution that is 10% w.r.t gum arabic. Butan-1-ol (10mls) was added and the mixture was gently shaken for 4 hours. Two distinct phases were allowed to separate in a separating funnel overnight. The sodium chloride serves to increase the density difference between the two layers and aids separation. The denatured protein should be present at the interphase. The lower aqueous layer, the upper organic layer, and the interphase layer which contained a brown precipitate were separated. The three layers were reduced in volume by rotary evaporation at 37°C to remove butan-1-ol. The three fractions were then dialysed against running tap water for 24 hours, then distilled water for 48 hours to remove sodium chloride. The three fractions were then freeze dried and their nitrogen content was determined.

The experiment was scaled up 4 fold (20g gum), to attempt to produce three fractions whose total carbohydrate and proteinaceous analytical parameters could be determined. The consistent result was a depleted nitrogen content in the fraction extracted in the lower aqueous sodium chloride layer, and an enriched nitrogen content in the fractions obtained from the upper organic butan-1-ol layer.

Deproteination of gum fraction depleted in protein.

The experiment was repeated on the nitrogen-depleted fraction (Dep I) from the first deproteination, but now using a 25% butanol/75% aqueous slurry, as opposed to a 20/80% slurry previously. Further nitrogen depletion of the aqueous phase material occurred, and a more highly enriched fraction came out of the butan-1-ol layer.

Treatment protein depleted gum arabic with a protease enzyme.

The experiment was repeated on the parent gum as described in the initial experiment, and again one nitrogen-depleted and two nitrogen-enriched fractions were obtained. The depleted nitrogen fraction (93% of the total weight of parent gum) was then divided into three (40g gum in each) to give three identical solutions. One depleted gum solution was treated as a control, as before, for a secondary deproteination. The second was treated with a live protease enzyme (0.1ml), and the third was treated with the same quantity of thermally denatured enzyme (0.1ml added to 100ml distilled water and heated to 80° for 60 minutes).

Two products a nitrogen-enriched fraction and a nitrogen-depleted fraction resulted in each of the three experiments, these fractions were analysed for protein and amino acid composition. The enzyme used in the determination was Neutrase (66), a

food grade proteinase made by Novo by submerged fermentation of a selected strain of Bacillus subtilis.

In a previous experiment the treatment of 100ml of 25% aqueous solution of gum arabic (Brookfield viscosity 110cps) with 1ml of Neutrase, the Brookfield viscosity of the gum dropped to 75 cps overnight. Therefore this enzyme was chosen in preference to other proteinases as it appeared to hydrolyse linkages involved in the overall macromolecular structure of the natural gum.

Treatment of Acacia seyal with butan-1-ol.

Acacia seyal Del. (10g), the major commercial source of gum tahla, was deproteinated by the same technique as that used for **Acacia senegal**, to enable comparisons to be made between the carbohydrate-protein complex in each gum.

III.3 (iii) RESULTS AND DISCUSSION

Initial deproteination

Results from the initial butan-1-ol deproteination of Acacia senegal (L.) Willd. are shown in Table III.8. Three fractions; a nitrogen-depleted fraction from the aqueous layer (Dep I), a soluble nitrogen-enriched fraction (En I sol) from the butan-1-ol layer and an insoluble nitrogen-enriched

TABLE III.8 Analytical data for gum arabic fractions from butan-1-ol deproteination.

Analytical Parameter	Control gum arabic	Dep I	Enrich I sol	Enrich I insol
Recovery (g)	35	29.3	2.6	0.12
Yield %	100	93	6.6	0.42
Moisture, %	9.8	3.4	3.6	3.1
Ash, % [a]	3.2	3.2	3.1	n.d
Nitrogen, % [a]	0.34	0.31	0.70	2.51
Nitrogen conversion factor (N.C.F)[b]	6.59	6.71	6.52	6.22
Hence protein, % (N.C.F X %N)	2.2	2.0	4.5	15.5
Specific rotation in water (degrees)[a]	-28°	-26°	-18°	n.d
Intrinsic viscosity, mlg^{-1} [a]	15	13	16	n.d
Equivalent weight [a]	1030	1120	960	n.d
Uronic anhydride, % [d]	17	16	18	n.d
Sugar composition after hydrolysis, % [c]				
(bracket value shows neutral sugar ratio)				
Glucuronic acid	17	16	18	n.d
Galactose	47(57)	51(61)	37(45)	(46)
Arabinose	25(30)	24(29)	31(38)	(44)
Rhamnose	11(13)	9(10)	14(17)	(10)

Notes:
[a] Corrected for moisture content.
[b] From table III.9
[c] Corrected for protein content.
[d] If all the acidity arises from uronic acids.

fraction (En I insol) from the interphase between the two layers were separated. All three have different analytical parameters to the control gum arabic sample.

The fractions separated in the butan-1-ol layer and at the solvent interphase both had enriched nitrogen contents and were termed En I soluble (sol) and En I insoluble (insol) respectively. Due to a very small recovery, and the insoluble nature of En I insol, only certain analytical parameters were determined for this fraction. Table III.8 indicates that En I sol, has a higher intrinsic viscosity than the parent gum arabic, a higher protein content, and a less negative specific rotation. Its carbohydrate composition differs from the parent with a higher glucuronic acid, rhamnose and arabinose content (i.e. increased proportions of those sugars known to be located in peripheral structural locations) and a correspondingly lower galactose content. The neutral sugar ratio of all three fractions and the parent gum arabic are also shown in Table III.8.

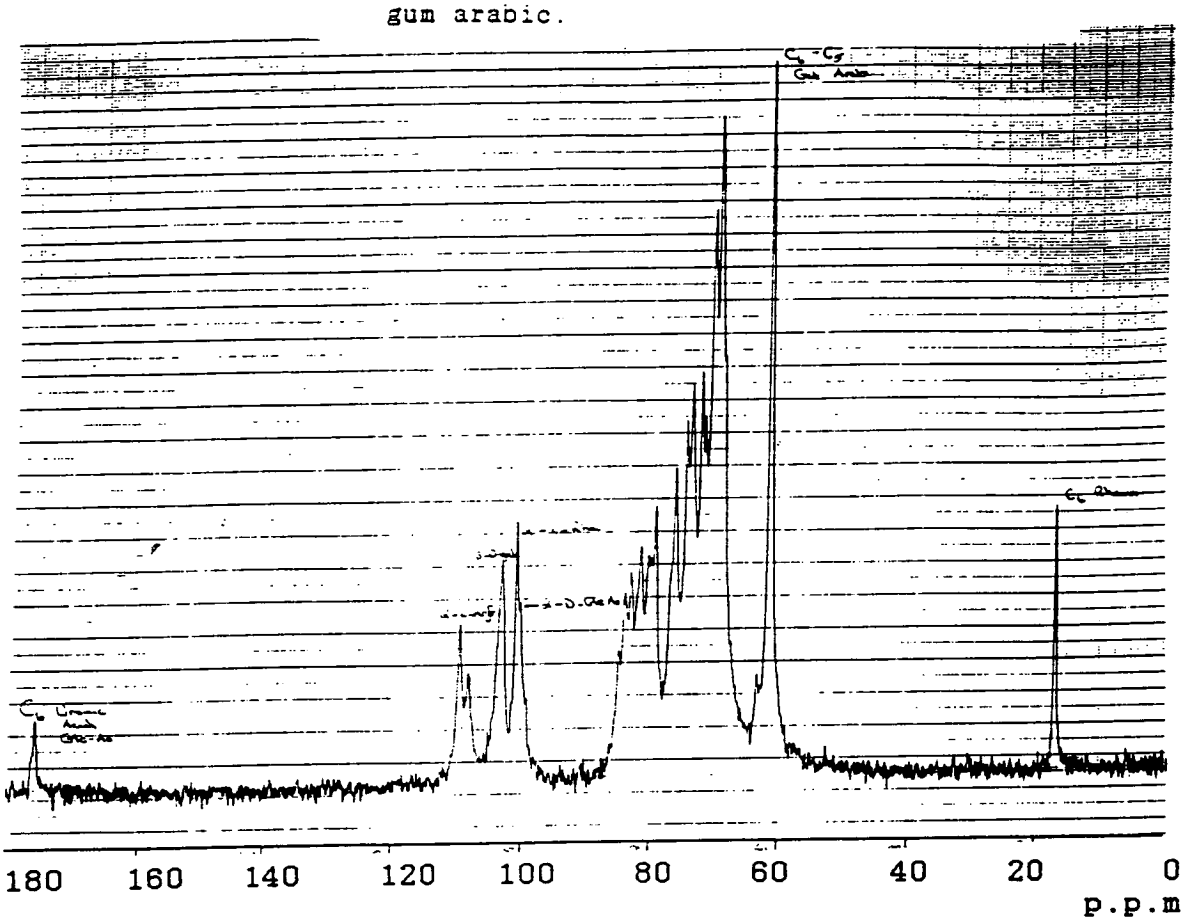
The major fraction (93% by weight of the parent gum arabic) separated in the aqueous layer and was termed Dep I, has a depleted protein content, a lower specific rotation, a higher galactose content and a lower rhamnose content, than the parent gum as shown in Table III.8.

Initial results indicate that two major fractions and a smaller highly proteinaceous fraction

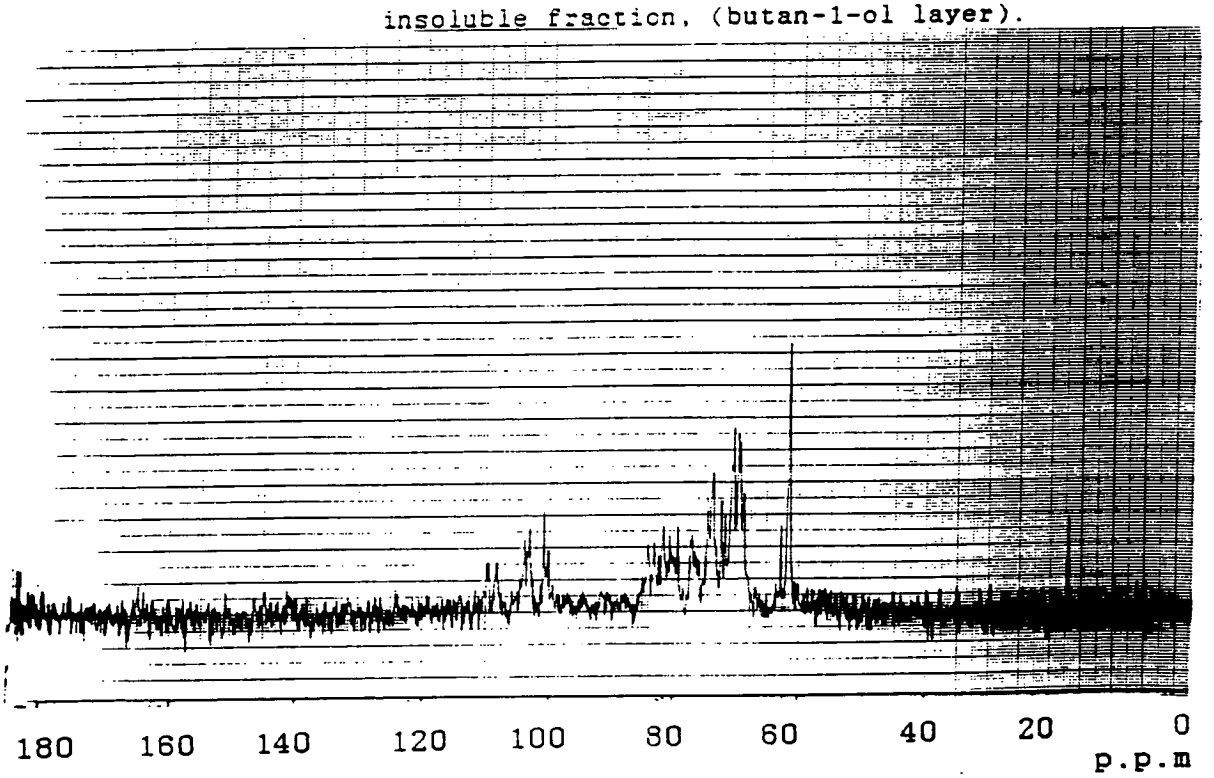
have been separated, the fractions differing greatly in nitrogen content; some differences also exist in the carbohydrate components of the fractions. ^{13}C Fourier Transform NMR spectra (46,3), were obtained for each fraction and compared to a reference spectra of the parent gum. The insoluble flocculant fraction (En I insol) was rendered soluble by adding small quantities of sodium borohydride to the gum. (Spectras III.13,14,15 and 16). The spectra of the two major fractions (En I sol and Dep I) of the gum show detectable differences in fine structural detail compared to the parent, for example involving the α-L-Arabinofuranosyl peak at 109.6 in the nitrogen-enriched En I spectrum III.15 and involving the α-D-glucuronic acid peak at 103.7 in the same spectrum (58), as expected from the differences in the sugar ratios of the fractions determined by paper chromatography. The NMR spectrum III.15 of the nitrogen enriched fraction En I sol also suggests higher glucuronic acid (175.6 p.p.m) and higher rhamnose (17.7 p.p.m) intensities than in the parent gum arabic, spectrum III.13 as indicated by the sugar ratios in Table III.8.

The conclusion, from the NMR spectra of the three fractions is however that only small differences exist between the carbohydrate components of the two main fractions, agrees with a previous publication which reported "no major differences" (29).

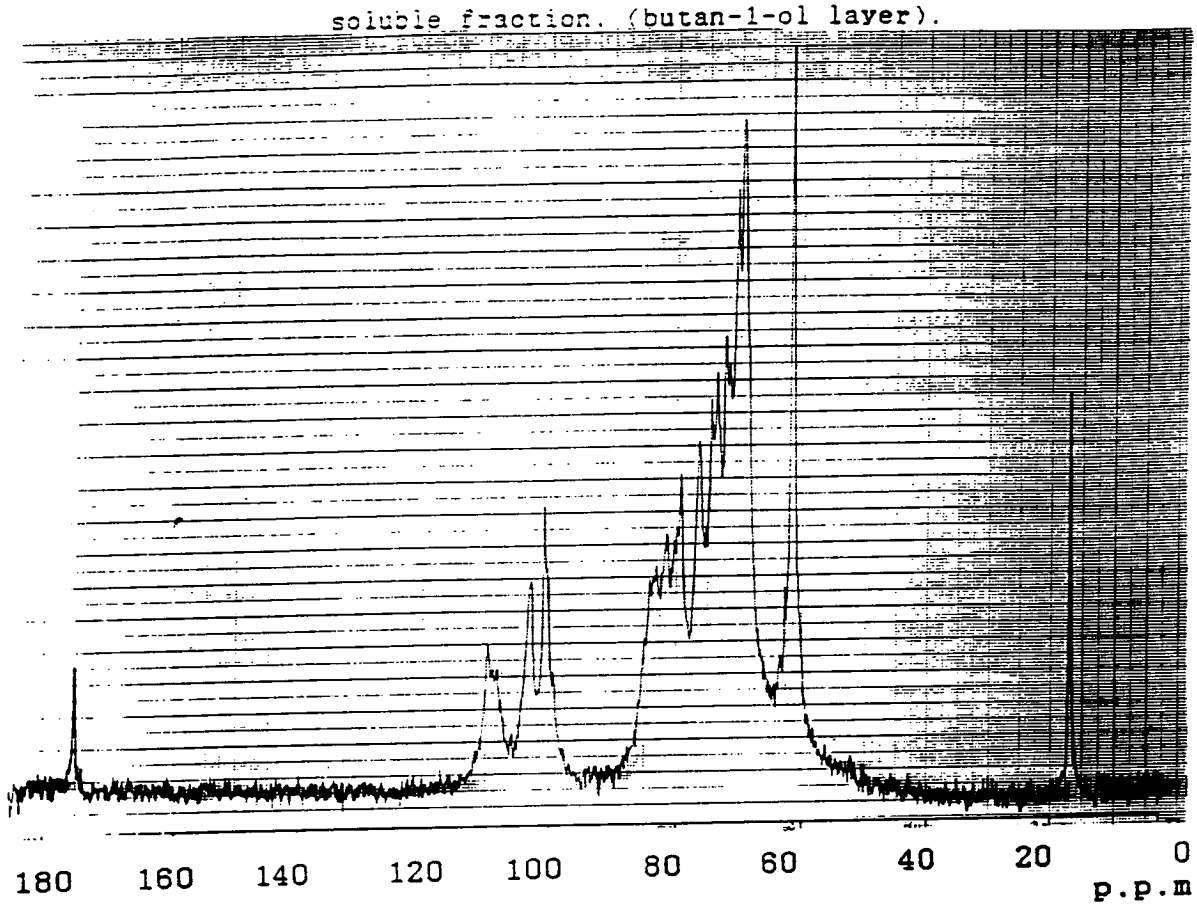
Spectrum III 13: 13C NMR spectra of parent (control) gum arabic.



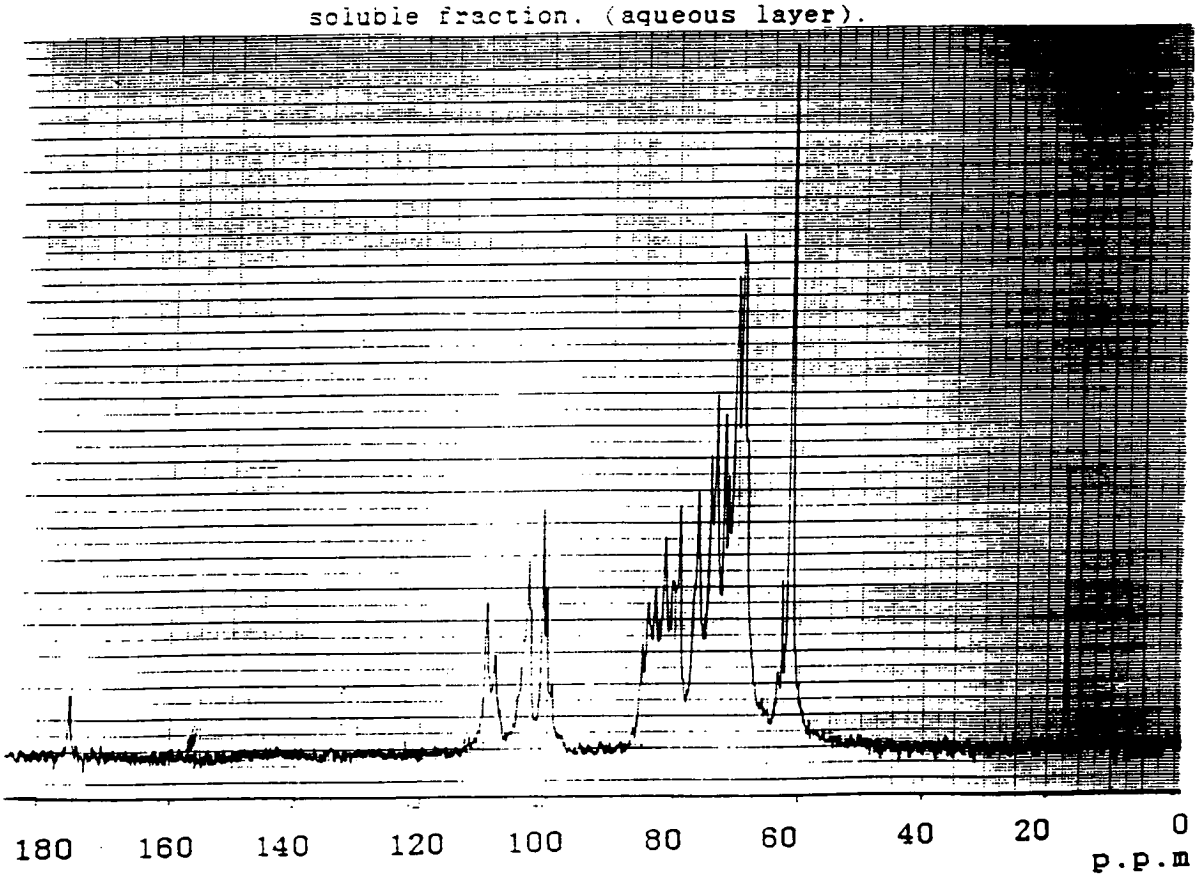
Spectrum III 14: 13C NMR spectra of nitrogen enriched insoluble fraction, (butan-1-ol layer).



Spectrum III 15: ^{13}C NMR spectra of nitrogen enriched soluble fraction. (butan-1-ol layer).



Spectrum III 16: ^{13}C NMR spectra of nitrogen depleted soluble fraction. (aqueous layer).



The clearly differentiated anomeric (100-120 p.p.m) signals, and that due to C-5 methyl of L-rhamnopyranosyl (17.6 p.p.m) are sharp and suggest that the structural environment for each constituent monosaccharide is likely to be repeated with a degree of regularity throughout the entire macromolecular structure. This reiterates previous suggestions that gum arabic is composed of regular polysaccharide subunits, linked to various degrees by various polypeptide chains (23,25,47).

The amino acid compositions expressed as residues per 1000 residues for each fraction (Dep I, En I sol and En I insol) are shown in Table III.9. The three fractions with various amounts of proteinaceous material show different amino acid compositions to that of the parent gum. The depleted fraction Dep I is enriched in hydroxyproline, serine and threonine. These three amino acids have been reported to be involved in glycoprotein linkages and in gum arabic have been found to be more heavily associated with the branched galactan core of the macromolecule rather than with its periphery (22,47). Akiyama and co-workers (24) have shown the presence of hydroxyproline-oligoarabinoside linkages and serine-carbohydrate linkages in unfractionated gum arabic. This nitrogen depleted fraction Dep I is depleted in alanine, isoleucine, valine, phenylalanine, lysine, leucine and tyrosine, i.e. the amino acids which have been shown to be

TABLE III.9 Amino acid composition of initial butan-1-ol deproteination of parent gum arabic.

	Control Parent Gum Arabic	Dep I gum	Enrich I soluble gum	Enrich I insoluble gum
% Nitrogen	0.34	0.31	0.70	2.51
Alanine	22	17	35	47
Arginine	10	7	13	23
Aspartic acid	55	52	62	53
Cystine	1	1	1	3
Glutamic acid	39	39	39	44
Glycine	59	54	62	70
Histidine	51	52	53	61
Hydroxyproline	270	339	220	141
Isoleucine	13	10	24	39
Leucine	75	48	91	102
Lysine	26	20	38	61
Methionine	2	2	2	3
Phenylalanine	39	30	45	57
Proline	89	81	76	75
Serine	126	136	103	66
Threonine	74	79	65	47
Tyrosine	10	9	15	27
Valine	39	24	55	83
Nitrogen Conversion factor	6.59	6.71	6.52	6.22

associated with peripheral chain-terminal locations in the gum structure (22,47). The findings from the sequential periodate oxidation study (Chapter III.2) agree with these results on the location of certain amino acids within the gum structure.

The nitrogen-enriched fractions show lower hydroxyproline, threonine, and serine values, and are enriched in alanine, arginine, isoleucine, leucine, lysine, valine and tyrosine.

Deproteination of degraded gum material Dep I.

In this experiment the nitrogen depleted fraction Dep I which consisted of 93% of the total weight of the first fractionation was further degraded by a second butan-1-ol/sodium chloride fractionation. In this experiment one part of the Dep I fraction was treated with a live protease prior to fractionation. Only the nitrogen contents and amino acid compositions of the degraded Dep I fractions were calculated and are shown in Table III.10 and 11.

One part of the Dep I fraction was deproteinated using butan-1-ol, and sodium chloride as before, one part with a small quantity of live proteinase enzyme followed by the butan-1-ol fractionation, and another part with the same quantity of denatured enzyme. Two fractions; a nitrogen-enriched fraction and a nitrogen depleted fraction were obtained

TABLE III.10 Amino acid composition of enzyme (protease) treated degraded gum (Dep I) from initial butan-1-ol deproteination.

	Dep I gum	Dep II gum control	Dep II live enzyme treated	Dep II denatured enzyme treated
% Nitrogen	0.31	0.28	0.25	0.30
% YieId	100	85	93	86
Alanine	17	15	10	16
Arginine	7	5	1	7
Aspartic acid	52	39	40	45
Cystine	1	2	0	0
Glutamic acid	39	29	26	32
Glycine	54	49	42	48
Histidine	52	49	25	38
Hydroxyproline	339	356	417	369
Isoleucine	10	7	4	9
Leucine	48	72	42	66
Lysine	20	18	15	16
Methionine	2	1	0	1
Phenylalanine	30	28	18	23
Proline	81	65	87	64
Serine	136	149	157	140
Threonine	79	84	94	85
Tyrosine	9	13	6	9
Valine	24	19	15	32
Nitrogen Conversion factor	6.71	6.70	6.97	6.77

TABLE III.11 Amino acid composition of enzyme (protease) treated degraded gum (Dep I) from initial butan-1-ol deproteination.

	Dep I gum	En II gum control	En II live enzyme treated	En II denatured enzyme treated
% Nitrogen	0.31	0.55	0.73	0.57
% Yield	100	15	7	14
Alanine	17	29	41	28
Arginine	7	10	16	12
Aspartic acid	52	55	52	51
Cystine	1	0	0	0
Glutamic acid	39	49	54	44
Glycine	54	55	62	53
Histidine	52	70	65	63
Hydroxyproline	339	290	238	291
Isoleucine	10	16	19	15
Leucine	48	38	65	49
Lysine	20	30	42	34
Methionine	2	1	0	1
Phenylalanine	30	36	46	38
Proline	81	96	77	94
Serine	136	98	94	104
Threonine	79	71	59	65
Tyrosine	9	8	15	10
Valine	24	48	55	48
Nitrogen Conversion factor	6.71	6.60	6.54	6.52

in the second deproteination in each of the three experiments. These fractions obtained from the Dep I starting material are termed En II, and Dep II respectively.

The fraction (Dep I) treated with only butan-1-ol is further separated into two fractions, one of which is enriched in protein (En II) and one which is depleted in protein (Dep II). The amino acid compositions of the two fractions are dissimilar as shown in Table III.10 and 11. The depleted-nitrogen fraction Dep II is deficient in alanine, isoleucine, valine, lysine, aspartic acid and glutamic acid, and enriched in hydroxyproline, threonine and serine. The enriched-nitrogen fraction En II is deficient in hydroxyproline, threonine and serine and enriched in isoleucine, alanine, valine, lysine, proline, glutamic acid and arginine compared to Dep I. This second fractionation did not use any enzyme and is termed the control fractionation.

The fraction (Dep I) treated with denatured enzyme, followed by the butan-1-ol fractionation shows similar amino acid composition, and % nitrogen values in its nitrogen-enriched fraction and its nitrogen-depleted fraction compared to the control fractonation. This indicates that the denatured enzyme is totally deactivated, and that the small contribution by the enzyme to the amino acid composition of the fraction is not significant. This fractionation is

termed the enzyme control fractionation.

The fraction treated with active proteinase enzyme separates into two distinct fractions, one depleted in nitrogen (Dep II Live) and one enriched (En II Live) in nitrogen. The amino acid compositions of both are shown in Table III.10 and 11. A lower yield of enriched material is obtained but the fraction (En II Live) is higher in nitrogen content than the two other control fractionations. The depleted fraction (Dep II Live) has therefore subsequently, the lowest nitrogen content obtained so far, i.e. 0.25%. It is greatly enriched in hydroxyproline, proline, serine and threonine, compared with the Depleted I (Dep I) fraction and compared to the parent gum arabic. The Enriched II (En II Live) fraction from the enzyme degradation is enriched in the following amino acids; isoleucine, alanine, arginine, phenylalanine and lysine and depleted in ; hydroxproline, proline, threonine, and serine. It is possible that the proteinase which is active at the serine amino acid site in a peptide has further degraded the proteinaceous core of the Depleted I fraction. However it still appears to be unlikely that gum arabic, because of its structure, can be deproteinated without degradation of the gum, and resulting loss of functionality (59).

Table III.12 compares the functionality of each gum arabic fraction from the butan-1-ol deproteination with respect to the "emulsification

TABLE III.12 Emulsification data for gum arabic and its butan-1-ol deproteination fractions.

	Control gum arabic	En I sol frac	Dep I sol frac	Dep II enzyme	En II enzyme
% Nitrogen	0.34	0.70	0.31	0.25	0.73
Emulsification activity 500nm	1.682	1.646	1.289	1.006	1.126
Emulsification stability 30 mins.	94%	98%	79%	46%	61%

TABLE III.13 Analytical data for Acacia seyal fractions from butan-1-ol deproteination.

Analytical Parameter	Parent gum	Butan-1-ol fraction	
		Depleted	Enriched
Yield %	100%	87%	13%
Nitrogen, % [a]	0.14	0.11	0.39
Specific rotation in water (degrees)[a]	+54°	+51°	+53°
Intrinsic viscosity, mlg^{-1} [a]	11	11	11
Equivalent weight [a]	1030	1120	1030
Emulsification activ 500nm.	1.21	1.14	1.03
Sugar composition after hydrolysis, %			
Glucuronic acid	17	16	17
Galactose	34	34	35
Arabinose	45	46	45
Rhamnose	4	4	3

Notes: [a] Corrected for moisture content.

activity" and "emulsification stability" of a limonene oil-in-water emulsion. The results indicate that the fraction Dep I from the initial deproteination, has poorer functionality than the parent gum arabic. The higher molecular weight fraction has similar emulsification activity, but enhanced emulsification stability, compared to the parent gum. Both fractions of the live enzyme-degraded gum Depleted II (Dep II Live) and Enriched II (En II Live) fractions, have poor functional characteristics (67).

Table III.13 compares the analytical parameters of the parent Acacia seyal gum, (the major botanical source of commercial gum tahla), with the two Acacia seyal fractions obtained by the butan-1-ol deproteination, carried out as previously described for gum arabic. The results from the nitrogen determinations of the two fractions suggest that Acacia seyal was deproteinated to a smaller extent than the Acacia senegal gum as no comparable highly protein-enriched fraction is obtained (c.f Acacia senegal enriched fraction [En I Insol] 2.51% nitrogen). The specific rotations of the two fractions and the sugar ratios are also similar to that of the parent gum. This differs significantly from the situation revealed for Acacia senegal gum and comfirms that there are extensive differences in the compositions and structures of the two gums (52).

The major analytical differences between

the Acacia senegal and Acacia seyal gums lie in their nitrogen content (0.33% and 0.14% respectively), rhamnose content (12-14% and 2-4% respectively and in their optical rotations; (-30±3°) for Acacia senegal, and +50 to +60° for Acacia seyal. Previous studies (52) have reported that the great difference in the functionality of these two gums may be related to the lack of peripherally located amino acid residues in Acacia seyal. Sequential Smith degradations studies (52) on Acacia seyal indicated that most of the proteinaceous component of the gum exists in the core structure of the complex macromolecules. The data from the experiments reported here are in broad agreement with these previous reports.

CHAPTER III.4 FRACTIONATION OF GUM ARABIC BY EXTRACTION WITH LIMONENE.

III.4 (i) INTRODUCTION

Gum arabic, the natural exudate from *Acacia senegal*, is used in large quantities by the soft drinks industry for the stabilisation of emulsions of flavour oils especially citrus oils (68). An attractive feature of the gum's unique functionality as an emulsifier is its ability to stabilise the flavour oil emulsions both as a concentrate and in the final highly diluted beverage (32). Its precise mode of action in the stabilisation of emulsions is not fully understood (39). The gum structure is complex, and regional and seasonal variation exists between gum samples. As discussed in Chapter III.3 the gum is not a homologous structure. It has been suggested that it is composed of three major fractions; [1] which is almost void of protein and consists of 88% of the total weight of the gum, [2] which consists of an arabinogalactan-protein complex and consists of 10% of the total weight of the gum and [3], a highly proteinaceous glycoprotein (47% protein) which comprises 1-2% of the total weight of the gum (14,25).

It appears that a high proportion of the protein in the gum therefore may be accounted for by a high molecular weight fraction of the gum (53). Recent

publications (34,39), have suggested that it is only this highly proteinaceous fraction that is surface active and is adsorbed at the oil-in-water interphase. A study by Dickinson and co-workers (30) on the emulsifying properties of the gums from different Acacia gum species having varying nitrogen contents, has suggested that a strong correlation appears to exist between the amount of proteinaceous material in the gum and its surface properties at the oil-water interface, although no simple relationship exists. This may partially explain why 5 to 12% solutions of gum arabic (0.33% nitrogen) are required to give stable 20% (w/w) limonene oil emulsions of small droplet size, as only 1-2% of this gum is actually adsorbed. The quantity of gum required for effective oil-in-water emulsification is five to ten times the amount required when a more proteinaceous emulsifier such as α-casein is used.

It is known that proteinaceous materials, of different origin vary immensely in their ability to stabilise emulsions (69,70), reflecting differences in composition, conformation and structural rigidity. The properties of a protein giving high "emulsification activity" characteristics does not ensure good "emulsion stability" (71). This suggests that different parameters are involved in initially forming an emulsion to those which stabilise an emulsion. A previous study has indicated that the

emulsification capacity of proteinaceous materials depends on a suitable balance between the hydrophilic and lipophilic characteristics, rather than merely on high values of each of these (72).

It has been suggested that certain amino acids in the gum arabic structure are peripherally (i.e. chain terminal) located, and others such as hydroxyproline, serine, threonine and proline are structurally important in sugar-amino acid linkages in the inner core of the complex macromolecules (2,22,47). The peripherally located amino acids, e.g. isoleucine, tyrosine, phenylalanine, alanine and valine, are all relatively hydrophobic in nature and may be predominately involved in the mechanism of adsorption at the oil interface in emulsion formulations with gum arabic (Chapter IV.I). This study investigates the participation of certain structurally important amino acids in emulsion formation.

III.4.(ii) MATERIALS AND METHODS

In this study a large volume of a D-limonene oil-in-water emulsion was made up as described in Chapter II. When the emulsion destabilised with respect to creaming (24 hours), the two layers were separated and freeze dried. Only the gum arabic molecules that were adsorbed at the oil interphase remain in the top oil layer and the aqueous layer

contains predominantly non-adsorbed gum.

The limonene was removed from each fraction by rotary evaporating to dryness at 40°C (reduced pressure), the fractions were then redissolved in water (100mls) and small amounts of dichloromethane was used to wash out any residual limonene. The fractions were washed three times with dichloromethane, the aqueous layer was separated and retained for analysis.

III.4 (iii) RESULTS AND DISCUSSION

Table III.14 shows the analytical parameters of the parent gum arabic and the two fractions obtained from the D-limonene emulsion destabilisation experiment. It can be seen that the predominant fraction (88%) is the fraction which is not adsorbed at the oil-water interface, and this fraction separates into an aqueous layer. This fraction is depleted in nitrogen content, and hence protein content; it also has a lower intrinsic viscosity which implies a lower molecular weight than the parent gum; and has inferior performance as a emulsifier. Its carbohydrate composition is similar to that of the parent, with a slightly higher galactose content and lower rhamnose content. The limonene extracted fraction amounting to 12% of the total weight of the gum, has a correspondingly higher nitrogen content, and a higher

TABLE III.14 Analytical data for gum arabic fractions from limonene emulsion destabalisation.

Analytical Parameter	Control gum arabic	Limonene extracted fraction	Emulsion destabil fraction
Recovery (g)	10g	1.0g	8.5g
Yield %	100%	12%	88%
Moisture, %	9.8	3.4	3.1
Ash, % [a]	3.2	n.d	n.d
Nitrogen, % [a]	0.34	0.52	0.31
Nitrogen conversion factor (N.C.F)[b]	6.59	6.35	6.64
Hence protein, % (N.C.F X %N)	2.24	3.30	2.05
Specific rotation in water (degrees)[a]	-28°	-26°	-27°
Intrinsic viscosity, mlg^{-1} [a]	16	18	15
Equivalent weight [a]	1030	1120	960
Uronic anhydride, % [d]	17	16	18
Emuls. activ., 500 nm	1.642	1.723	1.305
Emuls. stab., 30 mins	94%	98%	75%
Sugar composition after hydrolysis, % [c]			
Glucuronic acid	17	16	18
Galactose	47	44	49
Arabinose	25	28	24
Rhamnose	11	12	9

Notes:

[a] Corrected for moisture content.
[b] From table III.15
[c] Corrected for protein content.
[d] If all the acidity arises from uronic acids.

intrinsic viscosity, in comparison to that of the parent or the protein-depleted fraction. These results agree with previous publications on the properties of gum arabic responsible for its unique functionality as an emulsifier (14,20). The nitrogen-enriched fraction also has superior emulsification properties to those of the original gum arabic.

The amino acid compositions of the proteinaceous component of the parent gum and the two fractions are displayed in Table III.15. The protein-enriched fraction has relatively higher proportions of the amino acids; alanine, arginine, aspartic acid, isoleucine, phenylalanine, tyrosine and valine. As discussed in previous sections of the thesis, these amino acids have been reported to be located at peripheral positions on some gum fractions (1,22), and may be responsible for the gum's unique functionality. This fraction contains lower proportions of hydroxyproline, threonine, proline, histidine and serine. The depleted-nitrogen fraction is correspondingly enriched in hydroxyproline, proline, serine and threonine. As discussed previously these amino acids have been shown in previous studies (22,47) to be concentrated within the core of the polygalactan framework of the complex gum molecules.

TABLE III.15 Amino acid composition of gum arabic fractions obtained from emulsification destabalisation. (Limonene oil-in-water emulsion.)

	Parent control gum arabic	Limonene extracted fraction	Emulsion destabilised fraction
% Nitrogen	0.34	0.52	0.31
Alanine	27	46	20
Arginine	10	28	4
Aspartic acid	50	74	43
Cystine	0	0	0
Glutamic acid	52	45	56
Glycine	59	75	57
Histidine	49	42	48
Hydroxyproline	261	202	289
Isoleucine	12	23	6
Leucine	75	81	67
Lysine	26	29	25
Methionine	1	0	1
Phenylalanine	39	47	29
Proline	84	65	89
Serine	141	112	154
Threonine	74	61	79
Tyrosine	11	18	7
Valine	39	52	26
Nitrogen Conversion factor	6.59	6.35	6.64

CONCLUSION

These findings from Chapter III.4 agree with those conclusions drawn from Chapter III 1, 2 and 3, on the location of amino acids in the gum structure and their role in stabilising oil-in-water emulsions.

Previous studies (53,52) have shown that the proteinaceous component of the gum played an intégral role in the gums complex macromolecular structure. The findings from chapter III suggests that certain peripherally located, relatively hydrophobic amino acids also play an important role in the gums performance as an effective stabiliser in oil-in-water emulsions. The study has also shown that various fractions of the whole gum vary in their performance in emulsion stability.

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CHAPTER IV

AN ANALYTICAL STUDY OF GUM EXUDATES

CHAPTER IV.1. THE VARIATION IN GUM ARABIC SAMPLES COLLECTED BETWEEN 1958-1988.

IV.1 (i) INTRODUCTION

Gum arabic (*Acacia senegal* (L.) Willd.) was re-affirmed (1) as GRAS within the U.S.A in 1974. Following requests (2,3) for positive toxicological evidence of its safety, gum arabic was awarded the status "ADI not specified" by the FAO/WHO Joint Expert Committee on Food Additives (JEFCA) in 1982 (4), provided that the gum conforms to the established specifications for its identity and purity (5,6). The existing specifications for gum arabic were recently revised by JEFCA in 1990 as discussed in Chapter III.

Although other gum arabic reference samples have been characterised (7,8), data from a wider range of samples are desirable in order that acceptable average analytical parameter values, or range of values, can distinguish gum arabic unambiguously from other non-permitted water soluble gum exudates or polysaccharides (9,10).

The inadequacy of the present regulatory specifications for gum arabic based on viscosity and a simple optical rotation measurement have become increasingly more evident (11,12,13 and 14). Blending of two or more non-permitted gums by unscrupulous gum traders to achieve similar analytical parameters to "real" gum arabic is possible in order to produce a

product that meets the present regulatory specification for gum arabic although the blended product does not actually contain gum arabic at all. Besides the issue of these adulterant gums not being food permitted (12,15) they do not have the unique functional properties of gum arabic e.g. for the stabilisation of oil-in-water emulsions (16,17,18 and 19).

As a separate issue, there have been suggestions from a few gum traders that the severe Sahelian droughts of 1973-74 and 1983-85, which caused heavy losses of Acacia senegal trees, have led to physiological adaptations of the trees that survived, resulting in changes in the long established characteristic analytical parameters for gum arabic, particularly its specific rotation. Gum users have also speculated that, in recent years, gum arabic has shown decreased emulsification capacity and that a lower rhamnose content might be the cause of this. Thus it has been suggested that gums obtained from trees post-1985 may have different analytical parameters and functionality to those pre-1974.

The aim of this study was to verify or contradict these statements by studying twelve gum arabic samples available from a wide range of crop years. A paper has been published which compares the analytical properties of 22 gum arabic samples collected between 1904 and 1989 (20), and confirms that there is no evidence that the protein content has

decreased or that the specific rotation of gum arabic has become significantly less negative in recent years.

Even today, relatively little gum arabic is purchased on the basis of analytical specification. Criteria of quality is very dependant on the end-use to which the gum is put; solubility, viscosity or even taste may be the critical parameter for the basis of trade acceptance or rejection by gum dealers (9). However, complete chemical analysis should provide the only acceptable positive identification of gum arabic. Contrary to some traders suggestions or beliefs that the structure and rheological properties of gum arabic have altered as a result of the droughts, it is known that blending with adulterants such as the Combretum gums has become more widespread in recent years (14).

IV.1 (ii). MATERIALS AND METHODS.

Origin of gum samples.

The samples studied were natural Acacia senegal gum obtained from known, reputable sources. Some were obtained in natural lump form, others were kibbled. In all cases they were reduced to a powder by a pestle and mortar, to minimise structural degradation due to the heat generated by electric grinders. Spray dried samples were avoided, to eliminate the possibility of heat abuse or any form of blending or chemical pretreatment (eg bleaching) which certain processors incorporate into spray drying techniques.

Sudanese samples.

Sample S5 was supplied to this Department's gum research programme for reference purposes by Messrs Rowntree & Co. Ltd, York, U.K. in 1960 and was representative of a 25-ton shipment from the Sudan. Samples S6 and S7 were collected from individual trees by the late Mr M.P Vidal-Hall, Gum Research Officer to the Sudan. S6 was representative of the 4th picking of the 1960 season at Quala en Nehal, Sudan; S7 was representative of the 2nd picking of the season at Goz el Ganzara, Sudan. Sample S8 was collected at Goz Ashgar in 1970 by Mr A.G. Seif-el-Din, Sudanese Gum Research Officer at the time. Sample S9 was supplied in 1971 by a European gum importer. Sample S12 was provided in 1988 by a European user. Sample S13 was provided by a European importer, and was representative of shipments of the 1988/89 Sudanese crop received in March 1989.

Nigerian samples.

Sample N5 was provided for reference purposes by a U.K. user in 1958, and samples N6 and N7 by a U.K. importer in 1959 and 1960, respectively. Sample N8 was supplied for use in a research project in 1961 by Messrs Rowntree Ltd, York, U.K. Sample N9, from Maiduguri, northern Nigeria, was submitted to this department for evaluation in 1967 by the Tropical Products Institute, London.

It must be emphasised that these are

specially, selected Nigerian samples of the highest possibile quality: it is very common for greatly inferior grades, known commercially as "Nigerian II" to be offered by exporters at greatly reduced prices. Some such samples do not come from Acacia senegal, but from mixtures of tree species from other genera.

IV.1 (iii). RESULTS AND DISCUSSION.

The analytical data obtained for the seven Sudanese gum arabic samples are shown in tables IV.1(i,ii),3,5 and 7. The data for the five Nigerian gum arabic samples are shown in tables IV.2,4,6 and 8.

Comparing tables IV.1(i,ii) and 2 firstly, which show the data for 12 gum arabic samples collected over a wide range of years, it can be concluded that there are close similarities between the Nigerian gums and the Sudanese gums particularly with regard to their ash, nitrogen, methoxyl and specific rotation. The Nigerian samples tend on average however, to be slightly more viscous and to have slightly lower rhamnose contents.

Tables IV.3 and 4 show the data obtained for the amino acid compositions of the protein contents of the gums. For these parameters also, there is little difference between Sudanese and Nigerian gum samples. The close similarity between the Sudanese and Nigerian samples studied is seen by comparing the constancy of the nitrogen conversion factors for the twelve samples.

TABLE IV.1 (i) Analytical data for commercial samples of Sudanese gum arabic 1960-1989.

Analytical Parameter	S5 1960	S6 1960	S7 1962	S8 1970
Moisture, %	13.0	12.0	12.0	13.0
Ash, % [a]	3.7	2.8	3.6	3.4
Nitrogen, % [a]	0.32	0.36	0.36	0.31
Nitrogen conversion factor (N.C.F)[b]	6.54	6.44	6.47	6.71
Hence protein, % (N.C.F X %N)	2.1	2.3	2.3	2.1
Methoxyl, % [b]	0.33	0.22	0.28	0.25
Specific rotation in water (degrees)[a]	-32°	-29°	-31°	-31°
Intrinsic viscosity, mlg^{-1} [a]	19	14	14	16
Brookfield viscosity; 25% (cps)	85	60	70	80
pH, 25% aq soln, at 25°C	4.2	4.2	4.3	4.5
Equivilent weight [a]	950	1160	990	1120
Uronic anhydride, %	18	15	18	16
Sugar composition after hydrolysis, %				
Methylglucuronic acid [d]	2	1	2	1.5
Glucuronic acid	16	14	16	14.5
Galactose	48	50	46	45
Arabinose	23	23	20	23
Rhamnose	11	12	16	16

Notes:
[a] Corrected for moisture content.
[b] From tables IV.3 and 4.
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

TABLE IV.1 (ii) Analytical data for commercial samples of Sudanese gum arabic 1960-1989.

Analytical Parameter	S9 1971	S12 1988	S13 1989
Moisture, %	13.0	13.0	14.0
Ash, % [a]	3.4	4.1	3.6
Nitrogen, % [a]	0.38	0.32	0.32
Nitrogen conversion factor (N.C.F)[b]	6.66	6.70	6.57
Hence protein, % (N.C.F X %N)	2.5	2.1	2.1
Methoxyl, % [b]	0.32	0.21	0.29
Specific rotation in water (degrees)[a]	-31°	-30°	-32°
Intrinsic viscosity, mlg^{-1} [a]	15	14	20
Brookfield viscosity; 25% (cps)	60	75	100
pH, 25% aq soln, at 25°C	4.2	4.6	4.4
Equivilent weight [a]	1130	875	1090
Uronic anhydride, %	16	20	5
Sugar composition after hydrolysis, %			
Methylglucuronic acid [d]	2	1	2
Glucuronic acid	14	19	14
Galactose	48	50	47
Arabinose	23	17	21
Rhamnose	13	13	16

Notes:
[a] Corrected for moisture content.
[b] From tables IV.3 and 4.
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

TABLE IV.2 Analytical data for commercial samples of Nigerian gum arabic 1958-1967.

Analytical Parameter	N5 1958	N6 1959	N7 1960	N8 1961	N9 1967	Mean
Moisture, %	13.0	13.0	13.0	13.0	12.0	13.0
Ash, % [a]	3.6	3.8	4.0	3.6	3.9	3.8
Nitrogen, % [a]	0.39	0.31	0.32	0.31	0.29	0.32
Nitrogen conversion factor (N.C.F)[b]	6.52	6.51	6.63	6.56	6.59	6.57
Hence protein, % (N.C.F X %N)	2.5	2.0	2.1	2.0	1.9	2.1
Methoxyl, % [b]	0.25	0.20	0.19	0.25	0.18	0.21
Specific rotation in water (degrees)[a]	-32°	-32°	-32°	-29°	-29°	-31°
Intrinsic viscosity, mlg^{-1} [a]	20	17	19	18	16	18
Brookfield viscosity 25% (cps)	110	75	110	100	75	94
pH, 25% aq soln, at 25°C	4.2	4.3	4.3	4.3	4.2	4.3
Equivalent weight [a]	980	1090	960	930	960	980
Uronic anhydride, %	18	16	18	19	18	18
Sugar composition after hydrolysis, %						
Methyl-glucuronic acid [d]	1.5	1	1	1.5	1	1
Glucuronic acid	16.5	15	17	17.5	17	17
Galactose	51	52	51	51	45	50
Arabinose	19	21	22	18	22	20
Rhamnose	12	11	9	12	15	12

Notes:
[a] Corrected for moisture content.
[b] From tables IV.3 and 4.
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

TABLE IV.3 Amino acid composition of commercial samples of Sudanese gum arabic 1960-1989.

	S5 1960	S6 1960	S7 1962	S8 1970	S9 1971	S12 1988	S13 1989
% Nitrogen	0.32	0.36	0.36	0.31	0.38	0.32	0.32
Alanine	26	31	29	25	31	30	23
Arginine	12	12	10	13	7	11	12
Aspartic acid	77	63	69	72	73	69	71
Cystine	0	0	1	1	1	0	0
Glutamic acid	39	42	52	55	49	36	63
Glycine	47	48	51	48	53	47	51
Histidine	47	42	40	44	47	40	53
Hydroxyproline	311	296	318	335	290	304	269
Isoleucine	13	13	12	10	7	15	10
Leucine	69	57	61	65	65	61	73
Lysine	26	26	22	28	26	24	31
Methionine	3	4	3	1	0	0	3
Phenylalanine	33	27	33	35	37	31	38
Proline	55	66	65	48	68	90	49
Serine	126	132	124	124	144	122	138
Threonine	66	85	63	66	69	63	71
Tyrosine	10	16	9	13	9	11	12
Valine	40	40	38	17	24	46	33
Nitrogen Conversion factor	6.54	6.44	6.47	6.71	6.66	6.70	6.57

TABLE IV.4 Amino acid composition of commercial samples of Nigerian gum arabic 1958-1967

	N5 1958	N6 1959	N7 1960	N8 1961	N9 1967	n=5 Mean
% Nitrogen	0.39	0.31	0.32	0.31	0.28	0.32
Alanine	26	26	26	30	21	26
Arginine	12	11	12	15	10	12
Aspartic acid	70	78	72	65	67	70
Cystine	0	2	0	0	0	0
Glutamic acid	37	51	69	57	43	51
Glycine	53	52	53	53	49	52
Histidine	51	52	48	49	55	51
Hydroxyproline	284	297	251	287	306	285
Isoleucine	14	11	16	15	9	13
Leucine	76	74	69	78	73	74
Lysine	31	26	29	30	24	28
Methionine	0	2	1	2	1	1
Phenylalanine	43	12	39	36	35	33
Proline	56	49	59	43	42	50
Serine	129	140	133	124	153	136
Threonine	66	72	75	64	79	71
Tyrosine	11	13	12	12	8	11
Valine	41	32	36	39	25	35
Nitrogen Conversion factor	6.52	6.51	6.63	6.56	6.59	6.56

Tables IV.5 and 6 show the data obtained by atomic absorption spectroscopy for the cationic composition of the ash derived at 550°C. There are some differences in the amounts of the four major components (calcium, magnesium, potassium and sodium). The Nigerian samples tend to have higher than average aluminium, copper, lead and zinc contents (12). For some commercial purposes, the heavy metal content can be important ; the presence of traces of copper and lead can be detrimental when the gum arabic is used in emulsion polymerisation formulations. However there are no indications, from the data presented for samples produced over a period of about 80 years, of any self-consistent changes in the cationic contents evaluated.

There is no evidence therefore, from the data presented, that the specific rotation of gum arabic has become less strongly negative in recent years. Some traders have claimed that a change from a -30° to closer to -20° has occurred, suggesting that this has resulted from physiological adaptations of the Acacia senegal trees following the drought periods. It is extremely unlikely, however, that the physiological changes necessary in the trees would occur in as short a time interval. The specific rotations of all twelve gum arabic samples evaluated in table IV.1 and 2 lie between -29° and -32°, so the traders claims appear, as expected on physiological grounds, to be unsubstantiated.

TABLE IV.5 The cationic composition [a] of the ash from Sudanese gum arabic samples 1960-1989 (µg/g ash).

Sample No and year of origin.	S5 1960	S6 1960	S7 1962	S8 1970	S9 1971	S12 1988	S13 1989
% Ash [b]	3.7	2.8	3.6	3.4	3.4	4.1	3.6
Aluminium	219	222	172	194	183	111	119
Calcium X 10^3	246	238	306	268	232	328	238
Chromium	63	70	61	62	50	46	73
Copper	73	60	31	70	22	71	120
Iron	111	86	145	98	75	162	381
Lead	11	4	0	6	13	6	1
Magnesium X10^3	31	27	19	38	48	26	42
Manganese	91	84	27	90	87	36	31
Nickel	19	0	16	31	29	7	26
Potassium X10^3	218	302	268	262	254	222	186
Sodium X 10^2	152	53	78	47	52	100	88
Zinc	17	33	19	20	21	31	26

[a] For all samples, As, Cd, Co, Mo all < 1ppm.
[b] Table IV.1 (i and ii).

TABLE IV.6 The cationic composition [a] of the ash from Nigerian gum arabic samples 1958-67 (µg/g ash,550°C).

Sample No. and year of origin.	N5 1958	N6 1959	N7 1960	N8 1961	N9 1967
% Ash [b]	3.6	3.8	4.0	3.6	3.9
Aluminium	368	675	169	372	223
Calcium X 10^3	266	286	208	360	294
Chromium	50	50	67	50	61
Copper	50	225	26	19	110
Iron	75	172	139	99	117
Lead	12	13	3	11	0
Magnesium X10^3	24	32	29	56	39
Manganese	110	55	39	39	49
Nickel	43	28	0	35	0
Potassium X10^3	188	212	236	243	160
Sodium X 10^2	45	210	70	51	61
Zinc	12	159	24	21	18

[a] For all samples, As, Cd, Mo all < 1ppm.

[b] Table IV.2

When gum arabic is in short supply, surplus supplies of gum talha (Acacia seyal) with a strong positive specific rotation of +56° flood the market (21) and commercial pressures for blending operations then occur. Addition of 10% gum talha to a gum arabic sample lowers the specific rotation to from -30° to -21.° In addition there is a substantial increase in profitability as the cost of gum talha is only approximately 30% that of good quality gum arabic. Thus unscrupulous gum traders have claimed the existence of drastic changes in gum arabic's key analytical parameter, in order to disguise the deliberate blending of adulterant gums into gum arabic. Gum talha also has very poor functionality with respect to oil-in-water emulsion stabilisation (Chapter III.3). The poor emulsification stability and activity of gum tahla with D-limonene and parafin oil is shown in table IV.8.

Tables IV.7 and 8 show emulsification activities and emulsification stabilities at 30 mins for D-limonene and paraffin oil-in-water emulsions with the Sudanese and Nigerian gum arabic samples (22,23). As discussed in Chapter III, gum arabic, is used in large quantities by the soft drinks industry for the stabilisation of emulsions of citrus oils (24,25). Its precise mode of action in emulsion stabilisation is not totally understood. For a discussion on gum arabic's functionality with respect to emulsion stabilisation refer to Chapter III.4.

TABLE IV.7 Emulsification data for oil-in-water emulsions of commercial Sudanese gum arabic samples 1960-89.

Sample No and year of origin	S5 1960	S6 1960	S7 1962	S8 1970	S9 1971	S12 1988	S13 1989
Emulsification activity with limonene oil 500nm.	1.69	1.74	1.70	1.50	1.35	1.86	1.56
Emulsification stability with limonene oil 30 mins.	82%	74%	83%	87%	78%	77%	94%
Isoleucine content per 1000 Amino acids in gum.	13	13	12	10	7	15	10
Rhamnose content %	11%	12%	16%	16%	13%	13%	16%
Brookfield viscosity 25% cps	90	60	60	80	60	75	100
Emulsification activity with paraffin oil 500nm.	1.07	0.77	1.30	1.17	1.03	0.70	0.90
Emulsification stability with paraffin oil 30 mins.	75%	69%	73%	71%	69%	84%	84%

TABLE IV.8 Emulsification data for oil-in-water emulsions of commercial Nigerian gum arabic samples 1958-67, and comparison with two other Nigerian gums; *Acacia seyal* and *Combretum frommii*.

Sample No and year of origin	N5 1958	N6 1959	N7 1960	N8 1961	N9 1969	*Acacia seyal*	*Combrt frommii*
Emulsification activity with limonene oil 500nm.	1.65	1.60	1.89	1.83	1.45	0.91	1.17
Emulsification stability with limonene oil 30 mins.	93%	76%	79%	88%	87%	63%	70%
Isoleucine content per 1000 amino acids in gum.	14	11	16	15	9	17	50
Rhamnose content %	12%	9%	11%	12%	15%	4%	24%
Brookfield viscosity 25% (cps)	110	75	110	100	75	60	>1000
Emulsification activity with paraffin oil 500nm.	0.84	1.17	0.92	0.91	0.96	0.66	0.65
Emulsification stability with paraffin oil 30 mins.	81%	69%	85%	73%	83%	59%	68%

Notes [a] Refer to chapter IV.ii

It is evident, therefore that only highly proteinaceous gum material adsorbs on to the oil droplets. A feature of gum arabic is its ability to form a film at the oil-water interface, whose surface viscoelasticity is insensitive to dilution of the aqueous phase (19). It has been suggested that the surface activity and emulsifying properties of gum arabic require the gum to contain hydrophobic groups, which can reside near or penentrate the oil interface and hence anchor the gum structure to the surface of the oil droplet, and hydrophilic sugar groups which can protrude out into the aqueous phase and give a stable emulsion (16,29,30). It has been shown that a reduction in the molecular weight (31) of a gum arabic sample (the % protein remaining constant) gum arabic reduces its emulsion stabilising properties (refer to chapter III.1)

The emulsification properties shown in table IV.7 and 8 indicate that small differences in the gum arabic structure can give variable emulsification data. However no obvious reduction in emulsifying properties with time (1958-88) is apparent. It has been shown that D-limonene gives more representative results in emulsification tests than paraffin oil. As the results indicate, a different mechanism of emulsion formation and stabilisation may exist, therefore this study will concentrate on the results from the D-limonene emulsion tests.

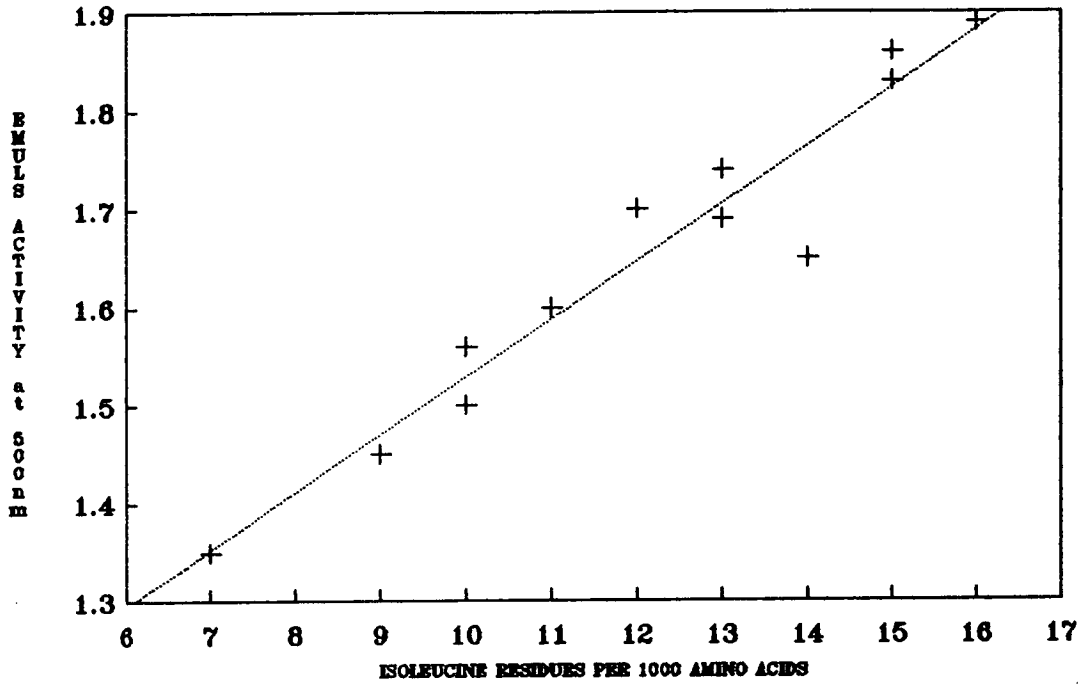
The mechanism of emulsion formation and

ultimately emulsion stabilisation by gum arabic is made more complicated by the finding that the initial fine droplet size distribution is formed by lower molecular weight fractions of the gum, which diffuse to the oil interface faster (16,31), although they are lower in protein. For long term emulsion stability, however, the highly proteinaceous, high molecular weight fraction of the gum diffuses to the interface eventually and is ultimately more effective, giving less droplet coalescence (16). Sequential Smith degradation studies on gum arabic (Chapter III.2) have shown that certain amino acid residues are associated with the structural core of the gum eg. proline, hydroxyproline, serine and threonine. Other amino acids, eg. alanine, valine, isoleucine, phenylalanine and tyrosine are more closely associated with the periphery of the gum molecules. These results agree with previous findings (32,33). Amino acids which are likely to improve functionality of oil-in-water emulsions would have hydrophobic side-chains. There appears to be no trend in the nitrogen and therefore overall protein content of the twelve gum arabic samples analysed with emulsification properties. However when the initial emulsification activity of a D-limonene oil emulsion is plotted against the number of isoleucine amino acid residues (per 1000 residues in the gum) a strong positive correlation emerges. A possible explanation may be that isoleucine is predominantly associated with the periphery of the gums structure (32), [refer to Chapter

III.1,2 and 3]; its non-polar hydrophobic side chain may be able to penentrate the oil interface, giving initial emulsion activity (graph IV.1). However little or no correlation appears to exist with valine, alanine or phenylalaline residues which are also peripheral and also contain relatively hydrophobic side-chains. This evidence reinforces results from the Smith degradation study (chapter III.2), where the loss of peripheral amino acids from the gum structure greatly reduced the emulsifying properties of the gum.

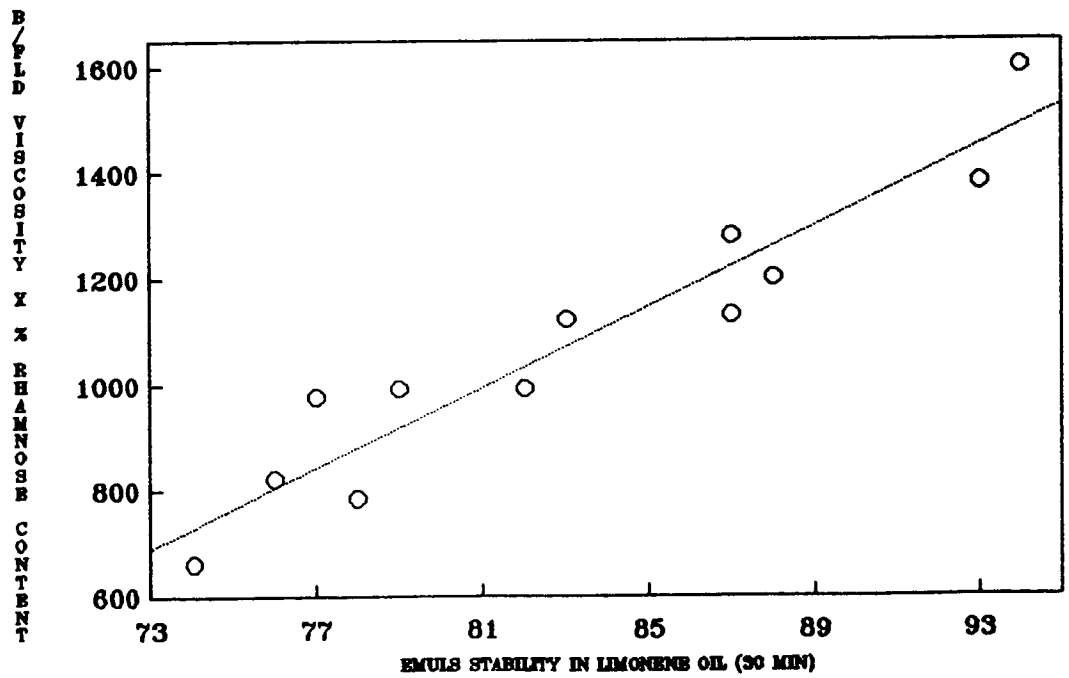
The superior stabilising and emulsifying powers of gum arabic may also arise from its significant proportions (9-16%) of rhamnose which has a hydrophobic methyl group attached to C-5 of the sugar ring. These rhamnose residues, from many previous structural elucidation studies on gum arabic, have been shown to occur at chain terminal, peripheral positions of the globular-shaped, highly branched molecules (34,35,36). Viscosity or molecular weight may also increase the emulsion functionality of the gum or assist in stabilising an emulsion once it has formed (30). Gum arabic has been reported to emulsify by the formation of a protective polyanionic film around each oil droplet (19); the oil droplets then mutually repel each other and so do not coallesce. The emulsification stability of D-limonene emulsions appears to increase as the product of the Brookfield viscosity and the rhamnose content of the gum arabic increases (graph IV.2). This agrees with previous observations that no

RELATIONSHIP BETWEEN EMULSIFICATION ACTIVITY AND ISOLEUCINE CONTENT IN GUM ARABIC IN LIMONENE/WATER EMULSION.



GRAPH IV.1

RELATIONSHIP BETWEEN EMULSIFICATION STABILITY/AND THE PRODUCT OF VISCOSITY AND RHAMNOSE CONTENT OF GUM ARABIC.



GRAPH IV.2
LIMONENE OIL-IN-WATER EMULSION

simple relationship exists between viscosity and emulsion stability (16,30).

However, although high isoleucine, high rhamnose content and a high Brookfield viscosity exists in Combretum frommi gum (Chapter IV.2) poor emulsion functionality results, as shown in table IV.8. There are, however, very considerable chemical and structural differences between Combretum gums and Acacia senegal, as discussed in the next section.

Further work is required with a larger range of gum arabic samples and partially degraded gums to understand fully the complex mechanism of emulsion stabilisation by gum arabic molecules.

IV.1 (iv). CONCLUSION.

Analytical data are presented for twelve authentic gum arabic samples obtained from a wide range of reputable sources. The data extend and strongly substantiate, those available previously for gum arabic (7).

There is no evidence to support recent traders suggestions that the structure, chemistry and emulsifying properties of good quality gum arabic have varied drastically as a result of the two Sahelian droughts through physiological adaptations by the Acacia senegal plant. On the contrary there is striking evidence that gum arabic, exuded by Acacia senegal (L.) Willd., has remained remarkably constant in analytical

parameters, over the past 40 years. Obviously climatic, seasonal and geographical variations may all occur, but each parameter is seen to lie within a small range. The small extent of variation first demonstrated by Anderson and his co-workers 20 years ago is confirmed in this study (7).

CHAPTER IV.II. AN ANALYTICAL STUDY OF SIX *COMBRETUM* GUM EXUDATES.

IV.II (i) INTRODUCTION

The family *Combretaceae* has been divided into two sub-families (37). One of these sub-families, *Combretoideae*, contains two tribes, of which one (*Combreteae*) contains three sub-tribes and these contain 16 genera in all. The other sub-tribe *Combretineae*, contains 200 species including the genus *Combretum*, of which six exudate gums are of interest in the present study.

The genus *Combretum* Loelf., cosmopolitan in the tropics (38) and sub-tropics except for Australia, is the largest and most complex in the Family *Combretaceae* (order *Myrtales*), as illustrated by the fact that around 180 African, and around 30 Asian species have been given over 600 different names by botanists over the years. Little was known about the Chemistry of this family of gums until an analytical study of *Combretum leonense* gum was made in 1959 by D.M.W.Anderson and co-workers (39). Examples of the extensive synonymy that exists in this Family, and a summary of the Taxonomic classification of the Family *Combretaceae*, has been reviewed by Anderson and his co-workers (14,40). In addition to chemical data for their characteristic gum exudates, flavonoids and terpenoids extracted from the leaves of certain

Combretum species (41,42) have been studied.

The first recent Sahelian drought (Chapter IV.1) in 1972-74 led to severe shortages of Acacia senegal, but not of Combretum gums. As a result the Combretum gums were used widely as adulterants of gum arabic. However they have very different molecular structures (9), the Combretum gums were very unsatisfactory substitutes, because they did not possess the unique functionality of gum arabic (16). Little analytical information was available at that time to identify these Combretum gums; and pressure from traders and industrial users led to subsequent structural data and analytical parameters being published by Anderson and co-workers (40,43).

A period of adequate supplies of gum arabic followed in 1976-1982, but another disastrous Sahelian drought struck between 1983-85 giving another international shortage of "real" gum arabic. However the International regulatory position regarding permitted food additives had changed since the 1970's; much more rigorous criteria of identity and purity, and demands for complete safety evaluations of food gums had been introduced (9,14).

Combretum gums are readily available at relatively low prices in East and West Africa, and are frequently offered for sale fraudulently as "gum arabic" in native markets. Vigilance on the part of buyers is necessary as the Combretum gums vary

widely in their quality, solution properties, chemical structure, and functionality. The gum nodules of Combretum species are, however, readily distinguishable from those of Acacia senegal. Combretum nodules tend to be smaller, darker, smoother on the surface, and the general shape is different to that of gum arabic whose nodules are pale amber, clear, heavily fissured on the surface and relatively large. Some species of Combretum can however give pale, clear gum nodules, but these are often too small and too smooth to be typical in appearance of "true" gum arabic. The possibility of distinguishing the two gums by nodule shape, colour, and surface appearance is lost, however, when the gum is kibbled or powdered; full chemical analysis must then be resorted to in order to differentiate either adulterated samples or inferior quality pure Combretum gums at the contract stage prior to purchase.

Aside from the fact that the Combretum gums are functionally inferior to gum arabic (refer to Chapter IV.1), Combretum gums have never been included in any of the international lists of permitted food additives. Toxicological safety evaluations have never been reported for any Combretum gum (14), and no orginisation has ever requested that they be evaluated for food use. Food manufacturers and regulatory authorities therefore require analytical data that characterise these exudate gums so they can be identified and their use in foodstuffs prevented. There have been official requests for the existing analytical

data available for Combretum gums to be extended to other species therefore this section of the thesis presents an analytical study of the gums from a further six Combretum species. As all of them have negative optical rotations similar to gum arabic this study recognised that this sole analytical parameter is no longer sufficient to confirm the identity of a gum. The additional methods of analysis to necessary to allow unambigious identification are discussed.

IV.II. (ii) MATERIALS AND METHODS.

Origin of samples.

One Nigerian gum sample (from Combretum sokodense Engl.) and five Tanzanian samples (from Combretum splendens Engl., Combretum pinpuriciflorum, Combretum apiculatum Sond., Combretum longispicatum and Combretum frommii Gilg.) were obtained by the courtesy of the Overseas Development Natural Resources Institute, Gray's Inn Road, London. The gum samples dissolved completely overnight to give viscous aqueous solutions, except those for C.pinpuriciflorum and C.apiculatum, which required the addition of trace amounts of sodium hydroxide and sodium borohydride (44), to ensure complete dissolution.

Analytical methods.

The analytical methods are described in

Chapter II.

IV.II (iii) RESULTS

The data for the general and polysaccharide-based parameters for the six Combretum gum exudates analysed are presented in table IV.9(i,ii). The data for the amino acid composition of the proteinaceous component of the gums (expressed as residues per 1000 residues), are presented in table IV.10 with the nitrogen conversion factors for each gum. Data for gum arabic ([8], and Chap III.2) are shown in tables IV.9(ii) and 10 for comparative purposes associated with identifying the analytical parameters which differentiate the Combretum gums from genuine gum arabic.

IV.II. (iv) DISCUSSION.

As mentioned previously, taxonomically the genus Combretum is complex and difficult, new chemotaxonomic indications are therefore useful. Combretum frommii Gilg. is regarded as a member (45) of the Combretum collinum Fresen. aggregate, yet there is little similarity in their gum chemistry; their specific rotations are -2° and -81° respectively (40). Another example involves the fact that the Tanzanian gum specimen from Combretum apiculatum studied here with a specific rotation -25°(table IV.9[i]) bears

TABLE IV.9(i) Analytical data for the gum exudates from four Combretum species.

Analytical Parameter	Combretum sokodense	Combretum splendens	Combretum pini-puriciflorum	Combretum apiculatum
Moisture, %	10.0	10.0	10.6	11.2
Ash, % [a]	4.5	2.1	3.8	4.1
Nitrogen, % [a]	0.13	0.14	0.17	0.45
Nitrogen conversion factor (N.C.F)[b]	5.96	5.98	6.55	6.17
Hence % peptide or protein (N.C.F X %N)	0.8	0.8	1.1	2.8
Methoxyl, % [b]	0.27	0.26	0.22	0.86
Specific rotation in water (degrees)[a]	-21°	-28°	-36°	-25°
Intrinsic viscosity, mlg^{-1} [a]	72	87	62	46
Tannin %	0.32	0.41	0.90	0.70
Acetyl %	0.8	1.8	1.0	0.6
Equivilent weight [a]	1040	1110	570	530
Uronic anhydride, %	17	16	31	34
Sugar composition after hydrolysis, %				
Galacturonic acid	4	9	14	17
Glucuronic acid	1.5	1.5	1	5
Methylglucuronic acid [d]	11.5	5.5	16	12
Galactose	26	42	25	12
Arabinose	26	15	24	25
Rhamnose	31	27	20	29
Xylose	tr	tr	tr	tr
Mannose	tr	tr	tr	tr

Notes:
[a] Corrected for moisture content.
[b] From tables IV.10.
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

TABLE IV.9(ii) Analytical data for the gum exudates two Combretum species and gum arabic.

Analytical Parameter	Combretum longispicatum	Combretum fronnii	Gum arabic
Moisture, %	12.5	13.9	6.0
Ash, % [a]	2.4	4.3	3.0
Nitrogen, % [a]	0.16	0.18	0.30
Nitrogen conversion factor (N.C.F)[b]	5.78	6.90	6.60
Hence % peptide or protein (N.C.F X %N)	0.9	1.2	2.0
Methoxyl, % [b]	0.03	0.42	0.20
Specific rotation in water (degrees)[a]	-21°	-2°	-30°
Intrinsic viscosity, mlg^{-1} [a]	53	64	17
Tannin %	0.09	0.75	0
Acetyl %	1.5	0.9	0
Equivilent weight [a]	1280	740	1020
Uronic anhydride, %	14	24	17
Sugar composition after hydrolysis, %			
Galacturonic acid	8	19	0
Glucuronic acid	tr	2.5	2
Methylglucuronic acid [d]	6	2.5	15
Galactose	45	21	45
Arabinose	29	28	24
Rhamnose	12	27	14
Xylose	tr	tr	0
Mannose	tr	tr	0

Notes:
[a] Corrected for moisture content.
[b] From tables IV.10.
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

TABLE IV.10 Amino acid composition of the proteinaceous components of six Combretum gums.

	Combretum sokodense	Combretum splendens	Combretum pin-puriciflorum	Combretum apiculatum	Combretum longispicatum	Combretum frommii	Gum arabic
% Nitrogen	0.13	0.14	0.17	0.45	0.16	0.18	0.34
Alanine	73	82	76	55	110	97	22
Arginine	75	54	23	30	51	46	10
Aspartic acid	80	104	112	89	96	31	55
Cystine	0	89	50	66	0	0	1
Glutamic acid	79	46	66	64	61	69	39
Glycine	135	103	101	86	179	123	59
Histidine	18	16	19	21	31	19	51
Hydroxyproline	0	0	73	74	0	0	270
Isoleucine	33	21	34	6	37	50	13
Leucine	47	34	57	49	52	68	75
Lysine	37	28	46	54	41	36	26
Methionine	12	26	8	8	0	16	2
Phenylalanine	29	34	36	73	55	29	39
Proline	175	162	55	139	50	71	89
Serine	82	71	93	78	53	66	126
Threonine	44	55	66	54	56	139	74
Tyrosine	30	20	36	37	54	60	10
Valine	51	53	51	54	56	54	39
Nitrogen Conversion factor	5.96	5.98	6.55	6.17	5.78	6.90	6.59

little resemblence to the analytical data for a Nigerian sample of the same gum (specific rotation +24°) published previously (40). It is possible that the samples may therefore correspond to different sub-species, but unfortunately the gum collectors did not supply such depth of information.

The data presented in tables IV.9(i,ii) and IV.10 support previously established generalisations (14,40), that Combretum exudate gums give rise to very viscous, unusually acidic solutions (pH 3.8-4.0 as a result of their acetyl content); their nitrogen contents tend to be low and their rhamnose contents after acidic hydrolysis are relatively high. The acetyl values for the six species reported here are lower than for similar studies carried out by Anderson and various co-workers in 1977 and 1986 (14,40). In contrast, the rhamnose contents reported here are on average considerably higher than for the species previously studied. It is of interest that trace amounts of the sugars xylose and mannose were detected by paper chromatography, traces of these sugars have never been detected in any Acacia gum species.

Extremely important is the confirmation of previous reports that Combretum gums contain characteristic proportions of galacturonic acid (14,40,43), ranging from 4% in C.sokodense to 19% in C.frommii. A further important confirmation to the identity of Combretum gums and their difference from Acacia senegal involves their low hydroxyproline

content. The present recorded values for hydroxyproline range from 0 to 74 compared to an average in Acacia senegal of 275 to 300 residues per 1000 residues. The Combretum gums also tend to contain relatively high proportions of alanine, glycine and aspartic acid (with the exception of Combretum fromii), Individual species, for example Combretum sokodense, also contain unusually large quantities of proline.

Combretum gums can therefore be detected as contaminants of gum arabic by means of their unusual values of certain analytical parameters. They can be differentiated from "true" gum arabic (Acacia senegal (L.) Willd.) by means of their sugar and amino acid compositions, by the presence of galacturonic acid, xylose and acetyl groups, by their greatly enhanced intrinsic viscosities, and much more strongly acidic solutions.

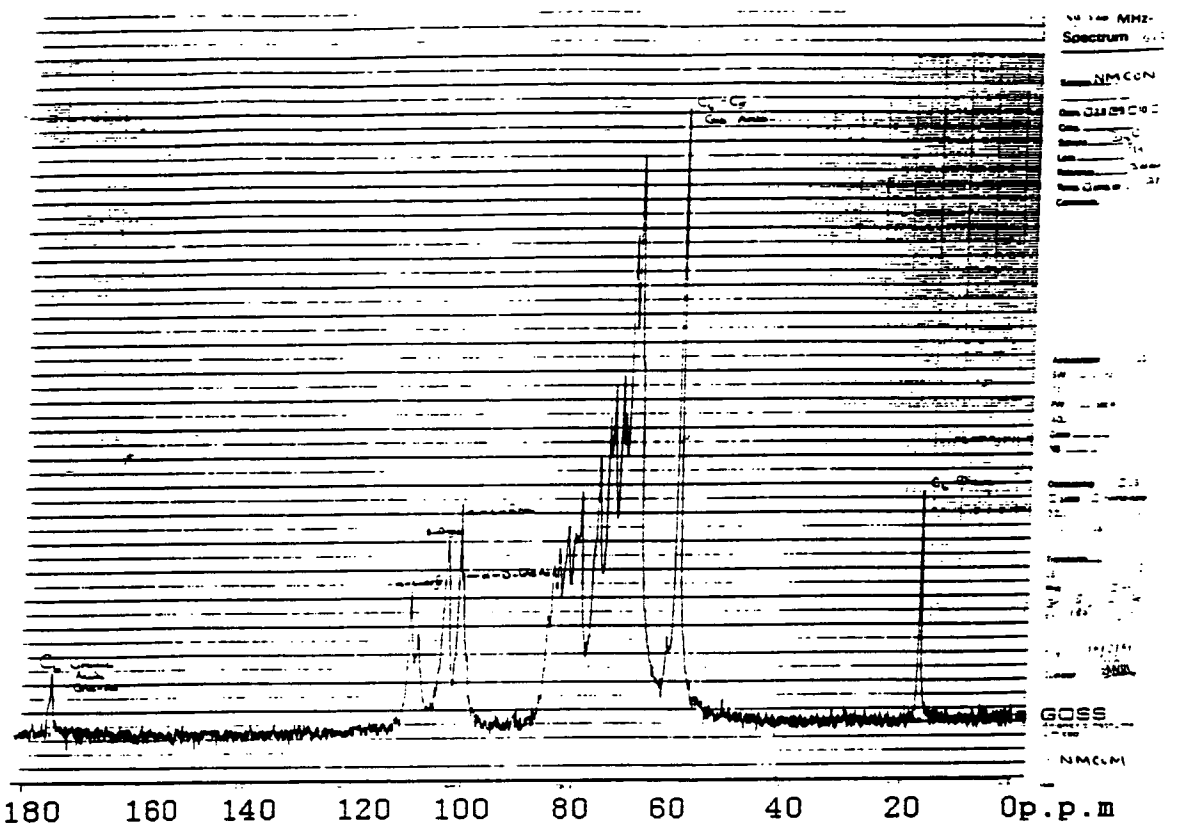
However full chemical analyses are rarely carried out on commercial gum samples; specific rotation has often been the sole analytical parameter on which the identity of gum arabic is based. For those concerned with identity and quality and food regulation compliance this is clearly no longer adequate. All six Combretum gums have negative specific rotations, moreover five of them have specific rotations close to that designated for gum arabic, for which the specified specific rotation is -30 ± 3° (8). Of the twenty Combretum gums for which analytical data are now available only eight give positive values for specific

rotation.

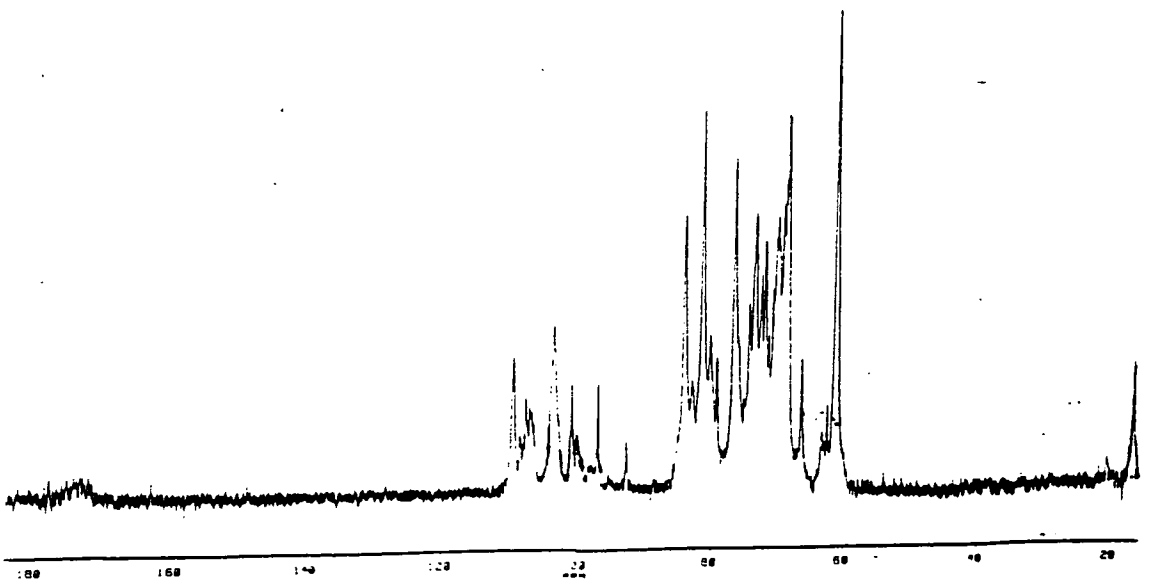
The data presented in this study suggest therefore that mere measurement of specific rotation is no longer adequate to ensure the identity, nor freedom from adulteration, of gum arabic. Many traders in the past relied heavily on this measurement, in isolation, as it was adequate to detect the presence of the inferior Acacia seyal gum (21), which is not permitted in foods, is functionaly inferior to gum arabic, and was previously the main commercial adulterant. Acacia seyal has a specific rotation of +55°, so its detection even as a minor component in a blend with gum arabic was possible: the addition of 10% of gum tahla had the effect of lowering the specific rotation from -30° to -20°.

There is no doubt that Combretum gums have some useful inexpensive technological applications. However their use in foods is not permitted, their deliberate introduction into the human food chain is in contravention of all existing food regulations, as currently there is a complete absence of any toxicological evidence of safety for any of the Combretum gums. Although there are usually some external differences in the physical appearance of gum arabic compared to most Combretum gums, value-added practices such as bleaching or chemical modification prior to the spray drying stage of gum processing are particularly cost-attractive for unscrupulous traders who can obtain these non-permitted poor quality gums in

Spectrum IV. 1: 13C NMR spectra of Control Gum Arabic (Acacia senegal).



Spectrum IV. 2: 13C NMR spectra of Combretum pinpuriciflorum.



the producing countries at low prices. There is no doubt from these results that unscrupulous gum vendors, through skillful selection of commercially available Combretum gums, can devise blends that would satisfy the Revised Specification for gum arabic in terms of optical rotation and nitrogen content (0.27 to 0.39%) fraudulently, through containing no gum arabic but only non-permitted gums (10). As Combretum gums are commercially available in Africa at a cost of around $500-600 per tonne compared to a price of $3200 a tonne for gum arabic (10), the great financial advantage is obvious.

The currently available, completely specific, unequivocal method of identifying gum arabic or a gum of Combretum origin involves Fourier transform ^{13}C NMR spectroscopy. However its high present cost makes this technique unreasonable except for settlement of legally based claims for breach of contract. Spectra IV. 1,2 show how the spectrum from Combretum pinpuriciflorum gum differs extensively from that for gum arabic (Acacia senegal). NMR provides unambigious identification of gum arabic or the detection of an adulterant non-permitted food gum exudate by comparison of the reference spectra now available (10,46).

CHAPTER IV.III AN ANALYTICAL STUDY OF FOUR PROTEINACEOUS *ACACIA* GUM EXUDATES.

IV.III (i) INTRODUCTION.

The genus *Acacia* (Family *Leguminosae*, sub-family *Minosoideae*) is one of the largest in the Plant Kingdom; the genus contains between 500 and 1100 species from various estimates (47), the exact number is not known but continues to be revised upwards from time to time. However only thirteen exudate gums from different *Acacia* species had been chemically characterised by 1963 (48). By 1969 some thirty *Acacia* gums had been analysed. To date the number characterised has reached 114 species (49,50). By far the majority of *Acacia* species are indigenous to *Australia*, where morphological divergance has occurred, although the genus is also important and widespread in Africa, where 130 species have been documented by Ross (47). Of particular importance is the exudate gum from *Acacia senegal* (L.) Willd. (51), which now occurs in four different varieties and has 12 other species related to it so closely that they are recognised as constituting the "*Acacia senegal* complex". These varieties and related species together form the internationally accepted sources of commercial gum arabic from a food legislative viewpoint (4,52,53,54).

It was quickly recognised for botanical purposes, as early as 1874 by Bentham when only 434

distinct Acacia species had been identified (51), that it was necessary to subdivide such a large genus into six sections. Of these six, two (Phyllodineae and Botryocephelae) classified the Australian species, and two (Gummiferae and Vulgares) classified the African species. Acacia senegal has been clearly established as belonging to the section Vulgares, whereas Acacia seyal (21), the major source of the distinctly different commércial gum tahla, is a member of the section Gummiferae.

The early distinction and classification made by Bentham was greatly strengthened when Vassal (55) proposed to divide the genus Acacia, into three sub-genera; subgenus Heterophyllum (which comprised Bentham's sections 1 and 2, the Australian species); subgenus Acacia (which included Bentham's Section 4 [Gummiferae]); and subgenus Aculeiferum (Bentham's Section 5 [Vulgares]).

This study analyses a further four proteinaceous Acacia gum exudates, all of which are African species from Bentham's Section 4 Gummiferae, in terms of their carbohydrate and amino acid components. None of the gums analysed are permitted as food additives, and are not included in major international regulatory lists. The established analytical parameters which distinguish the Acacia gums from the Gummiferae and Vulgares is important as gum species not related to Acacia senegal (L.) Willd., must be identifiable for food regulatory purposes. Although the sugar ratios and

amino acid compositions of the four gums are similar to that of Acacia senegal, in that they contain the same sugars as gum arabic, these four gums all have positive specific rotations and all give a positive reaction for the presence of tannin, a characteristic of species in Bentham's Gummiferae.

IV.III (ii) MATERIALS AND METHODS.

Origin of gum samples.

One sample of West African origin and three samples of East African origin were obtained throught the courtesy of the Tropical Products Research Institute, London. Acacia fischeri Harms is endemic in Tanzania, occurring on hard-pan grey soils in shallow drainage glades and on the fringes of large seasonal rivers (47); Acacia kamerunensis Gandoger is widespread in West Tropical Africa from Sierra Leone to the Central African Republic, in Zaire and Uganda (47); Acacia spirocarpa Hochst.ex A.Rich. is found in the Sudan, Somalia, Arabia and Israel (47); and Acacia stenocarpa Hochst. ex A.Rich. is widespread in East and North tropical Africa, extending to Egypt.

Analytical methods.

The analytical methods used to quantify carbohydrate and amino acid compositions for the four Acacia gums are described in Chapter II. The officially recommended qualitative method (52,53) for the

detection of tannin-containing gums was used as described. The method was converted to give quantitative values of the tannin content by means of colorimetry at 430nm. Tannic acid was used as the reference standard. After the ash content was determined by ashing the gums to constant weight at 550°C, the ash was dissolved in dilute hydrochloric acid and used for determination of cationic content by flame atomic absorption spectroscopy.

IV.III. RESULTS AND DISCUSSION.

Table IV.11 shows the analytical data obtained for the physico-chemical and carbohydrate parameters. Table IV.12 shows the amino acid compositions obtained from the proteinaceous components of the gums from Acacia fischeri, Acacia kamerunensis, Acacia spirocarpa and Acacia stenocarpa. Table IV.13 shows the compositions of the ash content obtained from the gums. Data for the gum from Acacia seyal var. seyal (56) and Acacia senegal are included in the tables for comparative purposes.

Of the four Acacia species studied, that from Acacia fischeri, most closely resembles that of Acacia seyal (21,56), the major contributor to commercial gum tahla, although Acacia fischeri has a higher nitrogen and methoxyl content. The gum from Acacia kamerunensis is considerably more acidic than the average Acacia species assignable to the

TABLE IV.11 Analytical data for Acacia gum exudates from the section Gummiferae.

Analytical Parameter	Acacia fischeri	Acacia kamerunensis	Acacia spirocarpa	Acacia stenocarpa	Acacia seyal	Gum arabic
Moisture, %	12.4	11.1	13.0	14.6	13.4	9.8
Ash, % [a]	2.1	5.9	1.7	3.4	2.87	3.2
Nitrogen, % [a]	0.46	0.13	1.27	0.38	0.14	0.34
Nitrogen conversion factor (N.C.F)[b]	6.15	6.83	6.31	6.67	6.25	6.57
Hence % peptide or protein (N.C.F X %N)	2.8	0.9	2.5	0.9	0.9	2.2
Methoxyl, % [b]	1.81	0.66	0.43	0.82	0.94	0.24
Specific rotation in water (degrees)[a]	+68°	+29°	+65°	+14°	+51°	-30°
Intrinsic viscosity, mlg^{-1} [a]	12	15	10	9	12	17
Equivalent weight [a]	1210	670	920	1045	1470	1020
Uronic anhydride, %	15	26	19	17	12	17
Tannin, % [c]	0.65	0.33	1.0	0.85	1.9	0.0
Sugar composition after hydrolysis, %						
Glucuronic acid	4	22	16	12	7	15
Methylglucuronic acid [d]	11	4	3	5	5	2
Galactose	36	26	26	41	38	48
Arabinose	44	36	47	32	46	24
Rhamnose	5	12	8	10	4	11

Notes:
[a] Corrected for moisture content.
[b] From tables IV.12
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

TABLE IV.12 Amino acid composition (residues per 1000 residues) for four *Acacia* gums (section *Gummiferae*) and gum arabic [a].

	Acacia fischeri	Acacia kamerunensis	Acacia spirocarpa	Acacia stenocarpa	Gum arabic
% Nitrogen	0.46	0.13	1.27	0.38	0.33
Alanine	30	35	47	36	27
Arginine	21	14	26	17	10
Aspartic acid	79	67	115	87	55
Cystine	12	0	43	43	0
Glutamic acid	37	32	58	30	42
Glycine	45	42	71	57	54
Histidine	29	28	21	30	49
Hydroxyproline	269	359	124	234	292
Isoleucine	25	17	38	16	12
Leucine	46	56	76	76	75
Lysine	15	16	27	18	27
Methionine	30	10	2	3	1
Phenylalanine	49	20	74	33	39
Proline	50	62	61	54	63
Serine	146	130	75	144	131
Threonine	59	60	51	51	74
Tyrosine	16	11	20	21	11
Valine	42	41	71	50	38
Nitrogen Conversion factor	6.15	6.83	6.31	6.67	6.59

[a] Refer to chapter III, Table III.1

Gummiferae subsection. The gum from Acacia spirocarpa is similar to gum tahla in terms of sugar ratios, optical rotation and intrinsic viscosity; however it is much more proteinaceous and its amino acid composition is unusually high in aspartic acid and low in hydroxyproline, features unusual for a species within the subsection Gummiferae. Such amino acid compositions have been reported previously for Bentham's section Phyllodineae (57), for example Acacia microbotrya (58), characterised by Anderson and co-workers and containing only 99 hydroxyproline residues per 1000 residues. This low hydroxyproline content may have some structural implications; there is evidence that suggests that this hydroxyproline is concentrated in the core of the gum molecules in both the positive optical rotation gum tahla (21), of the section Gummiferae, and in the gum from Acacia senegal (gum arabic), which has a negative rotation and is in the section Vulgares (32,33). The established analytical distinctions between gum species from the subsections Vulgares and Gummiferae are important as species like Acacia seyal, which is not permitted for use as a food additive, must be identifiable for food regulatory purposes (59,60).

According to Ross (47), Acacia stenocarpa can be regarded as a synonym of Acacia seyal. Comparison of the analytical data for the two gums in Table IV.12, suggests that the differences observed e.g. the much higher nitrogen content, the higher acidity and the higher rhamnose content of

Acacia stenocarpa, must be regarded as lying outside the ranges of possible analytical error.

The characteristic Acacia gum exudates are also interesting for the presence or absence of tannin, which is another secondary product of some Acacia plants. Several Acacia species for example Acacia mearnsii (Bentham's Botryocephalae) are widely grown in Africa for their commercial production of tannin which is used for leather curing purposes (61). All four proteinaceous Acacia gums analysed here and Acacia seyal (all section Gummiferae), give a positive reaction to the test for tannin. However Acacia species in section Vulgares for example Acacia senegal, yield only gum and no tannin.

The tannin-bearing gums are commonly recognised accordingly as conferring an unacceptably bitter, astringent taste to gum solutions. All of the major international specifications for gum arabic include a qualitative test to ensure the absence of tannin. Many food and pharmaceutical manufacturers rely on this test for acceptance or rejection of gum consignments that would otherwise lead to consumer-rejection of the end-product. The absence of tannin is also important in that tannins are established carcinogens (62,63), and non-mutagenic tannin can be converted into a mutagen by the presence of Mn^{2+} (63), which is present in quantiies of up to 220 p.p.m in gum arabic.

TABLE IV.13.The cationic composition [a] of the ash from some Acacia gum samples (µg/g ash,550°C).

	Acacia fischeri	Acacia spirocarpa	Acacia stenocarpa	Acacia seyal	Gum arabic
% Ash [b]	2.1	1.7	3.4	4.2	3.9
Aluminium	4650	870	1300	6100	171
Calcium X 10^3	224	335	266	260	235
Chromium	57	91	51	40	49
Cobalt	0	0	0	24	0
Copper	348	253	164	55	29
Iron	696	869	716	2861	105
Lead	301	53	19	4	3
Magnesium X10^3	26	55	36	28	48
Manganese	255	220	41	49	221
Nickel	5	17	31	10	5
Potassium X10^3	27	49	153	48	194
Sodium X 10^2	16	20	2	22	8
Zinc	202	140	73	23	10

[a] For all samples, As, Cd, Mo all < 1ppm.

[b] Table IV.11

The data presented for cationic contents of the ash component of the gums from three of the Acacia species studied and also the cationic composition of gum tahla and gum arabic are shown in Table IV.13. The data suggests that some of the differences in cationic composition observed are caused by the variation in the soil type on which the Acacia trees grew. All four Acacia gums from the section Gummiferae, contain relatively high levels of heavy metals, copper zinc and lead. They also contain higher values of iron content, and especially high aluminium contents compared to commercial gum arabic (section Vulgares). In view of recent medical suggestions of the involvement of high levels of aluminium in the diet as being linked to brain and dietary disorders (64,65), this may be additional evidence for the absence of dextrorotary gums (Bentham's section Gummiferae), from being blended with or sold as a substitute to replace gum arabic (Acacia senegal) as a food additive.

CHAPTER IV.IV. AN ANALYTICAL STUDY OF SEVEN *ALBIZIA* GUM EXUDATES.

IV.IV. (i) INTRODUCTION

The genus *Albizia*, (family *Leguminosae*, sub-family *Mimosoideae*, tribe *Ingeae*) comprises 150 distinct plant species (66), 72 of which are found widely in Africa. *Albizia* is a complicated, pantropical species, and is often mistaken for *Acacia* (Chapter IV.III). The main diagnostic difference between the two plant genus' involve the reproductive organs in the plants; the stipules (which are herbaceous and shed early in *Albizia*), and the stamens which are usually longer in *Acacia*, and united at the base into a tube (67).

The *Albizia* genus has been little exploited commercially compared to *Acacia* species. However, there have been recent official recommendations that *Albizia* species could be used as a source of rapidly growing firewood, and afforestation projects were promised. Therefore the increased use of *Albizia* species in agroforestry (68), for soil improvement and in projects to regenerate arid sub-tropical zones, may lead to the increased availability of *Albizia* gums in the future. As no toxicological data exists for any *Albizia* gum at present, they are not permitted as food additives. It is important therefore that analytical data are

available, so that their presence as adulterants or contaminants in a gum blend may be detected and prevented. The data augment those presented in chapter IV on Combretum gum species (15), and Acacia gum species (58), which are also not permitted in foodstuffs. Data for the gums from four Albizia species studied by Anderson and co-workers in 1966 (69), will be refered to for comparative purposes. The botanical synonymy for several of the more common Albizia species has been published in the Appendix to this paper (69).

Albizia species are abundantly nodulated, and have potential as soil improvers through nitrogen fixation. Other attributes of Albizia plant species, include their use as possible sources of tannin (66), poor quality exudate gums and possibly the extraction of insecticidal compounds from the barks of certain Albizia species.

IV.IV. (ii) MATERIALS AND METHODS.

Origin of gum samples.

The gum from Albizia anthelmintica was collected near El Obeid, Republic of the Sudan, by Mr. A.G. Seif-el-Din, formerly Gum Research Officer, Sudan. Gum samples for the following species; Albizia harveyi Fourn. (syns. Albizia hypoleuca Oliv., Albizia pallida Harv.); Albizia forbesii Benth.; Albizia amara (Roxb.) Boivin; Albizia lebbeck (L.) Benth.; Albizia samman

(Jacq.) F.Muell. (syns. Albizia gummifera, Albizia fastigiata, Albizia sassa), were obtained through the courtesy of the Overseas Development Research Institute, London.

Analytical methods.

The experimental methods used to determine various analytical parameters are described in chapter II. Tannin determinations were carried out by the addition of iron (III) chloride (12), to 0.5% gum solutions, rather than to 2% solutions to prevent gelation of the gums.

IV.IV (iii) RESULTS.

The data obtained for the general analytical parameters are presented in Tables IV.14(i,ii). Data for the amino acid composition expressed as residues per 1000 residues, and the nitrogen conversion factor for the proteinaceous component of the gums are shown in Table IV.15. The cationic contents obtained by Atomic Absorption Spectroscopy of the ash obtained at 550°C from the gum samples are shown in Table IV.16.

IV.IV (iv) DISCUSSION.

The data presented for the seven Albizia gums analysed supports the botanical observation (66)

TABLE IV.14(i) Analytical data for the gum exudates from four Albizia species.

Analytical Parameter	Albizia harveyi	Albizia forbesii	Albizia amara	Albizia lebbeck
Moisture, %	13.1	10.6	13.0	11.9
Ash, % [a]	4.5	9.9	7.6	6.0
Nitrogen, % [a]	0.46	2.17	0.50	0.24
Nitrogen conversion factor (N.C.F)[b]	6.11	6.75	6.24	6.49
Hence % peptide or protein (N.C.F X %N)	2.8	14.6	3.1	1.6
Methoxyl, % [b]	0.10	1.30	0.50	0.60
Specific rotation in water (degrees)[a]	-24°	-22°	-16°	+6°
Intrinsic viscosity, $ml g^{-1}$ [a]	33	19	62	142
Tannin %	0.5	0.6	1.0	0.7
Acetyl %	0	0	0	0
Equivalent weight [a]	620	1370	1090	1970
Uronic anhydride, %	29	15	17	9
Sugar composition after hydrolysis, %				
Glucuronic acid	28	7	14	5
Methylglucuronic acid [d]	1	8	3	4
Galactose	25	28	30	55
Arabinose	28	27	26	21
Rhamnose	17	29	9	9
Mannose	1	1	17	6

Notes:
[a] Corrected for moisture content.
[b] From tables IV.15.
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

TABLE IV.14(ii). Analytical data for the gum exudates from three Albizia species.

Analytical Parameter	Albizia saman	Albizia adianthifolia	Albizia anthelmintica
Moisture, %	13.5	14.8	12.8
Ash, % [a]	5.1	9.3	4.9
Nitrogen, % [a]	0.25	0.93	2.80
Nitrogen conversion factor (N.C.F)[b]	6.61	6.58	6.16
Hence % peptide or protein (N.C.F X %N)	1.6	6.1	17.2
Methoxyl, % [b]	0.23	0.55	0.91
Specific rotation in water (degrees)[a]	-27°	+22°	+18°
Intrinsic viscosity, mlg^{-1} [a]	171	38	19
Tannin %	0.9	1.0	1.9
Acetyl %	0	0	0
Equivalent weight [a]	1160	590	940
Uronic anhydride, %	16	32	19
Sugar composition after hydrolysis, %			
Glucuronic acid	15	29	14
Methylglucuronic acid [d]	1	3	5
Galactose	14	22	37
Arabinose	39	22	27
Rhamnose	25	12	7
Mannose	6	12	10

Notes:
[a] Corrected for moisture content.
[b] From tables IV.15.
[c] Corrected for protein content.
[d] 4-O-methylglucuronic acid.

that Albizia is a complicated genus. Thus Tables IV.14(i,ii) shows that four of the gums analysed have negative specific rotations and three samples have positive rotations. There are indeed wide variations for several analytical parameters: thus the protein contents of the gums vary from 1.6% to 17.2%. The methoxyl content varies from 0.1% to 1.3%. The intrinsic viscosities vary from 19ml/g for Albizia forbesii to the very viscous 171ml/g for Albizia saman. The carbohydrate composition of the gums also show large variations, for example 9% uronic anhydride content in Albizia lebbeck and 32% in Albizia adianthifolia. (This high figure may explain the cross-linked gel behavior of this gum in the presence of iron[III]). The galactose contents vary considerably from 55% in Albizia lebbeck to 14% in Albizia amara.

Although the wide range of values for the analytical parameters reported in this study and in previous studies on Albizia gums do not exceed those parameters established for Acacia gums, it is appropriate to recall that the Acacia genus comprises approximately 900 species whilst only 150 Albizia species have been identified. The data presented in Tables IV.14(i,ii) indicate however that certain Albizia species, for example Albizia lebbeck (142 ml/g), and Albizia samman (171 ml/g) have a much higher intrinsic viscosity than any Acacia gum exudate previously characterised (58). The highest intrinsic viscosity published for an Acacia gum is only 39 ml/g.

The rhamnose content of the carbohydrate component of certain Albizia gums is also higher than any previously published data for Acacia gums; e.g Albizia forbesii with 29% rhamnose, compared to the highest reported value for any Acacia species [23%],(58). Perhaps of greater diagnostic significance is the presence, in the seven Albizia gums analysed, of mannose, which ranges from 1% in Albizia harveyi to 12% in Albizia adianthifolia. Mannose does not occur in any published data on Acacia gums. The significant amounts of tannin present are also of diagnostic significance. Tannin has only previously been detected in the dextrorotatory Acacia gums belonging to Bentham's subsection Gummiferae (2,3,21)

The complex nature of the Albizia gums is also indicated by the wide range of their amino acids compositions. Previous results for Albizia gums for example Albizia glaberrima (17 hydroxyproline residues per 1000) have indicated that Albizia gums contain in general lower hydroxyproline levels than Acacia gum species, although low hydroxyproline values have been reported for certain Australian Acacia species [eg Acacia aestivalis 55 residues per 1000]). For the results presented in Table IV.15; Albizia anthelmintica and Albizia harveyi, tend to be low in hydroxyproline whereas Albizia lebbeck and Albizia forbesii give values more representative of what can be regarded as an intermediate value for an Acacia gum.

The overall amino acid composition for

TABLE IV.15. Amino acid composition of the proteinaceous components of seven *Albizia* gums.

	Albizia harveyi	Albizia forbesii	Albizia amara	Albizia saman	Albizia lebbeck	Albizia adianthifolia	Albizia anthelmintica
% Nitrogen	0.46	2.17	0.50	0.24	0.25	0.93	2.80
Alanine	55	58	61	52	54	53	46
Arginine	46	33	21	23	27	24	29
Aspartic acid	101	92	110	119	118	104	141
Cystine	6	0	1	2	0	1	13
Glutamic acid	54	67	74	65	58	69	69
Glycine	87	84	91	87	94	81	75
Histidine	22	18	18	23	14	25	58
Hydroxyproline	96	208	119	177	129	117	17
Isoleucine	33	29	28	26	28	31	50
Leucine	48	46	55	42	63	52	77
Lysine	57	39	73	96	44	65	47
Methionine	9	3	2	2	3	6	7
Phenylalanine	49	57	44	23	52	46	27
Proline	87	39	79	60	85	83	73
Serine	76	75	76	72	81	78	87
Threonine	65	52	64	42	50	64	69
Tyrosine	51	41	29	35	31	43	34
Valine	58	59	55	54	69	58	80
Nitrogen Conversion factor	6.11	6.75	6.24	6.49	6.61	6.58	6.16

all seven Albizia gums (Table IV.15), although variable in general, are all very different from the amino acid compositions previously reported for gum arabic. For example, the Albizia gums contain lower levels of hydroxyproline, serine and threonine, and higher levels of aspartic acid, glycine, isoleucine and alanine, than those reported for gum arabic samples (20). Amino acid compositions may therefore provide a simple yet useful analytical tool for helping to distinguish adulterant Albizia gums from food permitted gum arabic.

The data presented for the cationic compositions of the ash (Table IV.16) obtained from the gums at 550°C may also be of diagnostic value when comparing data available for Albizia gum exudates with those for gum arabic, (Acacia senegal (L.) Willd.). For example, Albizia forbesii gum contains relatively high levels of copper, aluminium and lead. Albizia samman contains relatively high levels of aluminium, copper and zinc. Five out of the seven species analysed contain much higher manganese levels than reported for gum arabic (30-100 μg/ml),(12). Some Albizia species also contain very low levels of calcium and magnesium and are relatively high in potassium levels compared to gum arabic. These cationic contents reflect the cations present as salt groups on the uronic acids present, and do not arise from contaminating sand or bark in the sample. The cationic compositions generally reflect the soil type on which the gum grew.

From a food safety viewpoint the high

TABLE IV.16. The cationic composition [a] of the ash from seven Albizia gum samples (µg/g ash 550°C).

	Albizia harveyi	Albizia forbesii	Albizia amara	Albizia saman	Albizia lebbeck	Albizia adianthifolia	Albizia anthelmintica
% Ash [b]	3.7	2.8	3.6	3.4	3.4	4.1	3.6
Aluminium	680	7880	2820	350	1260	73	n.d
Calcium X 10^3	196	129	57	8	277	31	84
Chromium	45	94	64	42	41	31	100
Cobalt	0	0	7	8	32	4	0
Copper	53	368	137	93	820	54	250
Iron	186	3450	1060	125	610	58	94
Lead	7	580	27	17	232	14	3
Magnesium X10^3	21	13	14	2	53	6	22
Manganese	665	245	576	24	89	1145	344
Nickel	8	20	32	23	64	17	7
Potassium X10^3	219	18	272	320	7	397	86
Sodium	943	1040	34508	360	11360	1240	1000
Zinc	39	113	70	101	540	25	48

[a] For all samples, As, Cd, Mo all < 1ppm.

[b] Table IV.14

levels of aluminium recorded for these samples is undesirable, as aluminium has been linked to certain brain and dietary disorders (65). Non-mutagenic tannic acid can be converted into a mutagenic form by the presence of small quantities of manganese (II), therefore the high levels of manganese reported in these species is also undesirable (63).

In conclusion it must be stated that the Albizia gums are harder to distinguish from permitted laevorotary Acacia species (58) than the Combretum gums (Chapter IV.I). In Combretum gums (9,31), the presence of galacturonic acid and acetyl groups (freshly powdered Combretum gum samples have a vinegar-like odour), gives additional analytical markers. There are several indications from the data presented that Albizia gums are undesirable and should not appear in food as contaminants or adulterants. As a result of increased cultivation of Albizia species (68,11) for ecological reasons, gum traders will have to increase vigilance to ensure that Albizia gums (especially those with negative specific rotations) are not mistaken at any time for "true" gum arabic samples.

The currently existing weak and archaic specification for the purity of gum arabic is presently being revised, as is desirable in the interests of food safety assurance for the consumer (13). As was stated in chapter IV.I, the ultimate but expensive analytical method of differentiating authentic gum arabic from samples contaminated with Albizia gums can best be

achieved by Fourier transform ^{13}C NMR spectroscopy.

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CHAPTER V

INTRODUCTION TO THE MECHANISM OF INTERACTION BETWEEN WATER-SOLUBLE CELLULOSIC POLYMERS IN SOLUTION.

CHAPTER V. INTRODUCTION

This study investigates the mechanism of interaction between water-soluble cellulosic ethers and galactomannan gums in aqueous solution for food application. Widespread technological usage of the synergistic interaction between unlike polysaccharides in solution, has been utilised in recent years (1). Due to processing costs and natural abundance of raw materials, cost effective blending of polysaccharides into a formulated product with no loss, or enhanced performance is commercially attractive. Understanding the mechanism of specific interactions may enable usage of these polysaccharides at reduced polymer concentrations in formulated food products.

Hydrocolloids, commonly referred to as gums in food systems are water-soluble. Their hydrophilic properties give important textural and rheological characteristics to a solution (2,3). These water-soluble polymers provide water control by thickening or gel formation. The polysaccharides hydrate in aqueous solution associating water molecules with its macromolecular structure. The volume of solvent that has to be regarded as bound to the polymer is several hundred times larger than the volume of a polymer molecule (4). The presence of very small quantities, (often less than 1% polymer concentration)

of these polymers can markedly alter the rheological properties of the solvent.

The hydrocolloids are commonly classified according to origin (5): the natural gums; seed endosperms (e.g guar and locust bean gum), gum exudates (e.g gum arabic and gum karaya), seaweed extracts (carrageenans and alginates), microbiological fermentation products (e.g xanthan gum) and the semi-synthetic cellulosic derivatives which are of primary concern to this study (the most commonly utilised cellulose ethers are sodium carboxymethyl cellulose and hydroxypropyl methyl cellulose). The rheological characteristics of each polymer are based on its viscosity producing capability when dispersed in aqueous solution. The solution properties of each polysaccharide depend on its molecular conformation, the molecular size and the molecular shape of the polymer.

These properties can vary widely between different polysaccharides; e.g chain branching effects. Some polymers are highly branched (e.g. gum arabic), others are based on a linear backbone (e.g. locust bean gum), whilst in other polymers the molecular conformation varies with temperature (e.g. methyl cellulose), thus the solution properties reflect these structural differences (6). Secondly, the polymeric chain stiffness plays a large role in determining the radius of gyration of a polymer molecule. This is

governed by the nature of the linkage between adjacent sugar residues and may explain why cellulosic derivatives have higher intrinsic viscosities compared to starch amylose. Another factor which influences the molecular dimensions of a polymer and which will be discussed throughout the text is charged polyelectrolytes. As a result of electrostatic repulsion between charged groups, if all other factors are constant an anionic polymer will adopt a more open structure than a non-ionic polymer (4).

Application of these hydrocolloids in food systems is wide and varied; as gelling, emulsifying, thickening, stabilising, binding, coating and suspending agents (7,8,9). These functional rheological properties are used in food applications such as; low calorie drinks, confectionary, dressings, sauces, frozen desserts and dairy products. (Industrial uses include fluid loss control in cement and gypsum based slurries (10), tablet coating and tablet disintegrators in the pharmaceutical industry, also in paper coating, oil well drilling, mining and ore flotation, detergents and in cosmetics applications). The solution properties of one gum can be modified by interaction or association with an unlike hydrocolloid.

This study will investigate the interaction mechanism between water-soluble cellulosic derivatives (cellulose ethers), both anionic and non-ionic, with non-ionic galactomannans. Previous

studies on water-soluble polymer-polymer interactions have often concentrated on gelling systems, two examples include; The interaction of kappa-carrageenan with locust bean (carob) gum (11,12,13), and secondly, the interaction of xanthan gum with polygalactomannans.

Carrageenans are gelling hydrocolloids consisting of sulphated polygalactan chains and are of seaweed extraction. The polymer is built up of alternating 1,3-linked β-D-galactopyranosyl and 1,4-linked α-D-galactopyranosyl units. The 1,3-linked units may occur as the 2- and 4-sulphates or unsubstituted. The 1,4-linked units can be sulphated or exist as an anhydride. The wealth of possibilities for substitution on the basic co-polymer therefore results in many carrageenan types. Various types of carrageenan exhibit different degrees of interaction with unlike polysaccharides. For example one carrageenan type, lambda-carrageenan being void of 3,6-anhydro-D-galactose units, and being highly sulphated, does not give synergistic association with locust bean gum. However kappa-carrageenan interacts strongly with xanthan gum and locust bean gum. It has been suggested that the structure of kappa-carrageenan allows segments of two molecules to form double helixes which bind the chain molecules in a three dimensional gel network. The structure of lambda-carrageenan does not allow such double helix formation. The double helix formation is thought to associate with smooth sections of the

polymannose backbone on locust bean gum.

Guar gum in comparison does not give synergistic gel formation with kappa-carrageenan. Gels can form in kappa-carrageenan/ locust bean gum blends at total polymer concentrations below those required for gelation of either individual component. The rheology and elasticity of the gel network can be altered to meet a particular application by varying the proportion of each component. The more carrageenan in the blend the more brittle the gel appears, whilst more LBG improves the elastic properties of the gel and also increases the breaking strength. Commercial blends of both components are available which make use of the contributing functional properties of each. One mechanism postulated, is that the random coil conformation of the galactomannan converts to a ribbon-like regular structure if it can be stabilised in this form by intermolecular association with the double helix of kappa-carrageenan (14).

Another well cited example is the synergistic interaction between the β-1,4 linked seed polygalactomannans with Xanthomonas polysaccharide, (15,16,17 and 18). Xanthan gum is an exo-polysaccharide extracted from a microbiological culture of Xanthomonas campestris. This water-soluble polymer consists of a cellulose based β-1,4 glucopyranosyl backbone with alternate O-3 substituted charged sidechains of 2 D-mannose residues (one internal and one terminal) and

a D-glucuronic acid residue. There are also acetyl and pyruvate residues on the side chains. The polysaccharide comprises D-glucose, D-mannose, D-glucuronic acid, acetate and pyruvate groups in the approximate ratio 2.8: 3.0: 2.0: 1.7 and 0.6. Xanthan is a non-gelling polysaccharide but forms a rubbery gel in a blend with locust bean gum at total polymer concentrations greater than 0.5%. It also interacts with guar but less strongly than locust bean gum resulting in synergistic viscosity enhancement.

This interaction is less well understood but an understanding of the mechanism is important due to the very high relative costs of xanthan gum (£5-6000 per tonne) compared to galactomannans (£8-1400 per tonne). Earlier investigators (18), proposed that the mechanism was based on interaction between the xanthan helix and unsubstituted regions of the galactomannan backbone. It was then suggested (19), that an association mechanism between the cellulose backbone of the xanthan and the mannan backbone was possible. Most recent studies (16), have suggested that the side chains of the xanthan and the backbone of the galactomannan interact. This was demonstrated by sequentially depyruvating and deacetylating samples of the polysaccharide, increased synergistic interaction was subsequently observed. This interaction has been of considerable interest from a commercial viewpoint and will be referred to throughout the text for comparison

with the mechanism of interaction of the cellulosic polymer blends investigated.

Relatively few studies have investigated the interaction of cellulosic derivatives with galactomannans (21,22). Mechanistic interactions between anionic and non-ionic gums in foods have received little attention although synergistic viscosity enhancement has been reported (23,24). The viscosity of mixed hydrocolloid solutions can be higher or lower than that of individual components at comparable concentrations. The mechanism of interaction between an anionic and non-ionic polymer which results in viscosity enhancement (synergism), and two non-ionic polymers which results in antagonism is discussed throughout this text. The interaction between gums is usually referred to as synergism if the result is an increase in the blend's viscosity and antagonism if the opposite results, ie the resultant viscosity is lower than the expected calculated viscosity.

The mechanism by which synergism exists is discussed at the molecular level and results from intermolecular hydrogen bonding between hydroxyls on the non-ionic polymer and "free" carboxyls on the anionic cellulosic ether. The amount of synergy also depends on the side chain configuration, fine structural differences, degree of substitution, relative degree of hydrophilicity of the polymer, competitive inhibition effects and polymer chain

length. All these factors are considered when investigating the mechanisms involved in the polymer-polymer associations.

Two co-existing mechanisms are proposed which contradict several previous publications on polymer-polymer blends (25). Walker and his co-workers in a recent publication (25), didn't account for variation in the "free" carboxyl content on sodium carboxymethyl cellulose and how this alters the synergistic interaction. These papers assumed hydrogen bonding could exist between a Na^+ COO^- on the anionic polymer with the hydroxyl on the adjacent non-ionic polymer. The mechanism proposed in this thesis indicates that this proposal is unlikely. The result may allow the usage of both hydrocolloids at reduced concentrations to optimise performance in formulated products. Due to functionality and cost considerations, blends of food gums are often used in commercial applications and a thorough understanding of the rheological properties of their blends is essential in maximising a polymer's performance in a certain application.

Three hydrocolloids which are commonly included in food application are; sodium carboxymethyl cellulose, guar gum and hydroxypropylmethyl cellulose. Their rheological properties are discussed briefly below.

Sodium carboxymethyl cellulose, an

anionic cellulose ether, is universally known as CMC and will usually be so designated in this text. CMC is probably the most diversified and important thickening agent in industrial and food applications (26,27). Production was developed into a commercial process during late 1943, with full scale production in 1946. Total worldwide sales of water-soluble cellulosic ethers are estimated to be over 400,000 tonnes per annum and CMC accounts for approximately 70% of this (4). Over 250 grades of CMC are now commercially available worldwide, as a colourless, odourless powder, which give specific rheological properties to various formulations (28).

Cellulose, a natural high polymer, is one of Nature's most abundant raw materials and constitutes the fundamental backbone of CMC. Cellulose in its natural state is insoluble due to strong intermolecular hydrogen bonding. Cellulose is regarded as a polycrystalline material (29). It has been suggested from X-ray data that the cellulose molecules align into crystallites only along a portion of their length, the rest consists of a entangled amorphous region which may fit into crystallites further along their length. The whole system therefore appears as a series of tightly bound crystalline regions and more loosely bound amorphous regions. It is this structural property of cellulose that plays an important role in the derivitisation reactions.

Cellulose is a polydisperse linear polymer of anhydroglucose rings bound through acetal (glycosidic) linkages (4,29). The repeat unit is ß-1,4-D-glucopyranosyl-D-glucopyranose (cellobiose). The anhydroglucose moiety contains three reactive hydroxyl functional groups, one primary at C-6 and two secondary at C-3 and C-2. These reactive functions provide the sites for the formation of cellulose ether derivatives. However the three sites are not equally reactive to substitution (29). C-6 is most accessible to bulky substituents for steric reasons whereas C-2 is most reactive to smaller substituents due to its close proximity with the acidic ring oxygen. In any etherification of cellulose however a mixture of C-2,C-3 and C-6 substituents exist.

CMC is produced by reacting cellulose (various wood pulps or cotton feedstock) with sodium hydroxide, to activate the hydroxyls and to achieve a uniform substitution pattern (4). The amorphous regions of the cellulose are more accessible to derivitisation than the crystalline regions. Various amounts of sodium chloroacetate are added, depending on the degree of substitution required, the slurry mixed is then left to age. The stoichiometry of the reactant mixture, the cellulose feedstock, the aging time and the amount of purification the product receives, gives a specific grade of CMC (7,19).

The extent of the reaction of cellulose

hydroxyls to form a derivative is called the degree of substitution (D.S), and is the average of the three anhydroglucose units which have reacted along the cellulose backbone (30). Commercial products of CMC have D.S values ranging from 0.4 to 1.4 but most common grades lie between 0.7 to 0.9. The D.S has an major influence on the CMC's rheological and solution properties. The higher D.S grades have greater salt tolerance (30). This is a result of the higher D.S grades having higher numbers of anionic sodium carboxylate groups which electrostatically repel each other and open up the CMC chain structure.

Various molecular weights and hence viscosity grades exist for CMC ranging from 10,000 cps at 1% solution to 5 cps at 2% solution. This corresponds to a degree of polymerisation (D.P) of 50 to 5,000 which equates approximately to molecular weights of 10,000 to 1,000,000 (31). CMC is a linear, anionic water-soluble polymer and usually exists as a carboxylic acid salt. The pKa varies with D.S but is commonly around 4.4. At pH values around 8 over 90% of the carboxylic acid groups exist in the sodium form and very few as "free" carboxyl COOH (32). The salt can be converted into the free acid form by dialysing and treating with a strongly acidic ion exchanger (33). However the free acid of CMC is rendered insoluble due to lack of chain-chain repulsion. This modification of the CMC structure has a major influence in the polymers

interaction properties and will be discussed in Chapter VII.

Solution viscosities increase rapidly with CMC concentration. All grades show almost Newtonian behavior at low shear rates. High molecular weight grades tend to be pseudoplastic at higher shear rates, whilst low degree of substitution grades tend to be more thixotropic (35,36).

Many non-ionic cellulosic ethers are commercially manufactured worldwide, but this thesis will be primarily concerned with the interactive and rheological properties of methyl cellulose (MC) and hydroxypropylmethyl cellulose (HPMC), as these are produced commercially by Courtaulds Fine Chemicals at their Spondon site near Derby (37). (Other non-ionic cellulosic polymers like ethyl hydroxyethyl cellulose and hydroxyethyl methyl cellulose will be discussed briefly for comparative rheological and interactive properties of polymer blends with CMC, and related to the performance of MC and HPMC). These non-ionic cellulosic polymers have useful thickening, reversible thermogelation, adhesive and film-forming characteristics which are applied in food formulations (38).

The non-ionic cellulose ethers are manufactured by Courtaulds Fine Chemicals by reacting a highly purified cellulose feedstock, which has been immersed in a caustic bath, then shredded, and is then

blown up into a reactor where it is treated with methyl chloride and/or propylene oxide in a pressurised vapour phase system. The cellulose ether then is passed to a centrifuge, then cooled in a gel precipitation stage, it is then dried, powdered and sieved to various particle sizes. The relative amount of each substituent on the polymer, is controlled by the stoichiometry of the reactant materials (4).

Both HPMC and MC form a thermally reversible gel network when heated and in the food industry it is this unique rheological property which is most commonly utilised. The gel point temperature is dependant on substituent levels (39). When heated to its gel point which usually lies in the temperature range 50-85°C, the kinetic energy of the water molecules increase and become less associated with the polymer structure, the hydrophobic methyl groups interact more strongly and closely associate to form a three dimensional gel network. By varying the amounts of methyl or hydroxypropyl substituents the gel point can be altered to fit a particular application. High methyl content HPMC tends to form a strong elastic gel with corresponding low gel point, whereas high hydroxypropyl, low methyl content HPMC tends to give a lower gel strength with higher gel point temperature (40). Due to the higher raw materials cost of the cellulose feedstock (a higher α-cellulose content), the high processing costs, and the inefficient gas phase

reaction, HPMC costs up to £4500 per tonne whereas food grade CMC sells for approximately £1400 per tonne. It is obvious therefore if CMC can be blended into HPMC with little reduction in gel strength performance, a considerable cost advantage is potentially attainable. Also by varying the proportion of CMC in a blend, the gelation characteristics of HPMC may be controlled.

As in the case of CMC, many grades of non-ionic HPMC and MC's are commercially available with a wide range of applications. Commercial HPMC samples have a D.S of between 1.5 and 2.0 and the hydroxypropyl M.S ranges from 0.1 to 1.0. Molar substitution can occur in HPMC resulting in formation of polymeric side chain branching within a molecule. Commercial products have a D.P in the range of 50 to 2000 corresponding to molecular weights of about 10,000 and 250,000 respectively (37). Grades are available with viscosities varing between 5 cps and 160,000 cps for a 2% solution. The molecular weight required in the end product, determines the source of the cellulose feedstock. For high viscosities/molecular weight, cotton-based cellulose is used, in other cases various wood-based cellulose grades are used.

D-Galacto-D-mannans are reserve carbohydrates found in the seeds of leguminous plants species in the Mediterranean and in parts of India and Pakistan. Two commercially important galactomannans are guar gum (Cyamopsis tetragonolbus) and locust bean gum

(Ceratonia siliqua) (4,41). Both are natural water-soluble gums originating in the Leguminoseae as seed mucilage and are based on a β-D-1,4 linked backbone of β-D mannopyranosyl residues having side branches linked α-D-1,6 and consisting of single α-D galactopyranosyl residues. On seed germination sugars are released by enzymatic (α-D galactosidase, β-D mannanases and β-D mannosidases) degradation of the galactomannan polymeric chain.

The polysaccharides listed above are both edible and have long been classified as a "generally regarded as safe" (GRAS), food additives by the American Food and Drug Administation and other regulatory committees around the world. It is estimated that 140 thousand tonnes of guar gum, with 20% of this value being used for food production and 7 thousand tonnes of locust bean gum are currently consumed worldwide. They are used extensively in the food industry as thickening agents and for their hydrophilic water binding properties (4). They are compatible with many other polysaccharides and solutes and have very useful rheological characteristics.

The production of both guar and locust bean gum is a series of crushing, sifting and grinding stages to separate the seed from the pod and then the valuable polysaccharide from the seed. The endosperm makes up approximately 42-46% of the total seed weight. Food grade guar is substantially but not purely endosperm material. This does not alter its suitability

as a food additive as the whole seed is edible. The proteinaceous component of guar gum (2.5-4.5%), although a contaminant, may have some structural significance in the rheology of the gum (42).

Guar gum is a cold water swelling polymer and is a natural alternating co-polymer. On average guar galactomannan has 64% of its D-mannosyl residues substituted with D-galactose, locust bean gum in contrast has 30% of the D-mannosyl residues substituted. Two examples were discussed above where LBG blends with kappa-carrageenan and xanthan gums gave stronger synergistic association than guar gum. It has been widely suggested that the fine structural differences of the two galactomannans is principally responsible for this phenomenon(43).

Locust bean gum has limited solubility when added to cold water at ambient temperature (44), and only fully hydrates if the solution is heated to 80°C. Recently published studies (45) on the fine structure of guar and locust bean gum indicate that the distribution of galactose residues on the polymannose backbone, suggests an irregular substitution pattern. However results from earlier studies indicated that the side chains of guar were alternatively disposed along the D-mannan backbone whereas those of locust bean gum are disposed in a block manner along the backbone (46).

The fine structural distribution of these D-galactose residues has received considerable

attention in recent years and makes an important contribution to the mechanisms of polysaccharide interaction discussed in this thesis. Galactomannans interact with many polysaccharides resulting in viscosity enhancement or gel formation (47), however guar gum shows limited synergistic interaction in association with polysaccharides like xanthan gum resulting only in viscosity enhancement. In contrast locust bean gum interacts strongly with xanthan resulting in a three dimensional gel-complex formation (48). The differences in synergistic interaction have been attributed to the differences in galactose content and the variation in fine structure of the galactomannans galactose substitution pattern along the polymannose backbone. The differences in the interaction properties of the two galactomannans will be discussed throughout the text.

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CHAPTER VI

EXPERIMENTAL METHODS

CHAPTER VI. EXPERIMENTAL METHODS

1. GENERAL METHODS.

Weighings. All accurate weighings were made within the range of the graticule scale (range 0-100mg) of a Stanton Unimatic Model C.L.1, single-pan balance, having an accuracy ± 0.1mg.

Moisture contents were determined by heating to constant weight at 105°C.

Brookfield viscometry determinations were carried out generally on 1% concentration polymer solutions using a RVF model Brookfield viscometer using various spindles at a shear rate of 20 r.p.m. at 25°C. The mean calculated viscosity (η_i) of the blends were calculated at a given shear rate using partial fractions.

ie $$\log \eta i = X_A \log \eta_i^A + X_B \log \eta_i^B \qquad (2)$$

Where η_i^A = experimental (apparent) viscosity of a 1% solution of component A at shear rate i; η_i^B = experimental viscosity of a 1% solution of component B at shear rate i; and X_A and X_B are the weight fractions of A and B respectively.

Viscosity enhacements were calculated by;

$$\% \text{ Visc enhan} = \left\{ \left(\frac{\text{Measured Viscosity}}{\text{Calculated viscosity}} \right) - 100 \right\} \times 100$$

Polymers were generally hydrated with mechanical shaker for 24 hours prior to viscosity measurement at 20 r.p.m on Brookfield viscometer (approx $5s^{-1}$ shear rate).

Reduced viscometry measurements were determined using Ostwald viscometer tubes grades E or G at 25°C. Flow times were measured to within 0.1 second with a digital stop watch and the viscosity calculated as (1);

$$[\eta] = 1/c\ (T/T^{\circ}-1)$$

T° = Flow time of solvent.

T = Flow time of polymer.

c = Dry weight concentration of polymer.

Fann viscometry was determined for higher shear rates between 3 r.p.m and 600 r.p.m on a 35SA model Fann viscometer at 25°C.

Acid washing. The structure of sodium carboxymethyl cellulose was modified by increasing or decreasing its free carboxyl content. Powdered solid CMC was added to a 70% ethanol, 30% distilled water slurry and stirred. Small volumes of concentrated hydrochloric acid was introduced to the flask and the pH of the slurry monitored. The pH of the wash liquor was reduced from 8 to 4 and the slurry maintained at this pH by addition of acid for six hours. The solid was then washed with ethanol then acetone then dried at 50°C. A similar procedure was used to remove all the free carboxyls in CMC and replace them with sodium ions. In this case small volumes of sodium hydroxide were added to the slurry and the pH maintained at around 12.2 until equilibrium was reached. The solid was then washed and dried in a similar manner (4).

pH Readings were determined using a Philips PW 9420 model pH meter was used at 25°C. The instrument

was calibrated daily using Fisons 4.0 and 7.0 buffer solutions.

2. ANALYTICAL METHODS.

Computerised Molecular Models were built using the Discover version 2.7.0 package (5). Glucose monomers were initially constructed then modified by derivitisation, this was β- 1,4 bonded to an identical unit and the structure's potential energy conformation minimised overnight. This was then polymerised to 4 then 8 then 16 sugar units. The final summation in the minimised molecules structure represents the total non-bonded interactions by a sum of repulsion, attractive dispersion forces and Coulomb like electrostatics as a function of the distance between atom pairs. The minimised structure represents a point of minimum free energy which the molecule might adopt in a vacuum with a dielectic constant equivalent to that of water (3).

Gel Permeation Chromatogram samples were prepared using double concentrated 0.2M sodium nitrate buffer solution and adding 50/50% to a 0.2% polymer solution. The samples were filtered using a Millex-HV 0.4µm filter unit attached between a 2ml syringe and 1.5 inch needle. The 0.1M sodium nitrate buffer solution was filtered daily and 1lb/in^2 of helium gas bubbled through it continually. The injection volume of 20µl was controlled by an injection loop (6).

The GPC apparatus consisted of a Waters 410 Differential Refractometer, a Waters 510 HPLC pump and a Waters 740 Data module. Two columns which were run in series were a Waters 1000 and a Waters linear ultrahydrogel column (linear first) at a flow rate of 0.5ml/min. The maximum molecular weight exclusion limits of the linear and 1000 column were 2 X 10^8 and 1 X 10^8 respectively (7).

Carbon 13 NMR. Guar gum samples were dissolved to 2.5% by weight in 3mls of deuterowater. After hydration for 3 hours, 60µl of a 100 times diluted solution of Novo mannase was added and shaken on a whirlometer. The samples were left overnight and the resultant partially depolymerised liquid poured into 10mm NMR tubes. Carbon spectra were acquired at 80°C using a 45° pulse flip angle and a 2.5 sec total relaxation time. The data were transferred to an offline workstation in order to carry out curve fitting and integration procedures. The NMR spectrometer used was a Brüker AC 300 at a frequency of 75 MHz (8).

Solid State Carbon 13 NMR. Solution samples were freeze dried, to maintain their integral solution molecular comformation in the solid state and analysed. The solid samples were compacted in solid state rotors and the standard magic angle spinning/cross polarisation technique was used to acquire solid state carbon spectra. A spinning speed of 4 KHz and a cross polarisation contact time of 1ms were used to aquire data. Proton Tle measurements were also carried out by

indirect observation of the variation of carbon intensities with cross polarisation delay. The spectometer used was also a Brüker AC 300 model run at 75 MHz (9).

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ABBREVIATIONS AND TERMINOLOGY.

CMC: Sodium carboxymethyl cellulose.

Acid washed CMC : Modified carboxymethyl cellulose with a high free carboxyl content.

Alkali washed CMC : Modified CMC with a zero free carboxyl content.

Courlose CMC : CMC Brookfield viscosity measured at 1% polymer concentration.

Celacol HPMC : HPMC Brookfield viscosity measured at 2% polymer concentration.

EHEC E411X : Bermacoll grade Ethyl hydroxy ethyl cellulose.

HEMC : Tylose grade Hydroxyethyl methyl cellulse.
2% viscosity = 15,000cps

Her 7M CMC : Hercules CMC, medium viscosity grade of low degree of substitution (0.6-0.8).

Her 7H CMC : Hercules CMC, high viscosity grade of low D.S.

Her 9M CMC : Hercules CMC, medium viscosity grade of medium degree of substitution (0.8-1.0).

Her 12M CMC : Hercules CMC, medium viscosity grade of high degree of substitution (1.1-1.4).

Hercules CMC grades

M - medium viscosity 2% Brookfield = 2700cps.
H - high viscosity 1% Brookfield = 1600cps

TH 100 HC Guar :

PUR 548 HF Guar : Commercial samples of guar gum.

PUR 547 MC Guar : F and C denote fine and coarse

Cerasol HF Guar : particle size respectively.

INTER 401 MC Guar :

PRE 401 MF Guar :

BDH Guar :

M : Medium viscosity grade (1% = 3000-3500cps).

H : High viscosity grade (1% = 4200-4800 cps).

NOTES

All samples were corrected for ash and moisture contents prior to weighing. Guar gum samples were washed with IMS/water (70/30%) to remove any salt present. Locust bean gum samples were hydrated in water at 80°C then cooled to 25°C prior to use. All CMC and HPMC grades used are EEC approved food additives and are 99.5% pure.

CHAPTER VII

RESULTS AND DISCUSSION

CHAPTER VII. RESULTS AND DISCUSSION

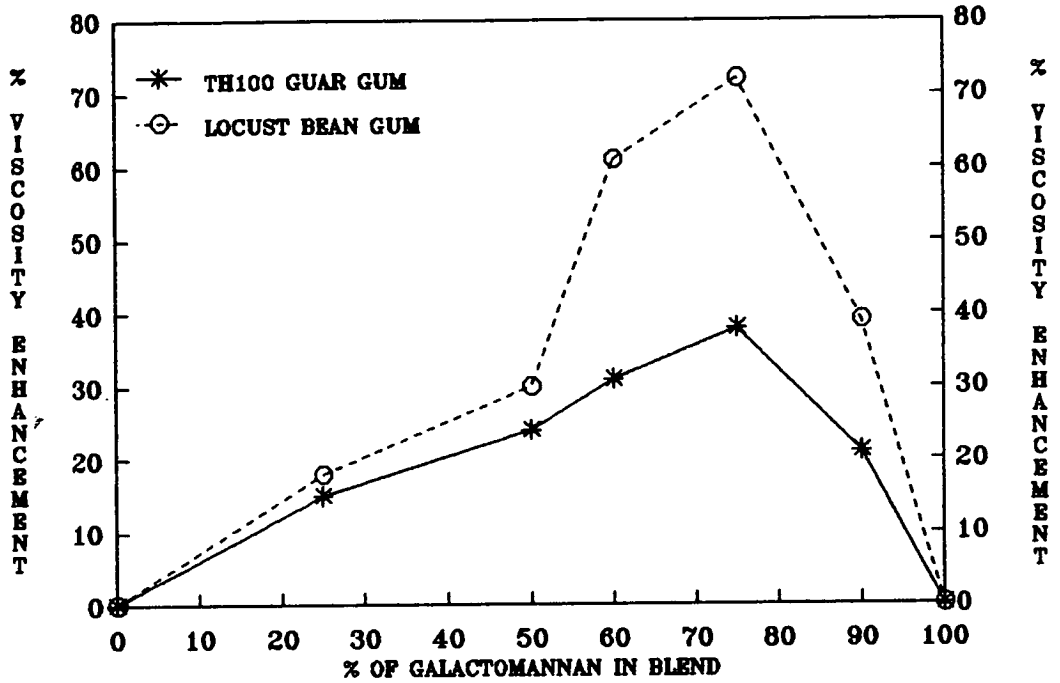
VII (i) INTRODUCTION TO SYNERGISTIC AND ANTAGONISTIC POLYMER BLENDS.

Initial research commenced by blending non-ionic galactomannan solutions with anionic CMC at various mixing ratios (1% total polymer concentration). The results are displayed in graph VII.1. A synergistic viscosity enhancement was observed which maximised for both galactomannans at the mixing ratio of approximately 75% galactomannan, 25% CMC. Resultant viscosities are higher than calculated viscosity at all blending ratios. A greater viscosity enhancement was achieved with a locust bean gum/CMC blend than with a guar/CMC blend at all mixing ratios (1).

Attempts to explain the marked differences in interaction properties of guar and locust bean gum with various polysaccharides have involved detailed structural elucidation of the galactomannan's fine structure by various studies including those of Dea and McCleary and their co-workers (2). One structural difference between the two galactomannans is that guar contains almost twice the number of galactose residues that locust bean gum contains. Secondly locust bean gum contains larger sections of "smooth" unsubstituted polymannose sections on the polymer backbone (3).

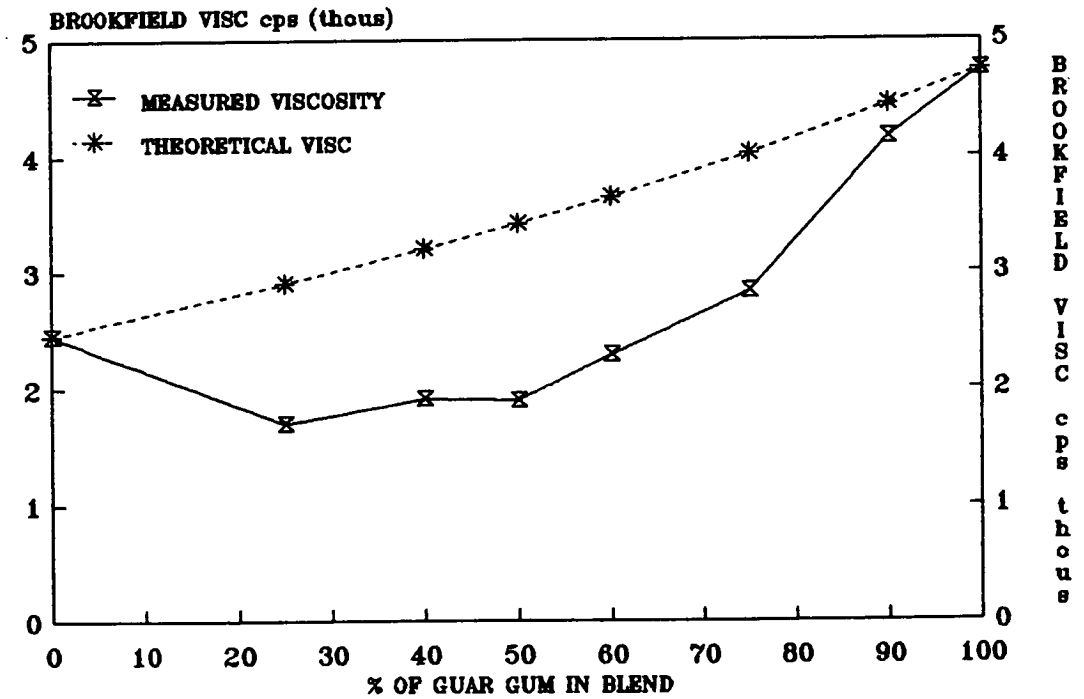
The 1% Brookfield viscosities of both galactomannans lie in a similar viscosity range

GALACTOMANNAN/CMC P1500P BLEND.
SYNERGISTIC POLYMER INTERACTION
1% TOTAL POLYMER CONCENTRATION.



GRAPH 7.1

GUAR GUM/HPMC 15,000P
ANTAGONISTIC POLYMER INTERACTION
1% TOTAL POLYMER CONCENTRATION



GRAPH 7.2

(3500-5000 cps) and the differences in interaction cannot solely be accounted for by differences in molecular weight. Literature studies (4) on the molecular weight of guar gum have resulted in a wide range of calculated values, depending on the method of analysis i.e. viscosity determination, gel chromatography or light scattering experiments (5). Studies have predicted values ranging from 532,000 to 2,360,000 for weight-average molecular weights (6). Molecular weight determinations(Mw) for locust bean gum have predicted values of around 1,350,000 for weight average molecular weight (7,8).

Early investigations into structural elucidation employing X-ray diffraction (9),chemical degradation (10) and enzyme techniques (11), indicated that the galactose distribution was uniform in guar and in contrast "blocky" in locust bean gum. More recent results however, applying highly purified enzyme biotechnology (12), periodate oxidation (13,14) and N.M.R. spectroscopy (15), suggested an irregular galactose substitution pattern in both gums. Evidence has shown that water-soluble polysaccharides can interact with galactomannans at regions in the D-mannan backbone which are unsubstituted or lightly substituted with galactose (16,17,18 and 19).

Research has indicated (20,21), that if the mannan backbone is substituted on only one side with galactose residues, gums like xanthan can interact with the smooth side. One study of a range of

galactomannans of varying galactose content (22), has shown that of the galactomannans with more than 40% of D-galactose, the ones with a high frequency of exactly alternating regions in the β-mannan backbone are most interactive. However of galactomannans with less than 30% galactose content, those that contain a high frequency of unsubstituted blocks of intermediate length in the β-mannan chain are the most interactive.

Another study using highly purified α-galactosidase enzyme (23), to remove varying amounts of galactose substituents, but maintain the original polymannose backbone has shown that as the galactose is removed the interactive properties with xanthan gum increase. For this method of enzyme biotechnology to be unambigious, it is necessary to employ highly purified, well characterised enzymes (24,25). The degradative products were systematically isolated and quantitatively analysed. The enzyme selected preferentially removed D-galactosyl groups separated by one D-mannosyl residue. There was no evidence of complete removal of galactosyl residues in a zipper-like manner leaving large sections of completely unsubstituted polymannose sections. Results indicate that an initial sample of guar increases its interactive and gelation properties until it reaches a galactose content similar to that of locust bean gum.

When two non-ionic polymers were dry blended together a different result is obtained (26). The two polymers were guar gum and the non-ionic

cellulose ether, HPMC 15,000P. Graph VII.2 shows antagonistic resultant viscosity at all mixing ratios. At a mixing ratio of 50/50%, a lower resultant blend viscosity is observed than for either individual guar gum or Celacol HPMC 15,000P component viscosities.

Although antagonistic blends have been observed in several systems (27), these have predominately been reported for a binary polymer blend of two anionic polysaccharides blended together, for example in the case of the CMC/Sodium alginate system (28). In this antagonistic blend although intermolecular interactive forces may still exist between unlike polymer chains the stronger electrostatic repulsion forces predominate.

In the case of a non-ionic polymer blend however there are no electrostatic repulsion forces present. Thus the antagonistic viscosity effect must be explained by a different mechanism. This mechanism will be discussed fully at a later stage in the text. It is proposed in this text that the observed antagonism results from differences in the molecular weight and the relative hydrophilic/lyophilic balance of the two non-ionic polymers being blended. This mechanism may also contribute to a proportion of the synergistic viscosity enhancement observed in certain polymer blends (e.g. graph VII.1).

Although synergistic molecular association was observed in graph VII.1, this may have resulted from several possible different effects e.g

competitive dehydration, specific intermolecular association or simply molecular entanglement (non specific physical entanglement). The third explanation may be eliminated immediately (29), because if this was the case and physical molecular entanglement of the polymers was the cause, synergy would be observed for all polymer-polymer blends. Also more synergy would be observed for a rapidly agitated blend of two polymer solutions than for a unstirred pre-mixed dry blend. For the CMC/guar blend identical resultant viscosities were achieved in each case within limits of experimental error.

It is convenient at this stage in the discussion to introduce the concept of molecular overlap in polymer solutions. The polymers being studied are long chain cellulosic derivatives and galactomannans which are relatively inflexible due to rigid β-1,4 bonds linking the backbone together and the steric hinderance created by bulky sugar residues. Thus resistance to flow and high intrinsic viscosities are not surprising. Dilute solution measurements of a polymer can yield intrinsic viscosity (η), which is a direct indication of the hydrodynamic volume of an isolated polymer chain. This fundamental parameter is related to the molecular weight M, of a polymer, by equation [VII.1], the Mark-Houwink Sakurada (MHS) relationship (30).

$$[\eta] = K M^{\alpha} \qquad [VII.1]$$

The parameters K and α are characteristic of a polymer under specific solvent conditions and temperature.

Competitive dehydration is however another possible mechanism to explain the observed viscosity enhancement in graph VII.I. All water-soluble polymers have varying degrees of hydrophilicity depending on their side chain configuration and the level of substitution (28,31,32 and 33). Alkyl groups tend to make a cellulosic ether more hydrophobic whilst hydroxyalkyl groups tend to heighten hydrophilic characteristics. When co-dissolved with another polymer or a solute there is competition for available water molecules for complete polymer hydration. Some polymers can tolerate less water being associated with its structure (due to hydrophobic alkyl moieties associating with each other in solution), than other more hydrophilic polymers. A rearrangement of water molecules may occur when two polymer solutions are blended, or two polymers hydrate together in competition, until equilibrium is achieved. The larger the differences in hydrophilicities of the two polymers in the system the larger the contribution from competitive dehydration may be.

VII (ii) HYDRODYNAMIC VOLUME STUDY ON POLYMER BLENDS.

Intermolecular molecular association is another possible explanation for the observed synergistic interaction in graph VII.1 (34). At present synergy has been observed at 1% total polymer concentrations but it was not known if it existed in a very dilute system. The concentration C* is the polymer solution concentration where there is a transition from dilute to concentrated behavior (17). At this point (35,36), plots of viscosity against concentration results in a large deviation in the gradient of the slope i.e a transition point. This sharp viscosity increase arises as a result of individual molecules coming into contact with one another, and is accompanied by a large change in flow behavior of the polymer.

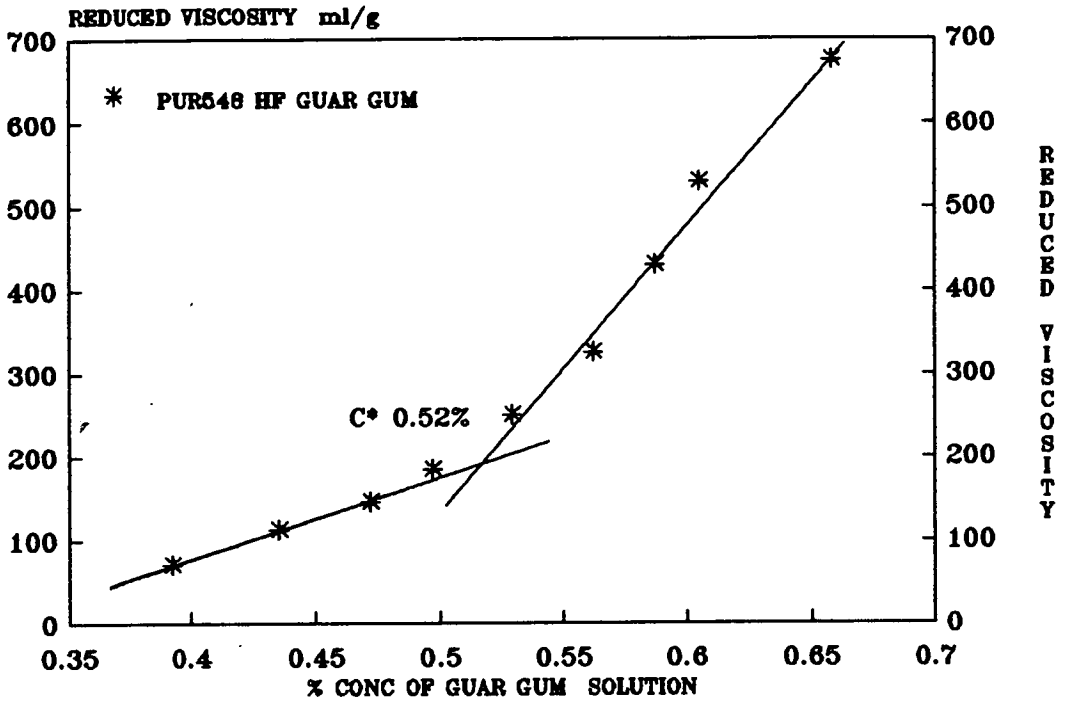
The concentration (C*) at which this occurs is inversely related to the hydrodynamic volume occupied by isolated polymer molecules. At the polymer concentration where $C < C^*$, the polymer chains are essentially separated from each other, intramolecular volume exclusion effects dominate and the molecules occupy volumes proportional to R^3 (radius of gyration). At the concentration C*, the solution is filled with hydrated chains, with no intervening regions of pure solvent (31,37), although interstitial solvent volume may still exist. Above the concentration C* the

hydrodynamic volume of all the polymer chains added together exceeds the volume of the solution (36).

Water-soluble polymers often have high solution viscosities at relatively low polymer concentrations. The two principal factors which determine the viscosity of a polymer are the molecular weight and the chain stiffness (37). Commercial polysaccharides have comparable average molecular weights to synthetic polymers, but are in general less flexible (32), and adopt a more extended coil geometry than synthetic random coil polymers. Due to the inherent stiffness of polysaccharides as a result of glycosidic bond linkages, formation of a highly entangled network structure occurs at much lower concentrations than for synthetic polymers (38).

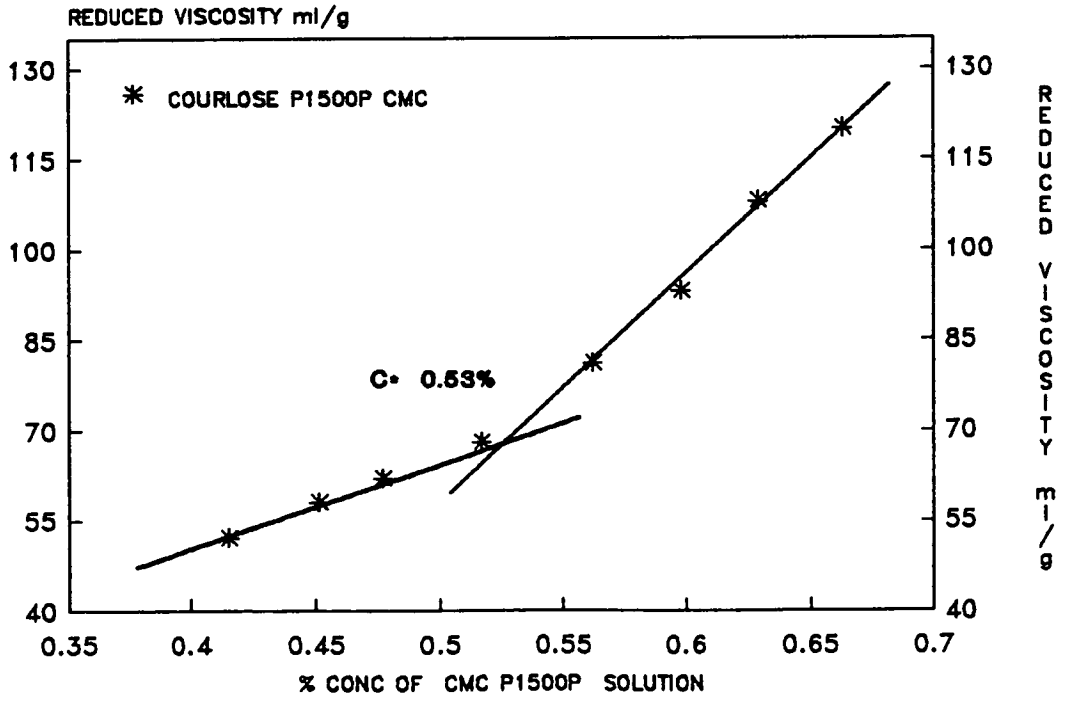
If specific intermolecular association between unlike water-soluble polymers occurs in solution, a viscosity enhancement results. It follows then, that there should be an increase in the overall hydrodynamic volume of an associated molecule in a polymer blend, thus the C* value of a polymer blend will be lower than either individual component. Graph VII.3 shows the Ostwald viscosity data curve to calculate the C* value for guar. The graph indicates the transition point (C*) to occur at approximately 0.52% polymer concentration. A similar set of data was calculated for a Courlose P1500P CMC and a C* value of 0.53% was obtained as shown in graph VII.4. If no intermolecular association occurred in a blend of the

OSTWALD VISCOMETRY DATA FOR GUAR GUM.
VISCOSITY AND HYDRODYNAMIC VOLUME
RELATIONSHIP TO EVALUATE C*



GRAPH 7.3

OSTWALD VISCOMETRY DATA FOR CMC P1500P
VISCOSITY AND HYDRODYNAMIC VOLUME
RELATIONSHIP TO EVALUATE C*



GRAPH 7.4

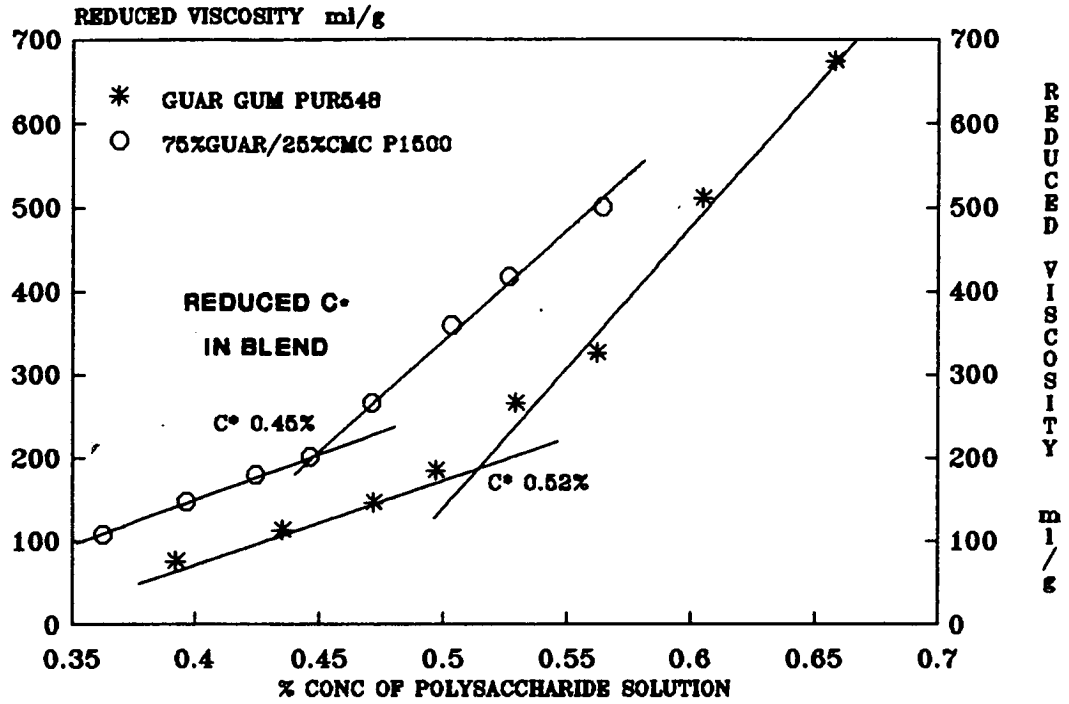
two polymers, the C* for the blend would have a similar value to the component C* values.

However graph VII.5 indicates that this is clearly not the case. Two interesting conclusions can be drawn from graph VII.5. Firstly there is a displacement of the polymer blends C* value to the lower figure of 0.45% polymer concentration. Secondly by comparing the blend viscosity/concentration data with that of the guar gum values, it is obvious that the blend has a higher viscosity than guar (or the CMC) above C* as was previously shown (graph VII.1), but more interestingly the synergy still exists below C*.

Synergistic interactions of two unlike polysaccharides in solution until recently (18) have been ascribed generally to competition for available solvent molecules by unlike polymer chains, and not by specific intermolecular association. The above results indicate that the molecules in a CMC/guar blend still associate even in dilute conditions i.e when sufficient solvent is present to ensure complete solvation of both polymers.

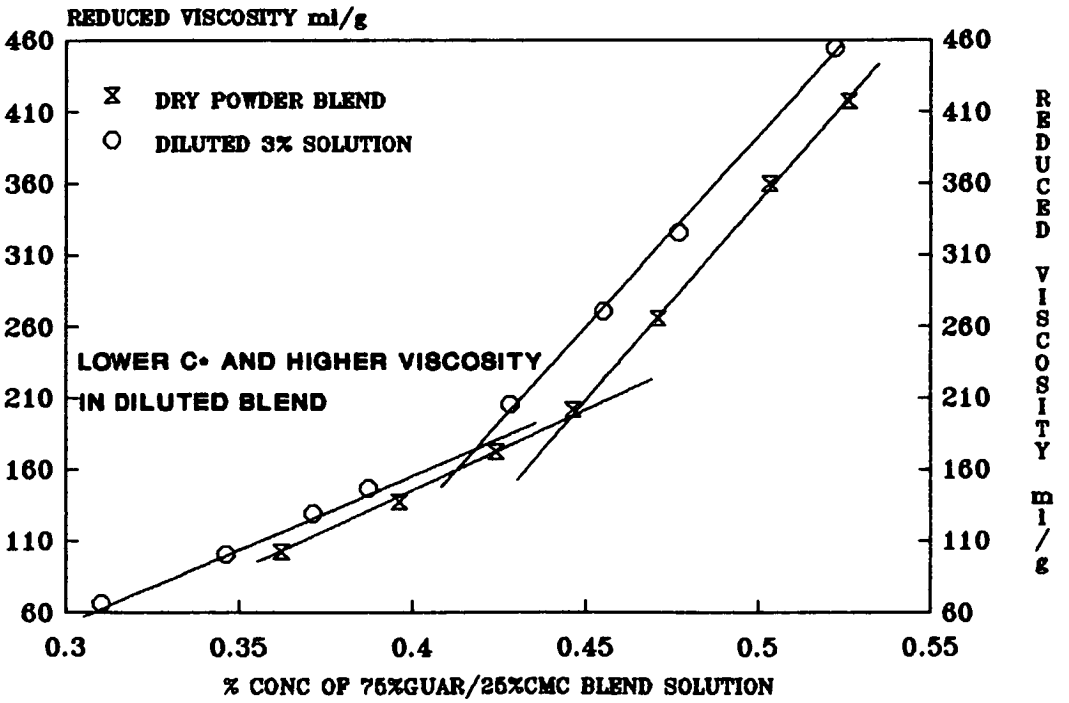
An interesting phenomenon occurs in graph VII.6 if the Ostwald viscometry of a diluted 3% blend is compared to a dry powder blend, the C* value of the 3% diluted blend is reduced, and the reduced viscosity of the diluted blend is higher at all polymer concentrations. This suggests that there is less available water for polymer solvation at 3% polymer concentration, with a consequent promotion of polymer

OSTWALD VISCOMETRY DATA FOR A COUGAR/CMC BLEND.VISCOSITY AND HYDRODYNAMIC VOLUME RELATIONSHIP TO EVALUATE C*



GRAPH 7.5

OSTWALD VISCOMETRY DATA FOR TWO BLENDS 75% GUAR GUM/25% CMC COURLOSE P1500P TO DETERMINE EFFECT OF DILUTION ON C*



GRAPH 7.6

association. It follows that the probability of polymer-polymer association is greater at 3% polymer concentration and the interchain junctions remain intact even when the polymer is diluted. This is further evidence that molecular association between unlike polymer chains is responsible for the viscosity enhancement observed in graph VII.1 for a guar/CMC polymer blend. This is a similar effect to a freeze-thaw cycle (17) where on freezing a mixed polysaccharide solution, ice formation raises the effective polymer concentration again promoting interchain molecular interaction.

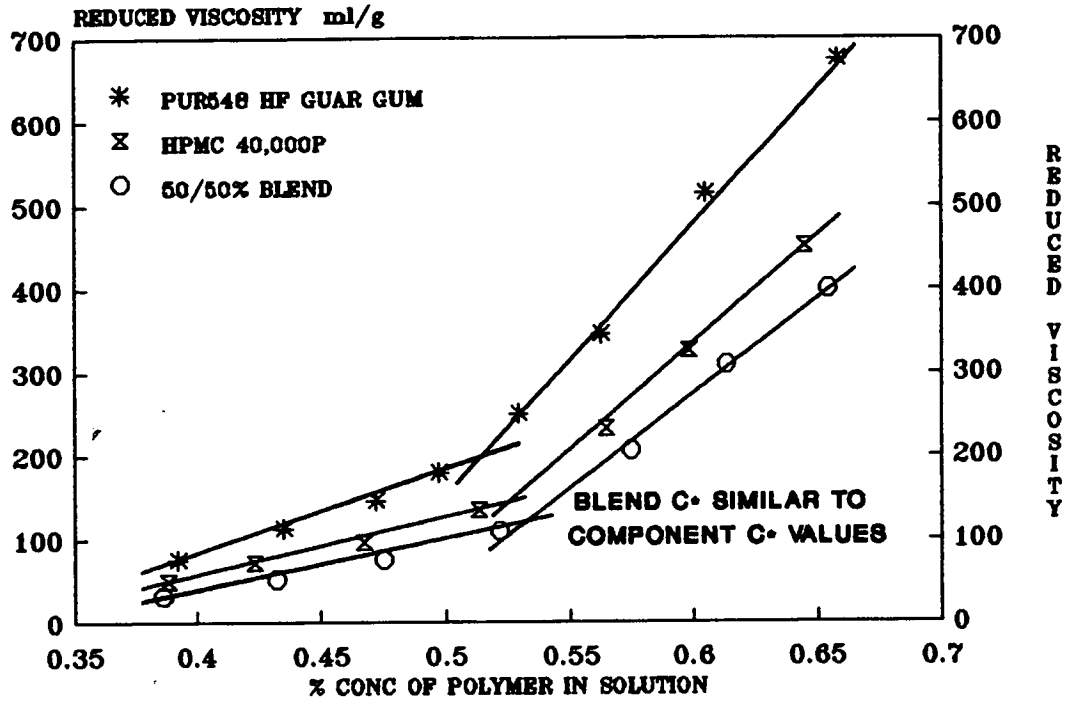
The C*, and Ostwald viscometry results obtained above (graph VII.5) were the first to indicate that the mechanism of synergistic interaction between the unlike polysaccharides being studied could be explained by a molecular association mechanism. Previous studies (28,38) of the interaction of CMC and methyl cellulose in a 0.1M NaCl solution suggested that the enhanced viscosities observed could be explained in terms of the coil expansion of the polyelectrolyte. It stated that an explanation to explain the interaction involving hydrogen bonding by molecular association was unnecessary. However these suggestions do not explain the interaction when no electrolyte is present and the above results indicate that molecular association is indeed occurring.

In a blend of two non-ionic gums it has been shown (graph VII.2) that antagonism occurs. The

Ostwald viscometry data to calculate C* values for guar gum, HPMC 40,000P and a 50/50% blend of the two are shown in graph VII.7. Unlike in the synergistic enhancement guar/CMC polymer blend, there is no displacement of C* when compared to the component C* values of guar and HPMC. The two component polymers have C* values of approximately 0.52%, and the blend C* is approximately 0.53%. It was observed however that the blend viscosity is lower than component viscosities above and below C*. It would appear that there is no specific intermolecular interaction between these two non-ionic polymer chains or synergy would result. There are no electrostatic repulsive forces between unlike chains to explain the antagonism as both polymers are non-ionic. A plausible explanation at present is that antagonism may be caused as a result of competitive dehydration due to differences in the relative hydrophilic/lyophilic balance of the two polymers.

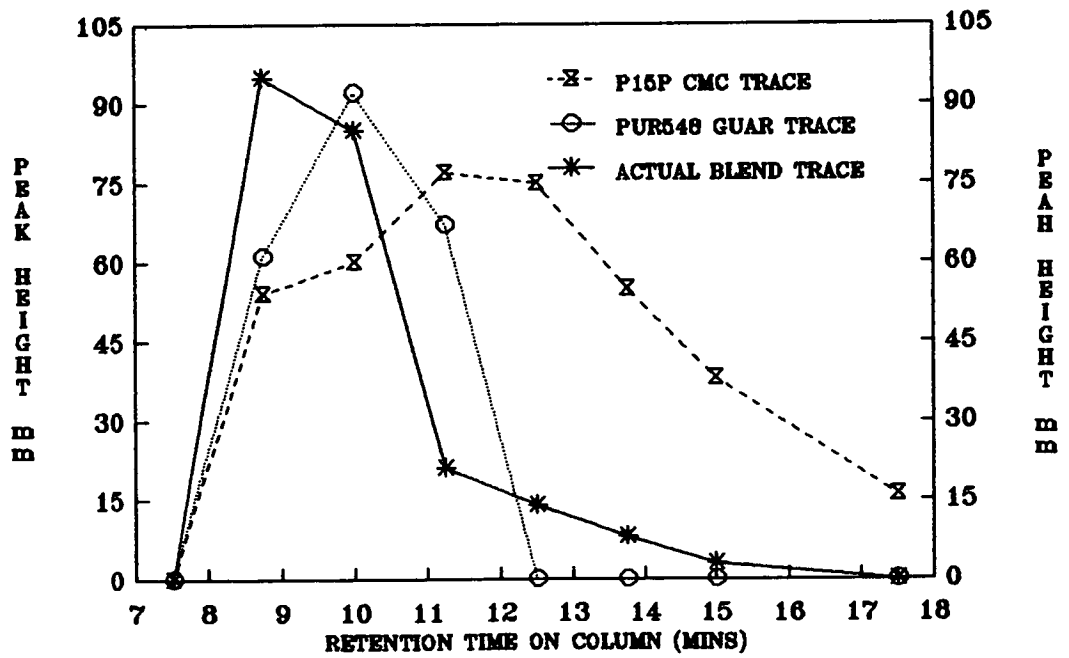
Gel Permeation Chromatography (GPC) or Size Exclusion Chromatography has been widely used for determining the molecular weight distributions, degree of polymerisation, and average molecular weights of polysaccharides due to its short analysis time and high efficiency (39,40 and 41). GPC is a form of liquid-partition chromatography based on the unique properties of the column packing material, for separating polysaccharides on the basis of molecular size (42). The technique is based on the principle that large molecular weight molecules are excluded from

OSTWALD VISCOMETRY DATA FOR GUAR/HPMC BLEND. VISCOSITY AND HYDRODYNAMIC VOLUME RELATIONSHIP TO EVALUATE C*



GRAPH 7.7

GEL PERMEATION CHROMATOGRAM OF A 75% GUAR/25%CMC BLEND LINEAR+ 1000 HYDROGEL COLUMN



GRAPH 7.8
0.2% TOTAL POLYMER CONCENTRATION

passing through the internal pore network of the gel system and are retained by the column for a shorter time than smaller molecules.

Samples were run on a Gel Permeation Chromatogram to reinforce the suggestion that in the guar/CMC blend, molecular association is occurring between unlike polymer chains resulting in an overall increase in hydrodynamic volume. If association is occurring between unlike polymer molecules some material should be retained on the column for a shorter time than either of the individual polymer component in the blend (43). Several previous studies have investigated intermolecular association in unlike water-soluble blends successfully using similar GPC techniques (44). Reproducable traces of a guar, a Courlose P15P CMC, and a 75% guar/25% CMC blend of the two are displayed in graph VII.8. Since higher molecular weight fractions in GPC are retained for a shorter time on the columns than smaller fractions, a shorter retention time indicates a larger hydrodynamic volume of a polymer species.

Graph VII.8 suggests that the polymer blend molecular weight profile is not a simple admixture of the two component polymers. Fractions of guar and CMC appear to interact by molecular association resulting in some very high molecular weight material not present in either component polymer, and correspondingly less lower molecular weight material being eluted. This is further evidence

of molecular association in a guar/CMC polymer-polymer blend. If no molecular association was occurring, the blend trace would have appeared as a combination of the two component traces. The blend was passed down the column at various mixing ratios and evidence of molecular association was indicated in each sample.

VII (iii) EFFECT OF MODIFYING SODIUM CARBOXYMETHYL CELLULOSE STRUCTURE BY VARYING THE FREE CARBOXYL CONTENT.

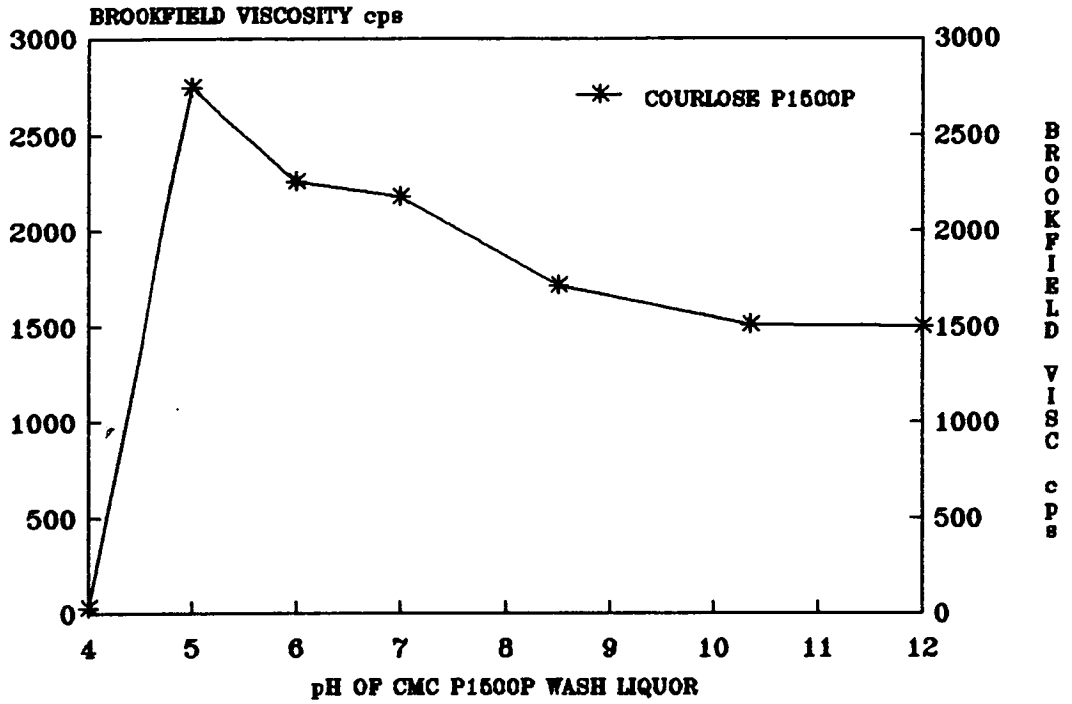
At present it has been shown that a blend of anionic CMC solution and galactomannans interact, which results in synergistic viscosity enhancement. However at present there is no evidence of a mechanism to support the suggestion that molecular association is occuring. Sodium carboxymethyl cellulose is the sodium salt of a weak carboxylic acid. A dilute solution of CMC with D.S 0.7 at pH 8.3 is at its equivalence point. At pH 7.0 approximately 90% of the carboxylic functional groups on CMC exist in the salt form (Na^+ COO^-), whereas at a pH of 5 approximately 90% of the CMC carboxyl groups (45), exist in the free acid form (COOH). Below this pH, CMC is rendered insoluble as the ionisation of the polymer is repressed. As the pH increases above pH 6, the repulsive forces of the anionic carboxylate groups uncoil the polymer chain. Increasing the pH of CMC uncoils the polymer chain due

to repulsion of sodium salt carboxylic groups, therefore it is unlikely that these groups could interact with uncharged hydroxyls on an unlike non-ionic polymer chain resulting in synergy.

The effect of increasing the free carboxyl content of sodium CMC on the synergistic interaction with non-ionic polymers was investigated. Graph VII.9 demonstrates the effect of adding small volumes of concentrated hydrochloric acid to a solid CMC sample (46), in a slurry with 500mls alcohol/water (70% alcohol) and allowed to equilibriate at a constant pH for 3 hours. To supress any esterification side reactions between hydroxyl groups and free carboxyl groups, the slurry is maintained at 5°C throughout. The modified CMC is then repeatedly acetone washed to remove sodium chloride formed in the process. The free carboxylic acid content is increased up to pH 5, below this value the CMC is rendered insoluble (47). The viscosity of the CMC reflects this change in free carboxyl content and is almost twice its original Brookfield viscosity at pH 5. The acid washed CMC was later prepared more precisely with narrow pH differences of the wash liquor (pH 5.2 to 5.9).

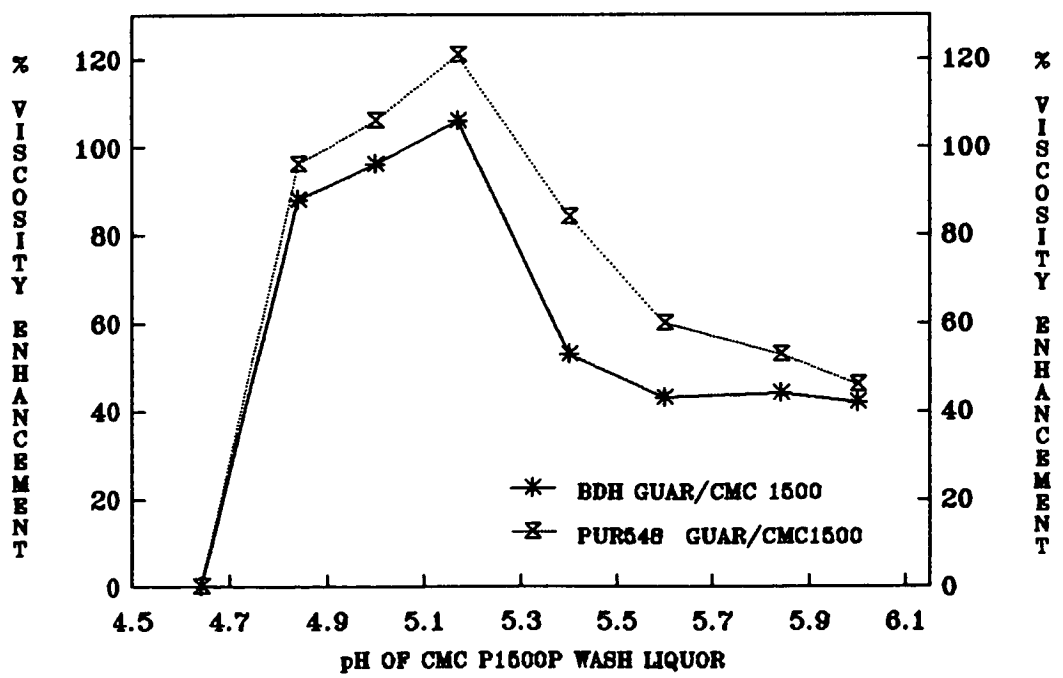
The modified CMC samples of varying free carboxyl content were blended with two guar samples and the synergistic viscosity enhancement measured (graph VII.10 and VII.11). It can be clearly seen that the maximum synergistic enhancement is at approximately pH 5.18 for both guar samples (the increasing CMC solution

EFFECT OF VARYING FREE CARBOXYL CONTENT
ON CMC P1500P SOLUTION VISCOSITY
1% TOTAL POLYMER CONCENTRATION



GRAPH 7.9

EFFECT OF VARYING FREE CARBOXYL CONTENT
ON % ENHANCEMENT OF 25%CMC.75%GUAR BLEND
1% TOTAL POLYMER CONCENTRATION



GRAPH 7.10

viscosities with increasing free carboxyl content [graph VII.9] has been taken into account in the evaluation of the blends overall % viscosity enhancement). An interesting point is that although both guars have almost identical component Brookfield viscosities they each give slightly different degrees of synergy. The reasons for this are discussed fully later in the text.

The synergistic association mechanism proposed for the polymer-polymer interaction between CMC and guar gum is by intermolecular hydrogen bonding between free carboxyl groups on the anionic CMC polymer and hydroxyls on the non-ionic galactomannan polymer. Only on the free carboxyl is there delocalisation of charge around the COOH group. A previous publication (48), on the rheological synergism between ionic and non-ionic cellulosic gums investigated the interaction between CMC and methyl cellulose. It concluded that the synergy could be accounted for by increased viscosity due to cross-linking of the two polymer chains. It stated that the cross-linking arises from hydrogen bonding on hydroxyls of the non-ionic gum with the carboxyl groups of the ionic Na^+ COO^-. It added that hydrogen bonding between a carboxyl (Na^+ COO^-) and a hydroxyl group on the non-ionic gum would give stronger interaction than between two hydroxyls on the same non-ionic molecule.

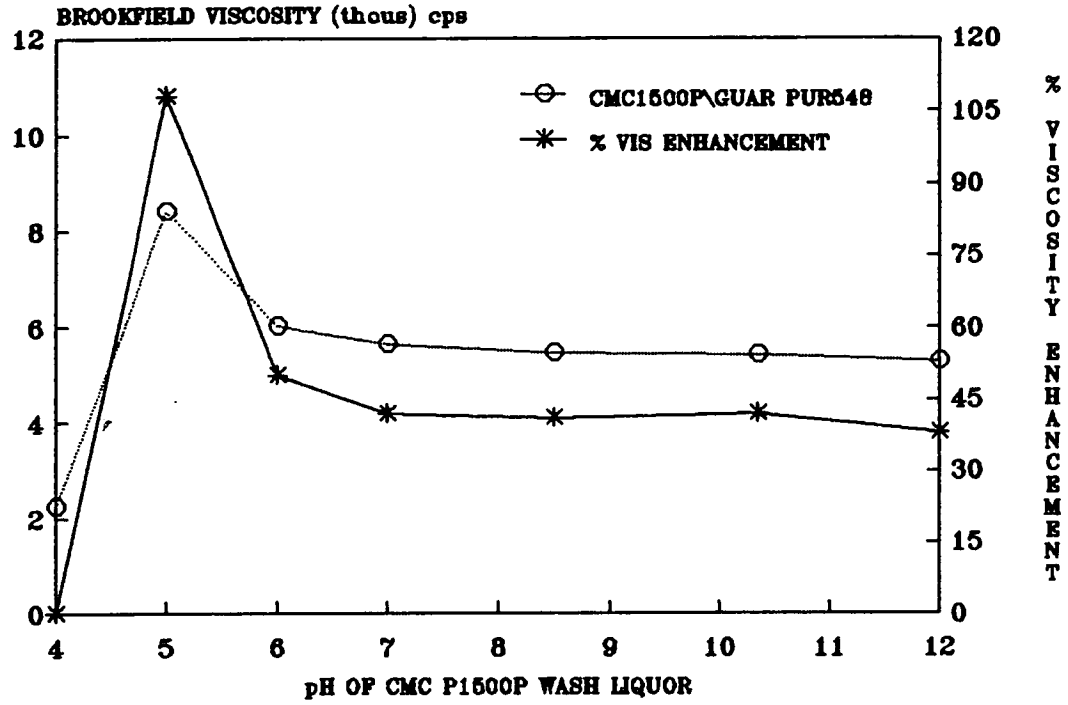
Hydrogen bonding is an associative interaction of two molecules of the same or different

substituents (49). Intermolecular hydrogen bonding is not limited to dimeric linkages and can produce 3-dimensional networks. A hydrogen bond exists only between a functional group A and an atom or group of atoms B in the same or on a different molecule. There must be experimental evidence of the bonds formation, and evidence of the new bond linking A-H to B specifically involving an hydrogen atom already linked to A. Hydrogen bonding is not possible between an ionic Na^+ COO^- group and a hydroxyl substituent on the non-ionic polymer.

An analogy of this is the formation of a dimer between acetic acid molecules but this phenomenon does not occur in the case of sodium acetate (50). In Na^+ CMC^- the positive charge on the sodium ion will reside close to the negative charge on the carboxyl and the non-ionic hydroxyls are unlikely to contribute a hydrogen bonding cross-link to this (51). The synergistic interaction observed in the publication discussed above (48), is due to hydrogen bonding between the few free carboxyls present at the pH studied and also a contribution from competitive dehydration (this co-existing mechainism will be developed later in the text).

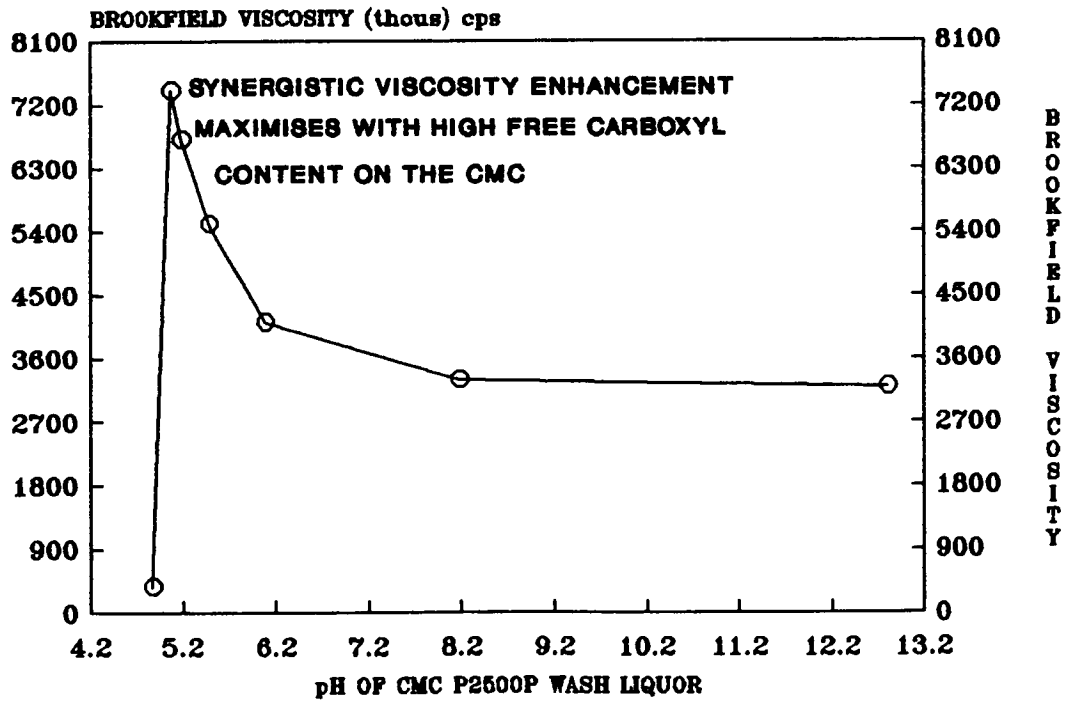
Graph VII.11 demonstrates how the degree of synergistic interaction in a CMC/guar blend varies as the pH of the wash liquor is made alkaline. Identical experimental conditions were selected but 35% sodium hydroxide was added to the CMC slurry and not

EFFECT OF VARYING FREE CARBOXYL CONTENT
ON % ENHANCEMENT OF 25%CMC.75%GUAR BLEND
1% TOTAL POLYMER CONCENTRATION



GRAPH 7.11

EFFECT OF VARYING FREE CARBOXYL CONTENT
ON % ENHANCEMENT OF 50%HPMC/50%CMC BLEND
1% TOTAL POLYMER CONCENTRATION



GRAPH 7.12

concentrated hydrochloric acid. This tests the suggestion (48), that association is possible between Na^+ COO^- groups on the CMC and hydroxyl substituents on the non-ionic polymer. At pH 12 all the carboxylate functional groups on the CMC molecules exist in the sodium form, thus from the association mechanism proposed above, no synergistic viscosity enhancement should occur at this pH. However this is clearly not the case. Synergy seems to fall between pH 5 and 8 then level off but it never falls to zero. Since no free carboxyl substituents exist on the CMC as a result of alkali washing, this viscosity enhancement is not a result of molecular association. Another co-existing mechanism is proposed which exists at acid and alkaline pH values and will be named Mechanism 2. It is therefore proposed that Mechanism 2 arises from the difference in relative hydrophilicities of the two polymers and arises from competitive dehydration between the unlike polymers, and in this example results in synergistic viscosity enhancement. Mechanism 1 is the molecular association mechanism.

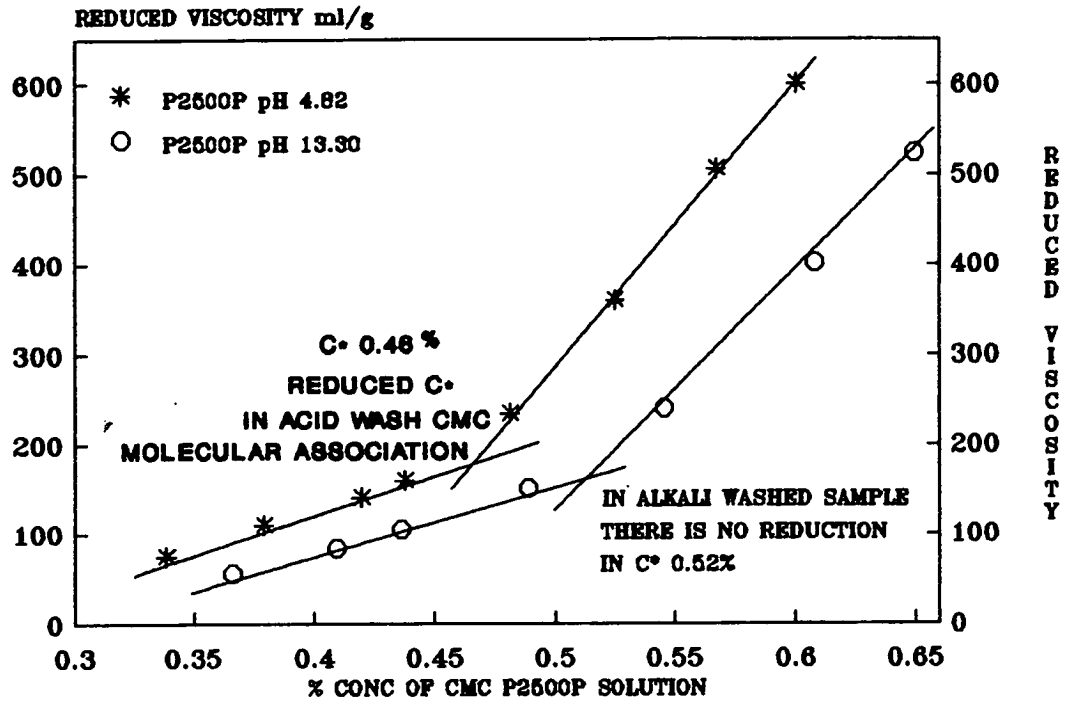
If the acid washed modification of CMC is repeated, the question arises if it can be applied to all blends of anionic CMC with non-ionic water soluble polymers. Graph VII.12 indicates that this may be the case where non-ionic HPMC is blended with CMC and a marked increase in viscosity enhancement is achieved by increasing the free carboxyl content of CMC. Graph VII.14 confirms the previous proposed

mechanism for a CMC/guar blend when applied to a CMC/HPMC blend. Again it can be seen that there is a large difference in the % viscosity enhancement of the blend when acid washed CMC is used compared to alkaline washed CMC.

Graph VII.14 suggests that as in the guar/CMC blend example synergy still exists with alkaline washed CMC/HPMC blend however. CMC is relatively more hydrophilic than HMPC (the methyl substituents in HPMC make it relatively hydrophobic for a cellulose ether) and when blended, competitive dehydration may contribute to viscosity enhancement, this is the basis of Mechanism 2 discussed in detail later in the text.

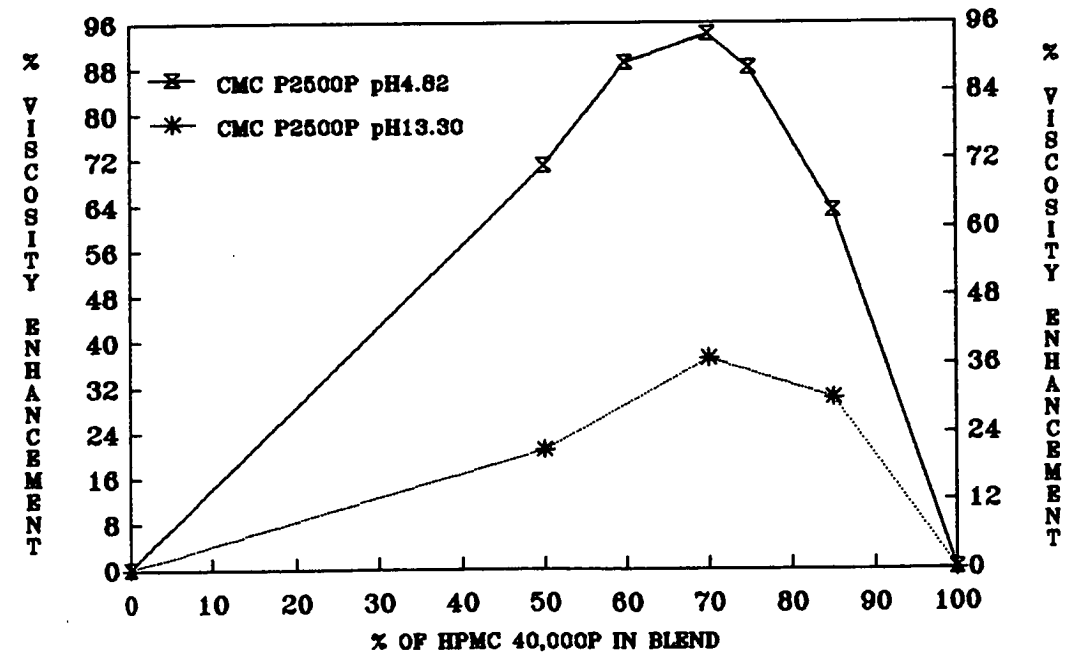
Ostwald viscometry data to evaluate hydrodynamic volume and the C* value of acid and alkali washed CMC shows two interesting results (graph VII.13). (These modified CMC polymers started as one material i.e. P2500P, by varying its free carboxyl content two distinct polymers are formed). Firstly the C* value is significantly lower for the acid washed CMC sample, this indicates a larger hydrodynamic volume in solution. Secondly the gradient of the alkaline washed CMC viscosity concentration slope is similar to the acid washed CMC slope below C* but less steep above C*. This suggests that as the concentration of the polymer increases the influence of the acid washed CMC on the overall solution viscosity increases. As the sodium ions in Na^+ CMC^- are removed and replaced with

VARIATION IN FREE CARBOXYL GROUP CONTENT
OSTWALD VISCOMETRY DATA TO EVALUATE C*
INTERMOLECULAR HYDROGEN BONDING EFFECT



GRAPH 7.13

VARY FREE CARBOXYL SITES ON COURLOSE CMC
% ENHANCEMENT OF CELACOL/COURLOSE P2500P
SYNERGISTIC BLENDS.



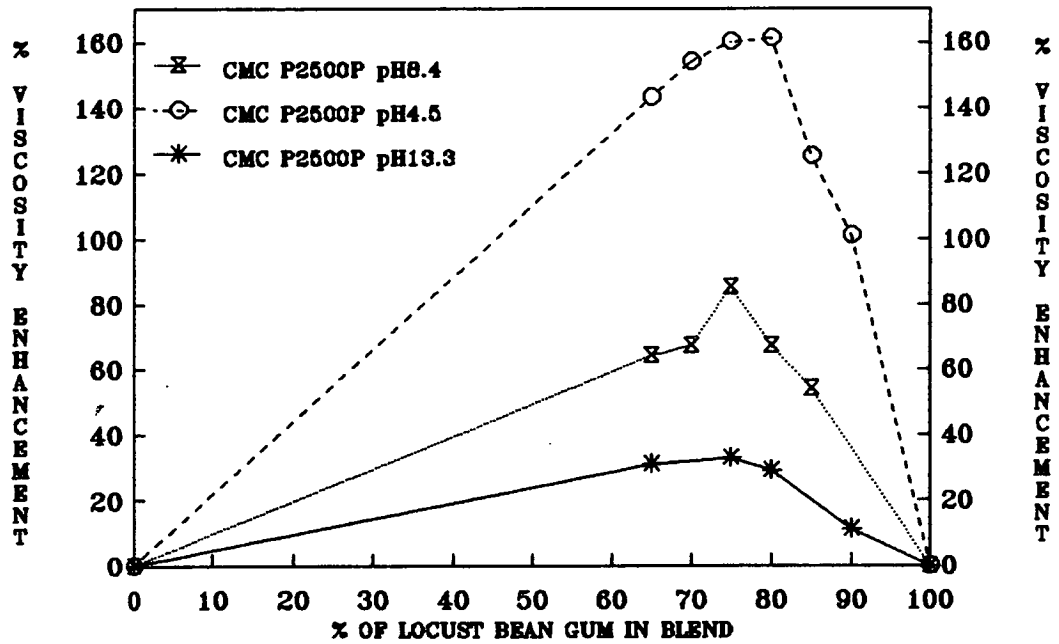
GRAPH 7.14
1% TOTAL POLYMER CONCENTRATION

hydrogen ions one would normally assume the structure would collapse resulting in a lower hydrodynamic volume and corresponding higher C* value. If the CMC wash liquor is lowered below pH 5 this is true but around pH 5-6 a different phenomena occurs. This effect may arise from intermolecular hydrogen bonding between free carboxyl (COOH) groups on adjacent polymer chains. The DS of this CMC is approximately 0.7 and assuming a relatively even substitution pattern of carboxyl groups, intramolecular hyrogen bonding between adjacent carboxyls on the same chain is unlikely. It is shown at a later stage in this thesis however that a CMC with a higher degee of substitution sample behaves differently.

A locust bean gum sample was blended with three initially identical CMC samples, structurally modified to give varying free carboxyl content. One CMC is the polymer that is commercially available (pH of slurry liquid was 8.4) where approximately 90% of the carboxyl functional groups are in the sodium form. The second is the alkali washed modified polymer (pH of slurry liquid was 13.3) where 100% of the carboxyl groups are in the sodium form. The third is the acid washed modified polymer (pH of slurry liquid was 4.5) where approximately 90% of the carboxyl groups are in the free acid (COOH) form. The results are displayed on graph VII.15.

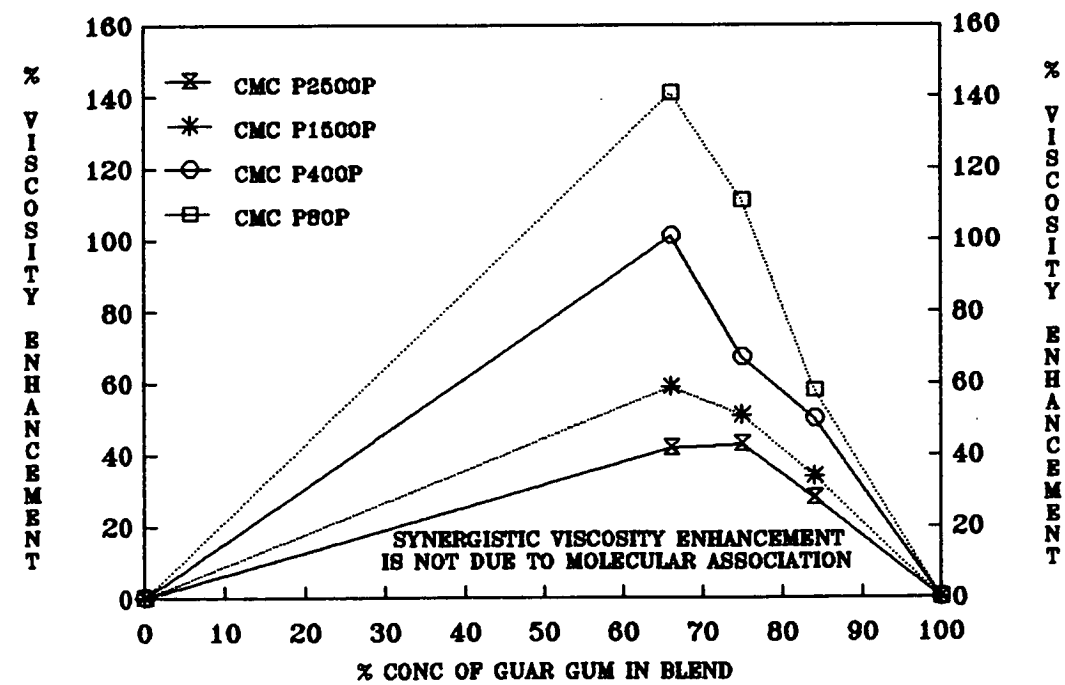
It is proposed that a similar interaction mechanism is occurring with this polymer

VARY FREE CARBOXYL SITES ON COURLOSE CMC
% ENHANCEMENT OF LOCUST BEAN GUM/CMC
SYNERGISTIC BLENDS.



GRAPH 7.15
1% TOTAL POLYMER CONCENTRATION

VARY MOLECULAR WEIGHT OF COURLOSE CMC
% ENHANCEMENT OF COUGAR TH100/CMC pH13.3
SYNERGISTIC BLENDS. 1% POLYMER CONCS.



GRAPH 7.16

blend as in the previous blends discussed. The strongest interaction occurs when acid washed CMC is blended i.e when maximum numbers of free carboxyls occur on the CMC chain to maximise intermolecular association. At pH 8 there is still a minor contribution from this molecular association mechanism (45). It is possible that this is partially responsible for the observed viscosity enhancement in previous reports (48). In the alkaline washed CMC blend the synergistic interaction arises from competitive dehydration due to the difference in relative hydrophilicities of the two polymers. Mechanism 2 contributes to all three synergy curves as competitive dehydration occurs in the acid and alkali washed CMC blends, and would also contribute to the synergistic viscosity enhancement observed in a previous publication (48).

VII (iv) MECHANISM 2 . COMPETITIVE DEHYDRATION.

At this stage it may be explained why competitive dehydration may occur when two unlike polymers are blended together and, if it occurs, why it appears to result in a positive contribution to viscosity enhancement in every example where an anionic polymer was blended with a non-ionic polymer. It is worth recalling at this point that a blend of two non-ionic polymers resulted in overall viscosity reduction i.e antagonism (graph VII.2). All

polymers have varying degrees of hydrophilicity (52,53), some are water-soluble others like polystyrene are totally water immiscible. From a cellulose ether point of view, the substituents affect the relative hydrophilicity of the polymer (54). CMC and HEC are two of the most hydrophilic whereas MC and HPMC are the most hydrophobic. Ethyl cellulose is even more hydrophobic but is not water-soluble, it is soluble in dichloromethane. It is this difference in hydrophilicity that many non-ionic cellulosic ethers exhibit the interesting phenomena of reversible thermogellation (HEC being relatively hydrophilic doesn't exhibit this phenomena). Nuclear magnetic resonance studies (55), have indicated the necessity of localised high concentrations of tri-methoxy anhydroglucose units for gelation to occur. The crystalline and amorphous regions in the structure of cellulose are responsible for this unique reversible thermogelation phenomenon. As the hydroxypropyl content of a HPMC sample increases its gel point increases and its gel strength decreases. This is a result of the overall hydrophilicity of the polymer increasing. In commercial HPMC grades usually a high hydroxypropyl content corresponds to a relatively low methyl content.

Much commercial exploitation has been made in modifying water-soluble polymers by selectively end-capping hydrophilic hydroxyl functional groups with hydrophobic moieties (56). An example of this is the conversion of guar gum into guar monoacetate by an

esterification route (57,58). The rheology of the polysaccharide is greatly modified and its interaction with minerals in the building industry enhanced, and its performance as an emulsifier in food formulations is improved (59).

Of the polymers being studied presently, CMC is likely to be the most hydrophilic followed by guar gum followed by HPMC (52). The molecular weight of a polymer does not affect its relative hydrophilic/lypophilic balance assuming similar substituent patterns in each case, and discounting end group contributions. Four molecular weight grades of Courlose CMC were each structurally modified by alkali washing in a ethanolic/water slurry as described previously. In a blend of alkali washed CMC, with a non-ionic polymer the possibility of molecular interaction by intermolecular association is eliminated. Therefore any synergistic viscosity enhancement observed is not due to molecular association. The four CMC grades were blended with guar gum at various mixing ratios and the % viscosity enhancement was calculated as before in each case (graph VII.16).

The results (graph VII.16) indicate that the maximum viscosity enhancement is achieved at approximately 70% of the guar component in each case. The lower viscosity CMC grades give maximum synergy at a mixing ratio of 65% galactomannan, the 2500P grade gives maximum enhancement at a mixing ratio of 75%

galactomannan.

It can be seen that the highest % viscosity enhancement is observed with the lowest viscosity grade (graph VII.16). Therefore this reinforces experimental evidence for Mechanism 2 assuming that the structure of CMC is more hydrophilic than guar gum. Although the largest viscosity enhancement observed for the alkali washed CMC/guar gum blends was with the low molecular weight CMC/guar blend, this is not repeated when an acid washed CMC of the same molecular weight is blended with guar gum. The viscosity enhancements observed for the alkali washed low molecular weight CMC/guar blend and the acid washed low molecular weight CMC/guar blend are similar in magnitude. This suggests that the contribution from the molecular association mechanism is small when a low molecular weight CMC is blended with guar gum. This indicates that although molecular association is possible, even if many small molecules associate along the length of a large molecule, less change in the hydrodynamic volume is likely, than if both polymers are similar in chain length (ie. a blend of acid washed CMC of high molecular weight with guar gum).

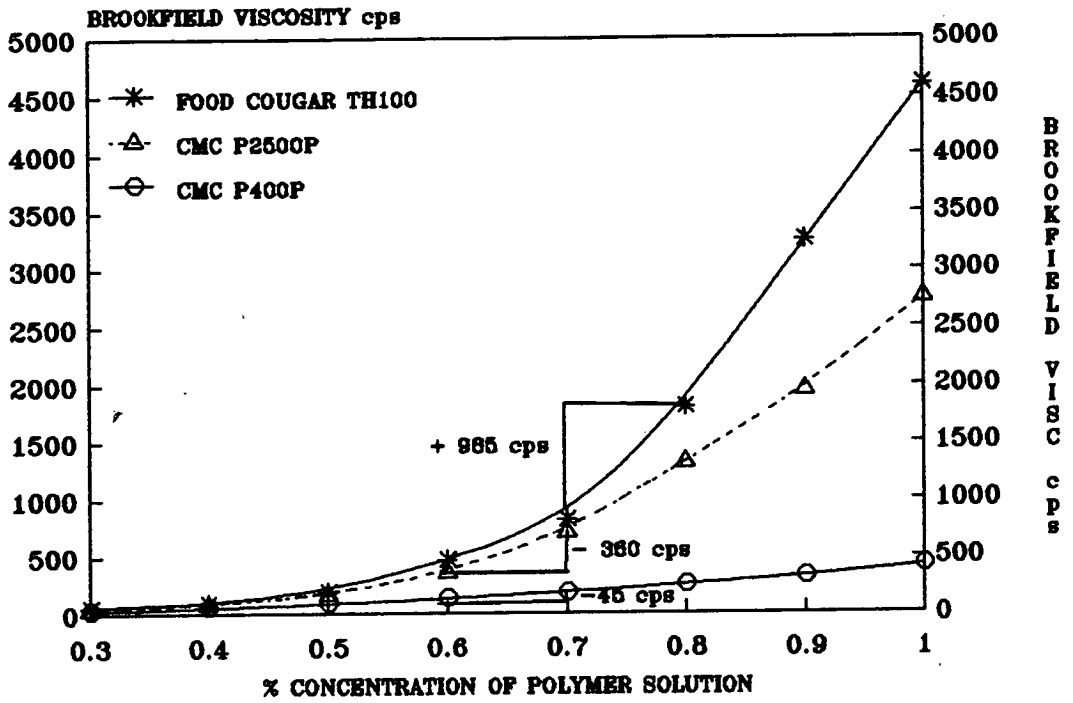
These findings agree with a previous report (60),on anionic polymer blends with a non-ionic polymer which indicated that maximum viscosity enhancement was achieved when two polymers of similar molecular weight were blended together i.e maximised when low molecular weight CMC was blended with low HPMC

and similarly for two high molecular weight samples. This line of thought was that if association occurred between two molecules the greatest change in hydrodynamic volume of a molecule would occur if the molecular weights of the two polymers were similar.

However this report did not account for the change in synergistic interaction with variation in the free carboxyl content of the anionic polymer. It also neglected the differences between hydrophilicities of the polymer components. Initially this may appear unimportant i.e. if two unlike cellulose ethers are blended it has been suggested in this text that the hydrophilic character of either polymer is independant of molecular weight. However the same viscosity enhancement is not achieved when a high viscosity CMC polymer is blended with a low viscosity HPMC polymer, in contrast to blending a low viscosity CMC with a high viscosity HPMC. This is shown to be the case at a later stage in the thesis.

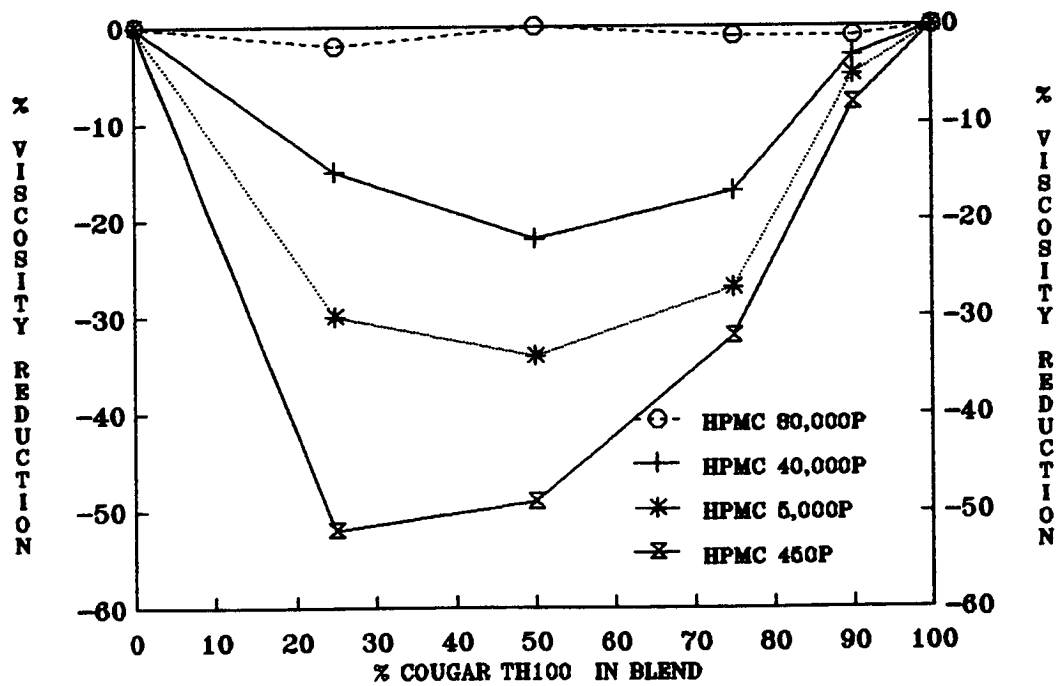
All water-soluble polymers have a certain degree of hydrophilicity and when hydrated in a blended system there is competition for available water molecules to ensure complete solvation (61). The more hydrophilic a polymer the greater its ability to remove water from the structure of a more hydrophobic polymer. Graph VII.17 shows the viscosity against concentration curves for guar gum and two CMC molecular weight grades. As the polymers move to a concentration above C* (approximately 0.5% polymer concentration)

EFFECT OF MOLECULAR WEIGHT AND RELATIVE HYDROPHILICITY ON SYNERGISTIC MECHANISM VISCOSITY AGAINST CONCENTRATION CURVES



GRAPH 7.17

ANTAGONISTIC POLYMER–POLYMER BLEND VARIOUS HPMC MOL WGT GRADES/GUAR GUM 1% TOTAL POLYMER CONCENTRATION



GRAPH 7.18

molecular overlap becomes possible; the amount of water molecules available for hydration is limited, and the viscosity increases rapidly with concentration. However the viscosity/concentration curve for low molecular weight CMC is less steep than the corresponding gradient of the higher molecular weight CMC curve. Therefore if two polymers are blended at the same concentration (50/50%) in a limited volume of water, a water balance equilibrium is reached due to differences in relative hydrophilicities of the two polymers. CMC will appear less concentrated as it has removed water molecules previously associated with the guar structure and consequently guar will appear more concentrated. Graph VII.17 indicates from a hypothetical starting point how this would alter the final blend viscosity. An overall resultant net increase in viscosity is achieved in each case but the increase is greater for the lower molecular weight CMC grade. This mechanism however doesn't account for the co-existing molecular association mechanism (Mechanism 1).

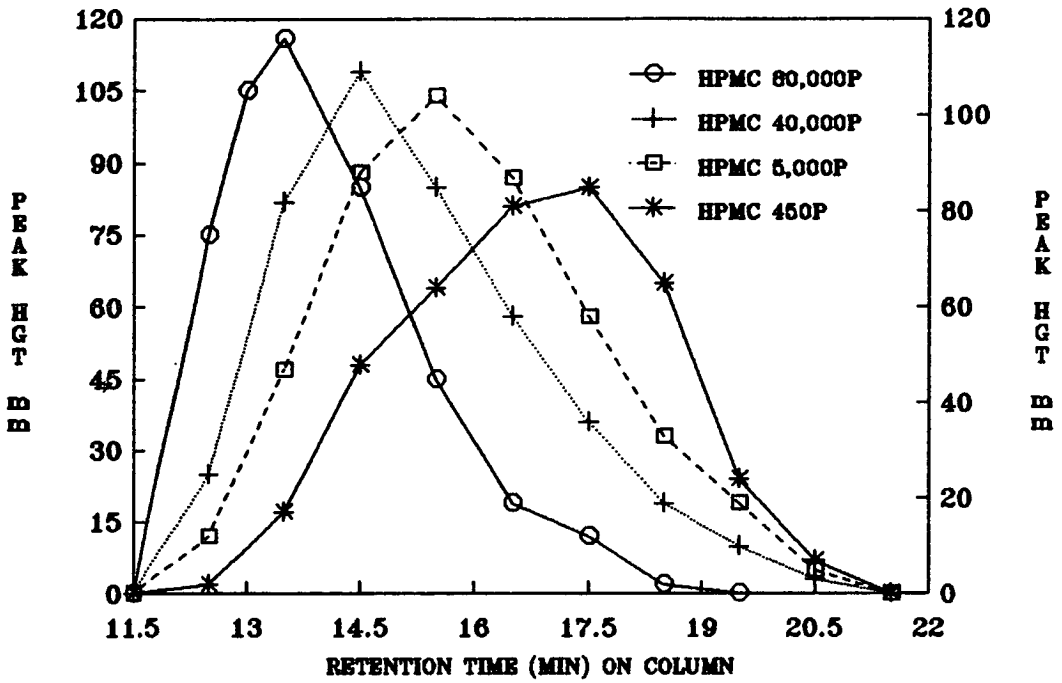
Polymer-polymer interactions will always compete with polymer-solvent association (62), reduction of water activity may increase synergistic viscosity enhancement of a polymer blend. Water activity may be reduced by addition of a low molecular weight hydrophilic molecule, for example sucrose, which binds water in competition with both polymer components (63). An example of this is the suppression of gel point when 20% sucrose is dissolved in a 2% methyl

cellulose solution, the monomer associates water molecules and the polymer appears more concentrated so methyl-methyl interactions occur at a lower temperature.

Various molecular weight grades of Celacol HPMC were blended at four mixing ratios with a guar gum (TH 100). Antagonistic viscosity reduction was observed for all grades at all mixing ratios. The results are displayed in graph VII.18. The largest % viscosity reduction was observed for the low molecular weight HPMC 450P, blend with guar gum. Little % viscosity antagonism was observed with the high molecular weight grade of HPMC 80,000P. In a polymer blend of this type, guar gum has a larger hydrophilic/lypophilic ratio than HPMC. Celacol HPMC therefore has a smaller affinity for available water molecules in a mixed polymer system than that of guar gum. However it is obvious from graph VII.18 that the molecular weight of the HPMC is a critical parameter in the final solution viscosity achieved.

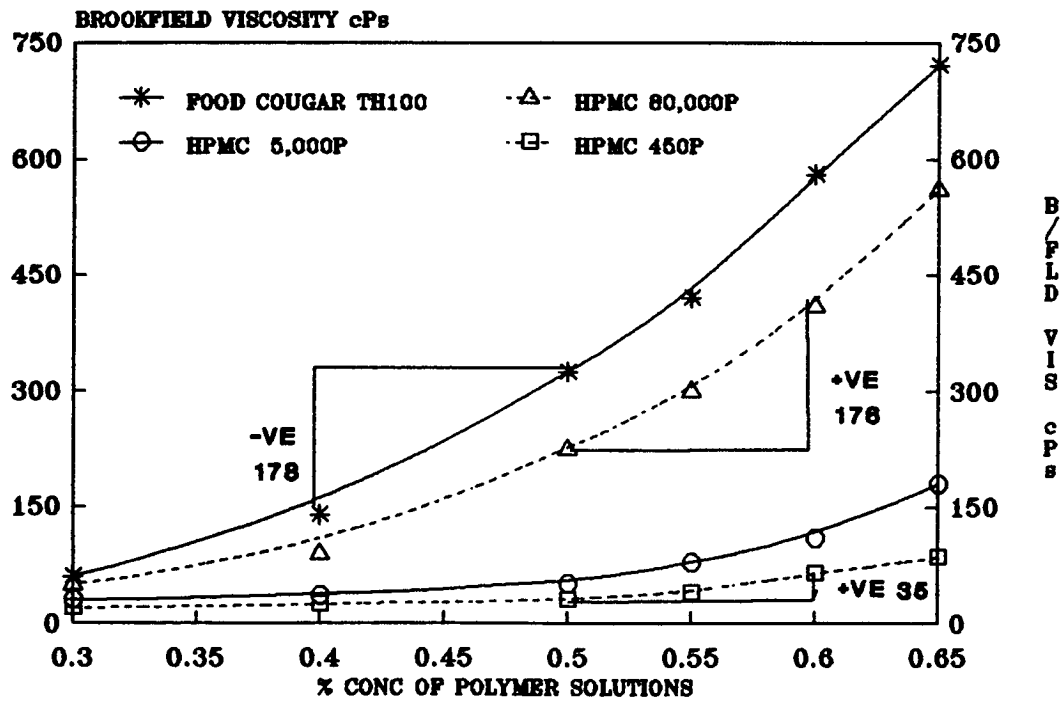
Graph VII.20 shows the actual viscosity concentration curves for various Celacol HPMC grades and for guar gum. From a hypothetical starting point of 0.5% total polymer concentration in a mixed system with a limited solvent volume, there is again competition for available water molecules for complete polymer hydration. The smaller the molecular weight of the HPMC the larger the extent of viscosity antagonism. In the

G.P.C. TRACES FOR CELACOL(HPMC) GRADES
OF VARYING MOLECULAR WEIGHT(0.2% CONC)
LINEAR+ 1000 HYDROGEL COLUMN



GRAPH 7.19

ANTAGONISTIC NON-IONIC POLYMER BLENDS
PLOT OF VISCOSITY AGAINST % CONC
OF HPMC MOL WGT GRADES AND GUAR GUM



GRAPH 7.20

example of the HPMC 80,000P/guar blend the viscosity concentration curves are very similar in shape and magnitude. The positive contribution from the HPMC component redistributing water molecules and appearing effectively more concentrated in the mixed system is almost exactly cancelled by the guar's apparent reduction in effective viscosity.

Celacol HPMC is not manufactured to an exact molecular weight or solution viscosity (53). Correct viscosity ranges are achieved by a computerised blending model and the way the blend is composed can ultimately affect the overall performance of the polymer. Gel permeation chromatograms were run for each Celacol HPMC grade on two Waters Hydrogel columns (in series) and the results are displayed in graph VII.19. All four molecular weight grades have fairly polydisperse molecular weight distributions, however none of the peaks are split into two distinct molecular weight bands. It can be concluded that the viscosity reductions observed in the HPMC/guar blends (graph VII.18) are as a result of the proposed mechanism (Mechanism 2) and not caused as a result of high and low molecular weight HPMC grades having been blended to achieve an intermediate viscosity.

In commercial practice Celacol HPMC or Courlose CMC viscosity grades are sold as blends of low and high molecular weight components to achieve the desired intermediate viscosity. These results suggest that this polydispersity should be minimised if at all

possible otherwise the exact predicted viscosity enhancement will not be achieved.

The full effect of blending low and high molecular weight components is shown in section (xiii), where a low molecular weight HPMC is blended with a high molecular weight CMC and an overall antagonistic viscosity results, whereas if a high molecular weight HPMC is blended with a low molecular weight CMC, synergistic viscosity enhancement results.

Another factor worth considering in blending unlike molecular weight polymers is that often a polymer like Celacol HPMC is sold commercially on its performance; as a water retaining agent in the building industry or as a gelling agent in the food industry, and not solely on its viscosity characteristics. Although blending extreme molecular weight polymers can achieve the desired viscosity, it often can have a detrimental effect on polymer performance.

VII (v) EFFECT OF VARIATION IN DEGREE OF SUBSTITUTION.

It was demonstrated with Courlose CMC P1500P and P2500P samples that increasing the free carboxyl content of the polymer resulted in an increase in the synergistic interaction when the modified CMC was blended with guar gum (graph VII.1). Both of these CMC grades have an average degree of substitution (D.S) of approximately 0.7. The degree of substitution and

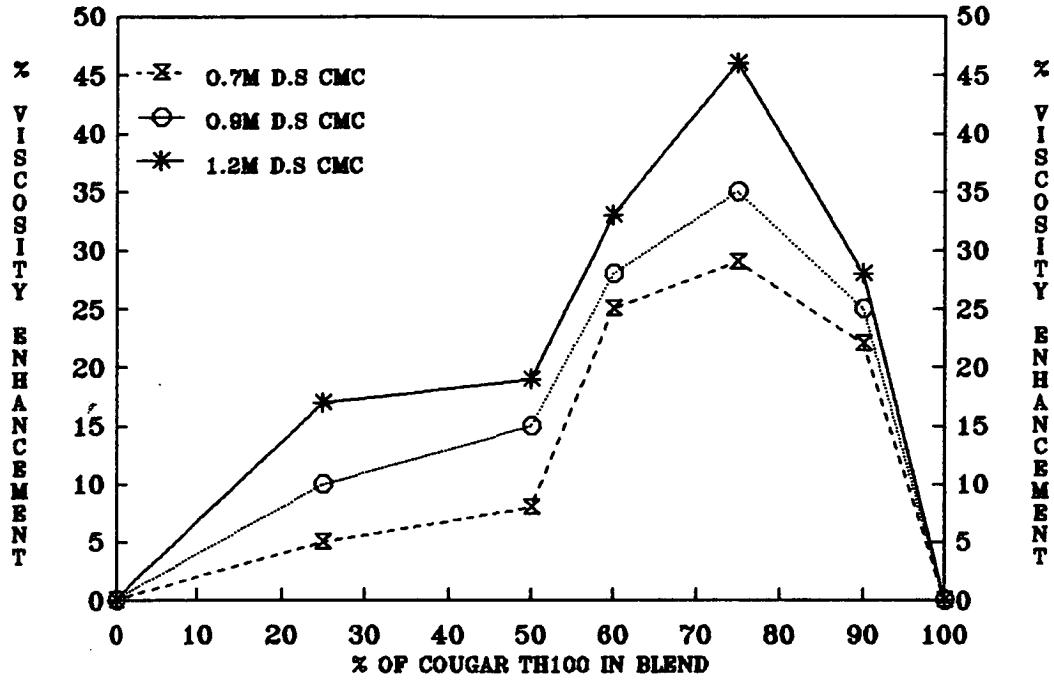
uniformity of substitution (64), has a profound effect on the solution properties and rheological characteristics of sodium carboxymethyl cellulose. As the D.S of the polymer increases, the solubility of the CMC increases and the polymer becomes more tolerant to acidic systems and dissolved electrolytes (65,66). The D.S commonly encountered with commercial grades of CMC lies between 0.4 and 1.2. Below 0.4 CMC is insoluble as a result of strong intramolecular hydrogen bonding between underivatised anhydroglucose units, as a result of the crystalline nature of the starting material. Degrees of substitutions above 1.0 are not permitted in food grade material and D.S values above 1.2 are unusual in industrial grade material. Rederivatisation of CMC is necessary to achieve this high D.S (above 1.2) and excessive molecular weight degradation of the polymer backbone subsequently occurs. Hercules (a French CMC producer), manufacture various degree of substitution grades of CMC, which may be of three similar molecular weight ranges and therefore viscosity. Three Hercules samples of medium viscosity (approximately 3200 cps at 2% polymer concentration), were blended with guar gum at four selected mixing ratios and the viscosity enhancement measured (67). The three grades had varying degrees of substitution of 0.7, 0.9 and 1.2 .

Since a higher degree of substitution CMC contains more potential free carboxyl sites (66), it was initially assumed that a high D.S CMC would

result in an even greater viscosity enhancement as a result of acid washing CMC in an alcohol/water slurry, when the modified CMC was blended with guar gum. The results with unmodified CMC of varying D.S values reflect this assumption in that, at a certain pH of 8.3, approximately 90% of the carboxyl groups will exist in the sodium salt form. Thus the higher degree of substitution CMC will have a greater number of free carboxyls and the % viscosity enhancement would be greater (graph VII.21). A similar trend but with larger viscosity enhancement was observed when locust bean gum was blended with the three differing degree of substitution CMC grades.

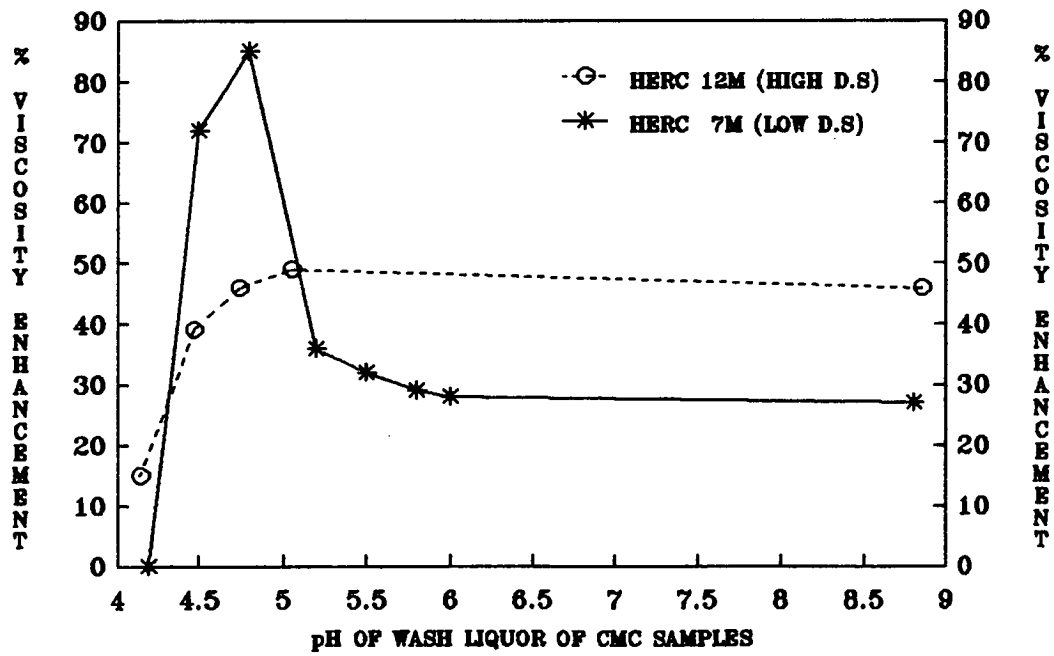
However when the high and low D.S CMC samples were acid washed in an alcohol/water slurry as before, and the structurally modified CMC polymer was dry blended with guar gum, a very different result is evident (graph VII.22). It can be seen that a cross-over effect has occurred, and the low DS acid washed carboxymethyl cellulose now gives a larger percentage viscosity enhancement, than does the high D.S CMC. The cross-over point occurs around the pH value of 5.3. At this point when the acid washed CMC is hydrated many of the previously Na^+ COO^- substituents exist in the free acid form and can interact by molecular association with a hydroxyl on the non-ionic polymer (graph VII.10) or a hydroxyl on an unsubstituted group on the CMC backbone. The free COOH groups can also intermolecularly with another free

VARIATION IN DEGREE OF SUBSTITUTION
OF THREE HERCULES CMC SAMPLES
GUAR/CMC POLYMER INTERACTION



GRAPH 7.21

VARIATION IN FREE CARBOXYL CONTENT
OF VARYING D.S HERCULES CMC SAMPLES
75/25% GUAR/CMC SYNERGISTIC BLENDS

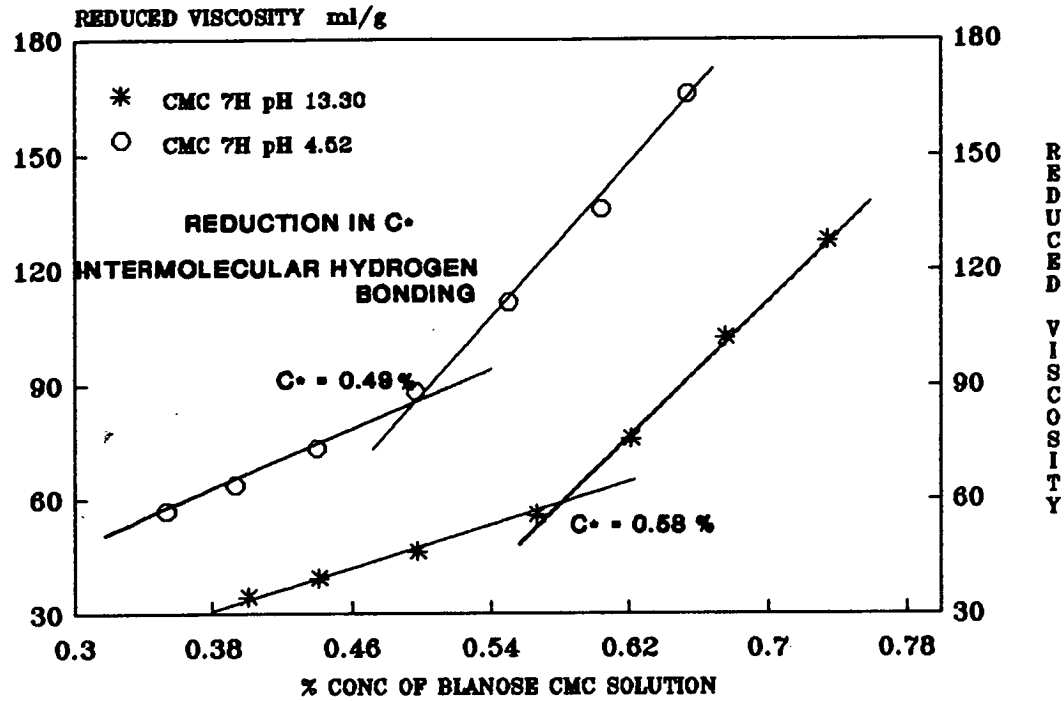


GRAPH 7.22
1% TOTAL POLYMER CONCENTRATION

carboxyl group, on another CMC polymer chain, which was observed for the CMC P2500P sample (graph VII.13). This appears to occur in the 0.7 D.S example. The Hercules low D.S sample has a similar D.S value to the Courlose P2500P sample.

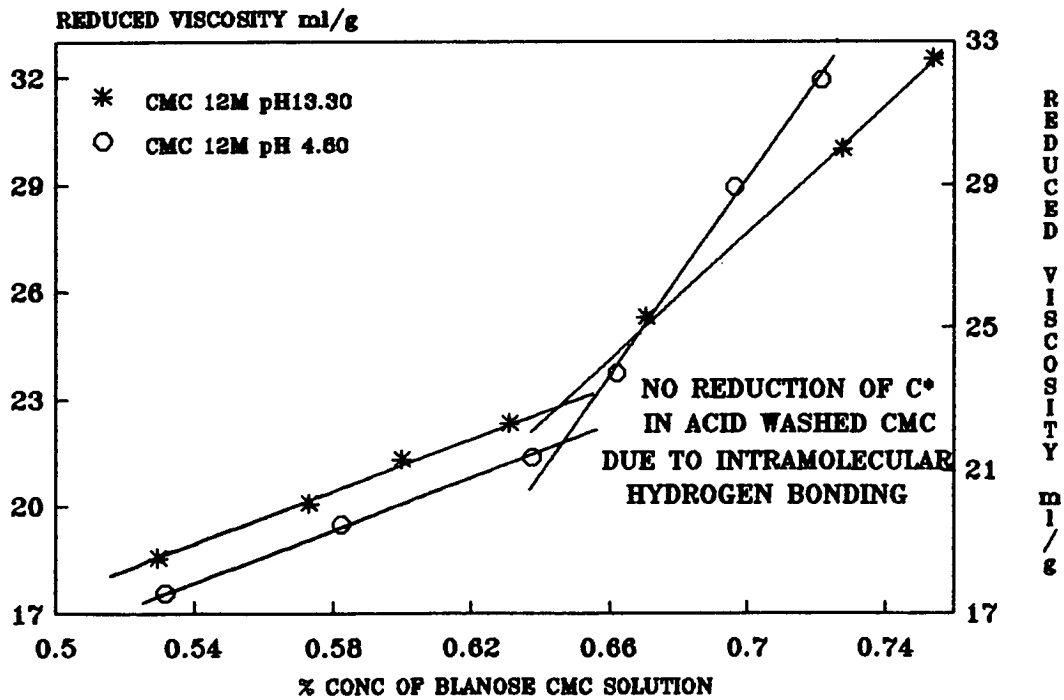
Ostwald viscometry data were collected at this stage for acid and alkali washed Hercules CMC samples of low and high degrees of substitution. Graphs VII.23 and VII.24 indicate a possible explanation as to why the D.S grade of 1.2 did not give high synergistic enhancement for the acid washed sample. In the low DS example, the acid washed sample shows a greater hydrodynamic volume, greater solution viscosity at any concentration and a reduced C* value when compared to the alkali washed sample. This may be a result of increased intermolecular association, between free carboxyls on different chains. In the high D.S example no enhancement of solution viscosity and little change in the C* value is apparent as a result of acid washing. However many free carboxyls must exist on the acid washed polymer.It may be noted that below C* the solution viscosity of the low D.S acid washed CMC sample is of similar magnitude to that of the alkali washed high D.S CMC sample. If intermolecular hydrogen bonding is not occurring between chains it is a possibility (due to the high D.S and the close proximity of free carboxyls in the C-2, C-3 and C-6 positions, on adjacent derivatised anhydroglucose units), that intramolecular hydrogen bonding between

OSTWALD VISCOMETRY DATA FOR HERCULES
DS 7H CMC SAMPLES WITH DIFFERENT FREE
CARBOXYL CONTENT TO EVALUATE C*



GRAPH 7.23

OSTWALD VISCOMETRY DATA FOR HERCULES
DS 12M CMC SAMPLES WITH DIFFERENT FREE
CARBOXYL CONTENT TO EVALUATE C*



GRAPH 7.24

adjacent residues, on the same molecule, predominates over intermolecular association between unlike CMC molecules. This possibility of intramolecular hydrogen bonding is also possible in the low D.S sample but is much less probable due to the large number of unreacted hydroxyls on the cellulose backbone (68).

At this stage it was necessary to provide evidence that molecular association was occurring by intermolecular hydrogen bonding on acid washed samples of low D.S carboxymethyl cellulose and predominately by intramolecular hydrogen bonding in high D.S grades. Several studies have been published in which simulated computerised modelling was used to investigate polymer configurations and specific interaction with an unlike polymer (69,70). Computerisised molecular models on a "Discover" program (71) were constructed as described in the experimental methods section. "Discover" is a molecular simulation programme by Biosym Technologies that performs molecular mechanics and dynamics. The findings confirmed the conclusions drawn from the previous hydrodynamic volume results.

From this molecular modelling study, energy minimisation and calculated interaction energies of polymer association is possible. Two CMC molecules with differing degrees of substitution (0.7 and 1.2 on average and randomly distributed on the cellulose backbone) were constructed from linking two glucose sugar monomers together, and then polymerising the

dimer. This was repeated and a polymer of 16 glucose units long was finally constructed. Appropriate levels of substituents were added and the molecules' structural conformation was minimised overnight, through thousands of iterations by a computerised mathematical model which locates positions of local minimum free energy for each molecule. This is an approximation to the structure which the molecule would adopt in a vacuum which has a dielectric constant equivalent to that of water.

Although the aim of minimisation can be stated simply, difficulties arise for a large system, for example a polymer chain. Generally, several local minima points exist where the atomic forces are zero, although the global minimum is usually of interest. The actual coordination of a molecule combined with the potential energy surface gives an energy expression for a molecule. This is an equation which describes the potential energy surface of a molecule as a function of its molecular co-ordinates and is discussed in the experimental methods section.

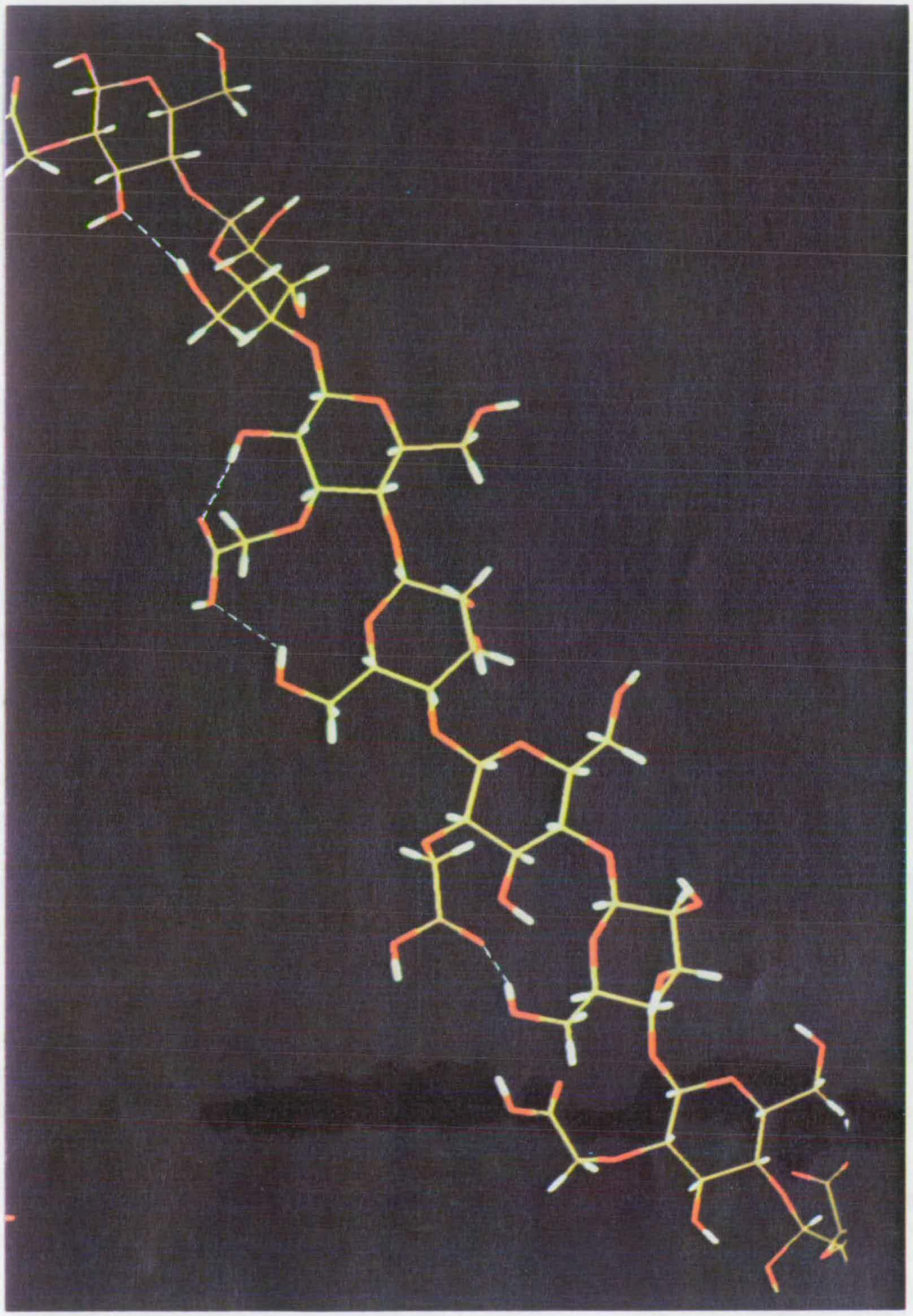
Three assumptions made throughout this modelling work were that firstly the polymers could exist in a vacuum which had the same dielectric constant as water. The second was that molecules of D.P (degree of polymerisation) equal to 16 would behave in a similar comformational manner to macromolecular CMC polymers. The last assumption was that CMC has all its carboxyls existing as COOH free carboxyl to maximise

the possibility of observing hydrogen bonding within the same molecule. The program could predict which units lay close enough in conformational distance to allow measurable hydrogen bonding energies. The results (photographs 1,2 +3) indicate that, in the low degree of substitution case, the probability of a free COOH on one glucopyranosyl unit interacting intramolecularly with another on the next unit is unlikely. It is more likely to occur in the higher D.S molecular model where a C-6 on one unit may interact with a C-2 unit on the adjacent glucopyranosyl residue. The models allowed confirmation of the possibility of intramolecular hydrogen bonding in an acid washed high D.S sample of CMC.

At this stage a mechanism has been proposed where molecular association only exists in a blend of an anionic polymer and a non-ionic polymer by intramolecular hydrogen bonding between free carboxyls on the anionic and hydroxyls on the non-ionic polymer. It has been stated that viscosity enhancement between a zero free COOH content CMC and a non-ionic polymer arises by a co-existing competitive dehydration mechanism. Therefore in a blend of alkali washed CMC (D.S 0.7) and guar gum, no reduction in C* should occur compared to component values. This prediction is confirmed in graph VII.25 where little viscosity enhancement and no change in hydrodynamic volume occurs in the blend. The C* of the polymer blend as predicted is similar in magnitude to the C* values of the two

PHOTOGRAPH VII.1

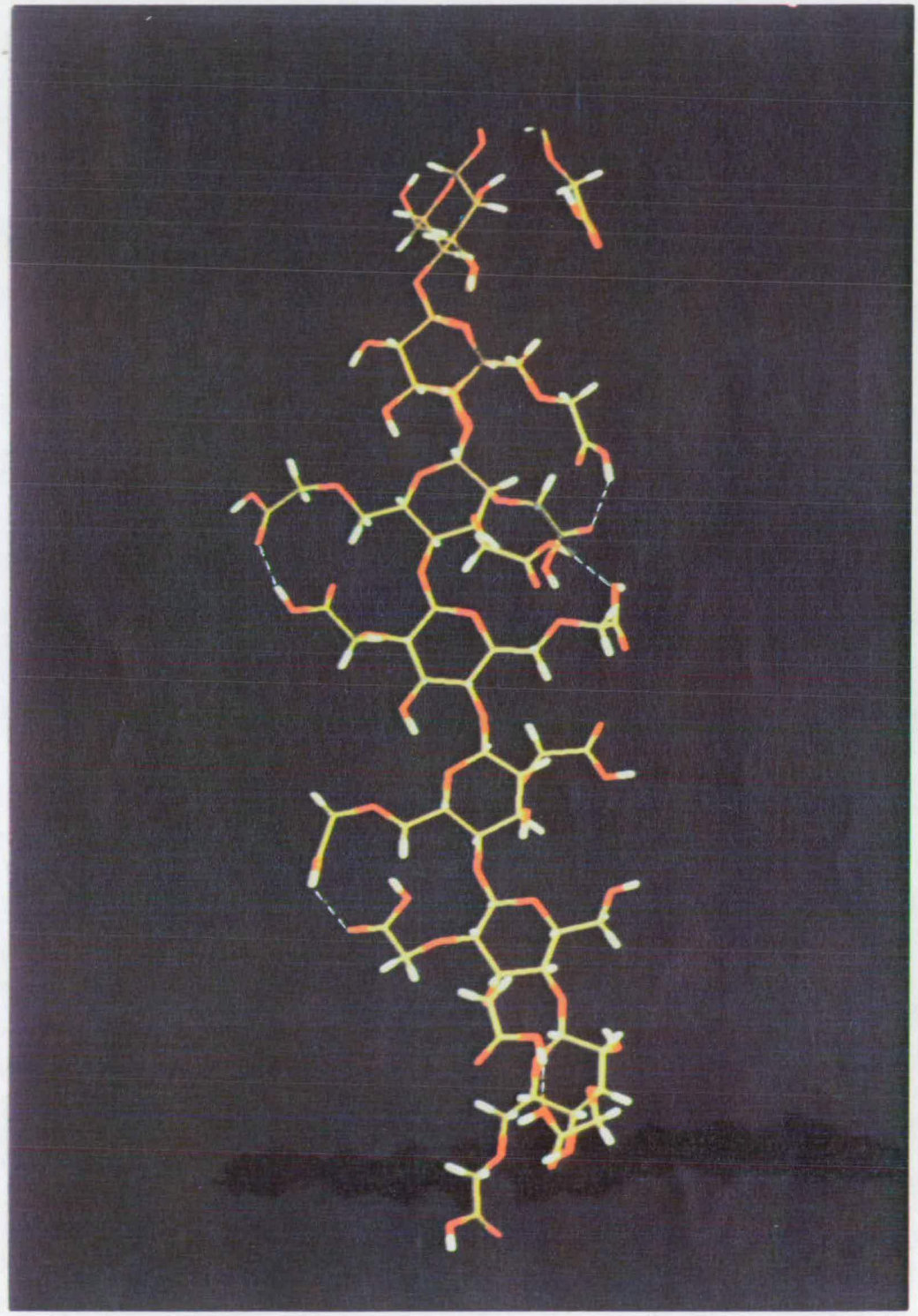
PHOTOGRAPH 1 SHOWS A MOLECULAR MODEL OF A LOW D.S CMC. HYDROGEN BONDING (DOTTED LINE) EXISTS BETWEEN FREE CARBOXYLS AND HYDROXYL GROUPS ONLY.



RED LINES - OXYGEN ATOMS
GREEN LINES - CARBON ATOMS
WHITE LINES - HYDROGEN ATOMS

PHOTOGRAPH VII.2

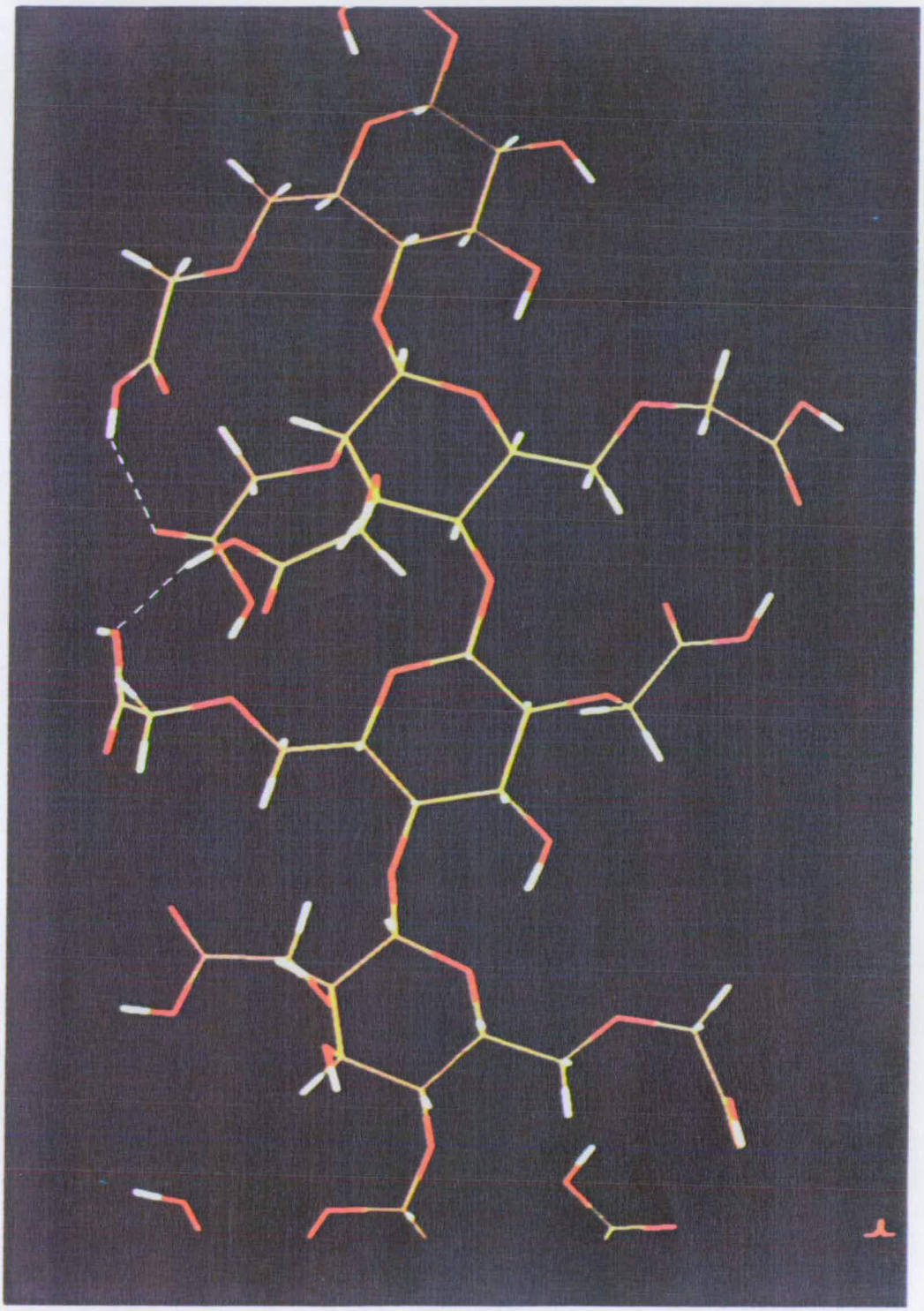
PHOTOGRAPH 2 SHOWS A MOLECULAR MODEL OF A HIGH D.S CMC AND SHOWS THE EXISTANCE OF HYDROGEN BONDING.



RED LINES - OXYGEN ATOMS
GREEN LINES - CARBON ATOMS
WHITE LINES - HYDROGEN ATOMS

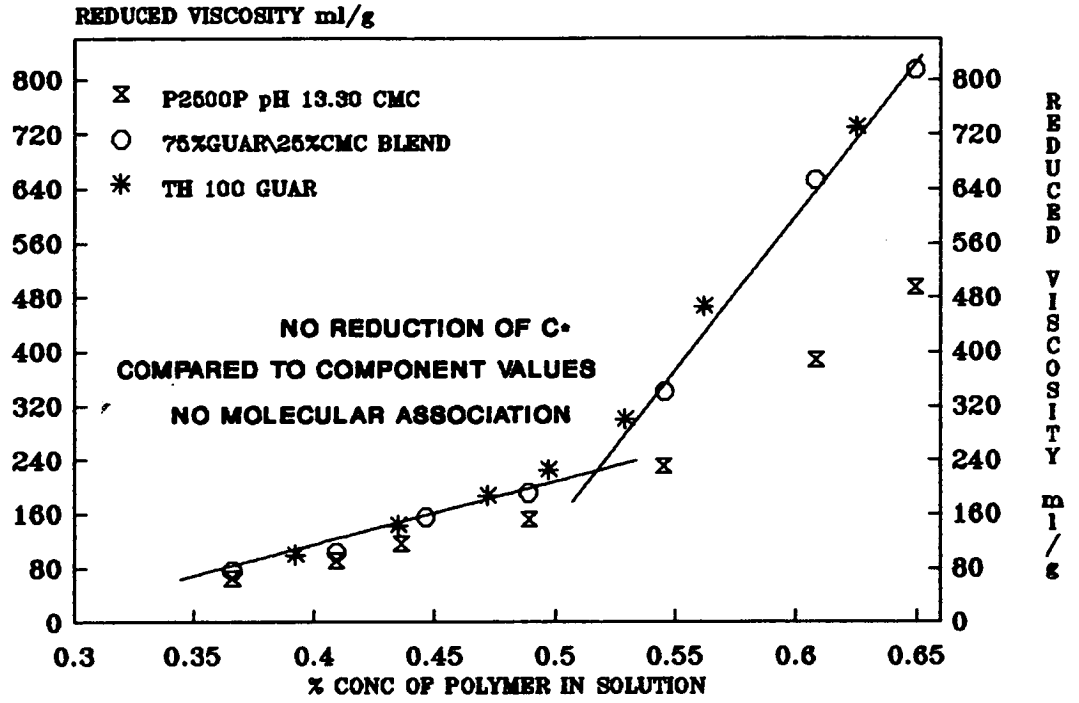
PHOTOGRAPH VII.3

PHOTOGRAPH 3 SHOWS A MORE DETAILED REPRESENTATION OF PHOTO 2 AND SHOWS THE EXISTANCE OF HYDROGEN BONDING BETWEEN ADJACENT FREE CARBOXYLS GROUPS (INTRAMOLECULAR).



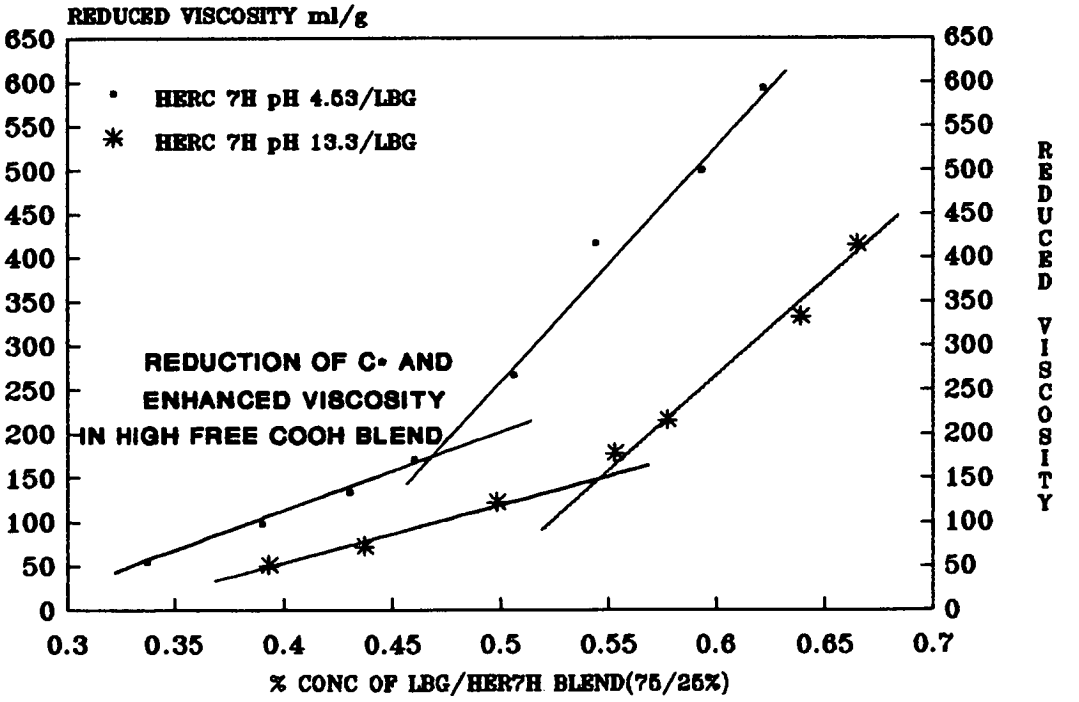
RED LINES - OXYGEN ATOMS
GREEN LINES - CARBON ATOMS
WHITE LINES - HYDROGEN ATOMS

OSTWALD VISCOMETRY DATA FOR A COUGAR/CMC BLEND. EFFECT OF ZERO FREE CARBOXYL CONTENT ON C* AND HYDRODYNAMIC VOLUME.



GRAPH 7.25

OSTWALD VISCOMETRY DATA FOR A LBG/CMC BLEND.EFFECT OF VARIATION IN FREE CARBOXYL CONTENT ON C*



GRAPH 7.26

component polymers.

Graph VII.26 shows the difference between the acid washed and the alkali washed CMC blends with locust bean gum (LBG) and reinforces the above proposed mechanism. Again in this example synergistic viscosity enhancement still exists in the alkali washed CMC/LBG blend but this may result from a competitive dehydration mechanism (Mechanism 2) as previously discussed.

VII (vi) VARIATION IN FINE STRUCTURE OF GUAR GUM.

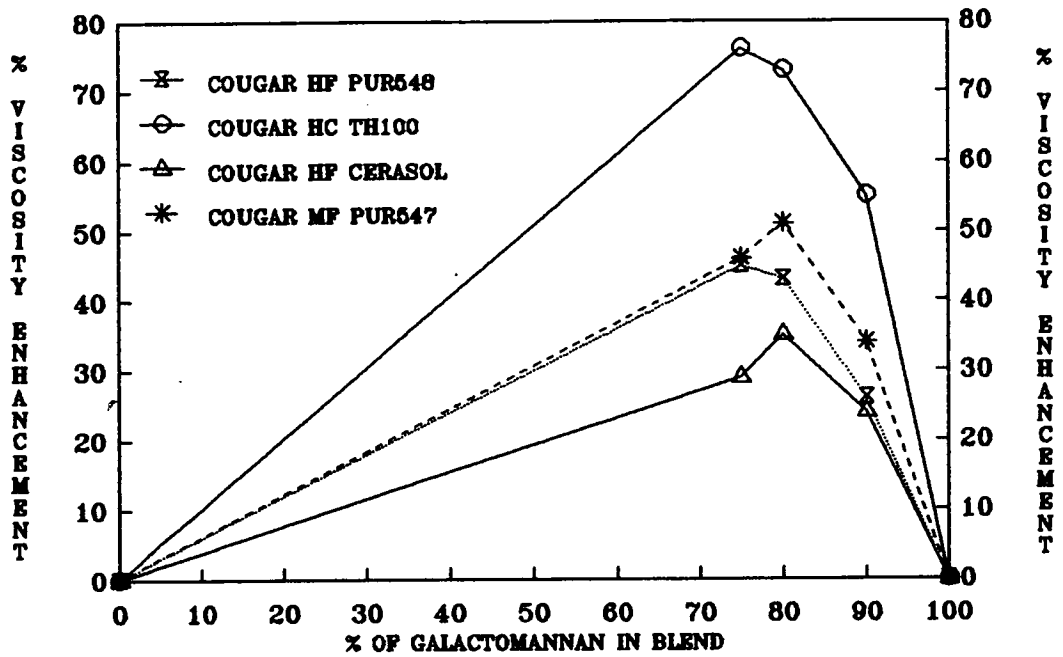
There has been great debate over the fine structure of guar gum (72). The fine structure of guar gum, from different sources and its varying interaction properties in CMC polymer blends is now investigated. In the Xanthan\galactomannan interaction (discussed in chapter V) it had been suggested that blocks of unsubstituted mannose backbone on the galactomannan could form cross-links by interacting with the helical structure formed by xanthan (73,74). Extending this further it follows that the lower the galactose content, the greater the interaction. This appears to be reflected in the comparitive behavior of guar and locust bean gum (graph VII.1) in CMC polymer blends. A substantial amount of effort has been focused on treating guar gum with various galactosidase enzymes to produce a LBG "lookalike", even to the extent of genetically engineering a yeast to express the correct

galactosidase by various workers (23,24).

It appears however that the complete mechanism is not at all simple. It is also possible that evenly substituted regions of the galactomannan can interact strongly with xanthan gum, providing that the galactose units lie on exactly alternating mannose residues and hence tend to protrude on one side of the backbone only, leaving the other side free for interaction (19). Hence galactomannans with relatively high galactose contents such as that extracted from Leucaena leucocephala, can possess stronger interaction properties with xanthan gum, than galactomannans with similar galactose contents, such as guar gum from Cyamopsis tetragonolbus but with differing galactose distributions. This may explain differences in the interaction properties between galactomannans of various species (e.g. guar and LBG).However graph VII.27 and VII.28 indicate that substantial differences in the degree of synergistic interaction exist between various guar grades, all from the same species but from different geographical sources.

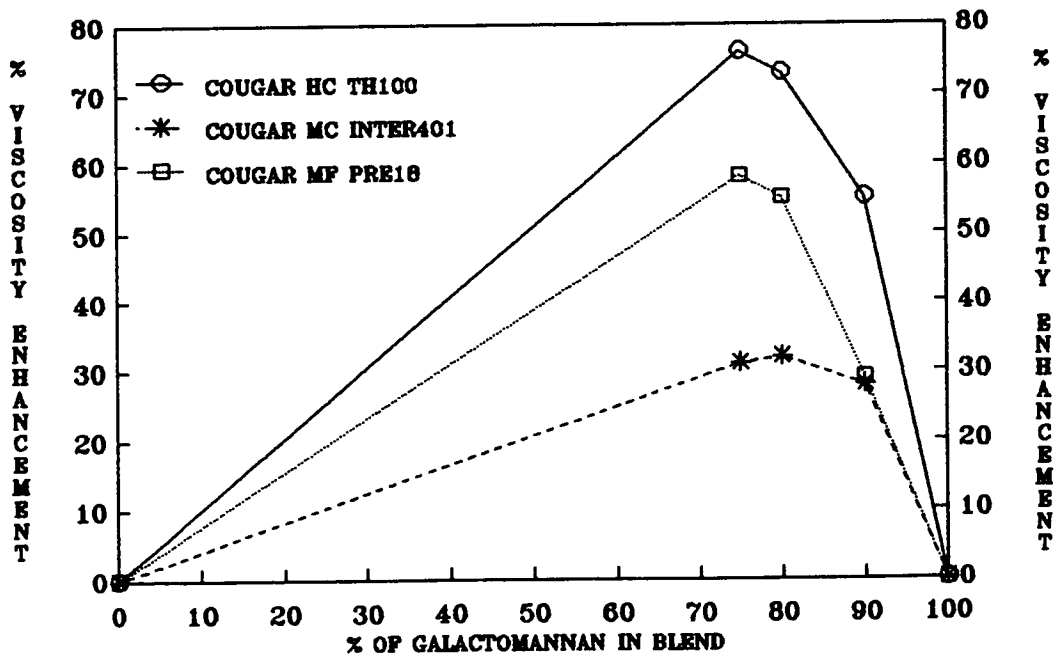
Experiments were undertaken to investigate if the variation in viscosity enhancement of the guar/CMC blends could be linked to the molecular weight differences or fine structural distribution differences between various guar samples. Interestingly graph VII.28 indicates that the synergy maxima is not consistent for all samples but varies from a blend containing 75% guar (TH100) to a blend containing 80%

COMPARATIVE ANALYSIS OF FINE STRUCTURE
% ENHANCEMENT OF COURLOSE P2500P
SYNERGISTIC BLENDS WITH VARIOUS GUARS



GRAPH 7.27
1% TOTAL POLYMER CONCENTRATION

COMPARATIVE ANALYSIS OF GUAR FINE
STRUCTURE %ENHANCEMENT OF COURLOSE
CMC P2500P SYNERGISTIC BLENDS



GRAPH 7.28
1% TOTAL POLYMER CONCENTRATION

guar (INTER 401). Secondly the resultant blend viscosity achieved varies considerably depending on the source of the guar and does not correlate directly with the guar's molecular weight.ie the assumption that the larger the guar's initial viscosity the greater the degree of interaction. On the contrary in some cases greatest synergistic enhancement in a blend with CMC was achieved with lower molecular weight guars.

At a later stage in the study various molecular weight guars (gamma irradiated) were blended with CMC and the viscosity enhancement observed decreases with a reduction in the molecular weight of the guar. This assumes that the fine structure of galactose substituents is unaltered by the degradation of the mannose backbone. The explanation for the above seemingly contradictory results, may be accounted for by the possibility that differences in the fine structural galactose distribution on the guar backbone contribute to a greater extent to synergy than simple molecular weight differences. Work with galactomannan and agarose blends arrive at similar conclusions (17).

Two guars (TH100 and PUR 548) which had displayed different degrees of enhancement with CMC in its natural state graph VII.27 (ie. with approximately 90% of its CMC's carboxyl functional groups in the sodium salt form), were now blended with high free carboxyl and zero free carboxyl CMC. The structure of CMC was modified as in previous experiments with acid and alkali washing in a ethanolic slurry. The results

are shown in graph VII.29. The two guars show almost the same degree of enhancement with alkali washed CMC. Both guars had similar initial Brookfield viscosities of approximately 4600cps. Since no free carboxyls can exist on the CMC chain, molecular association by intermolecular hydrogen bonding is not possible. Therefore the enhanced viscosities observed are as a direct contribution from the competitive dehydration mechanism due to CMC being relatively more hydrophilic than guar gum (graph VII.16). In the example of the acid washed CMC blends a similar result is not evident. Here guar TH100 appears to interact to give larger viscosity enhancement than guar PUR548. In this case there is still a small contribution from competitive dehydration, but this will be similar for both guars as was observed in the alkali washed CMC/guar blends. The difference in the interactive properties of the two guar samples may be due to differences in the galactose fine structural distribution on the polymannose backbone. It appears that the fine structure of guar TH100 favours stronger association with the cellulosic polymer's free carboxyl groups than the PUR548 guar does.

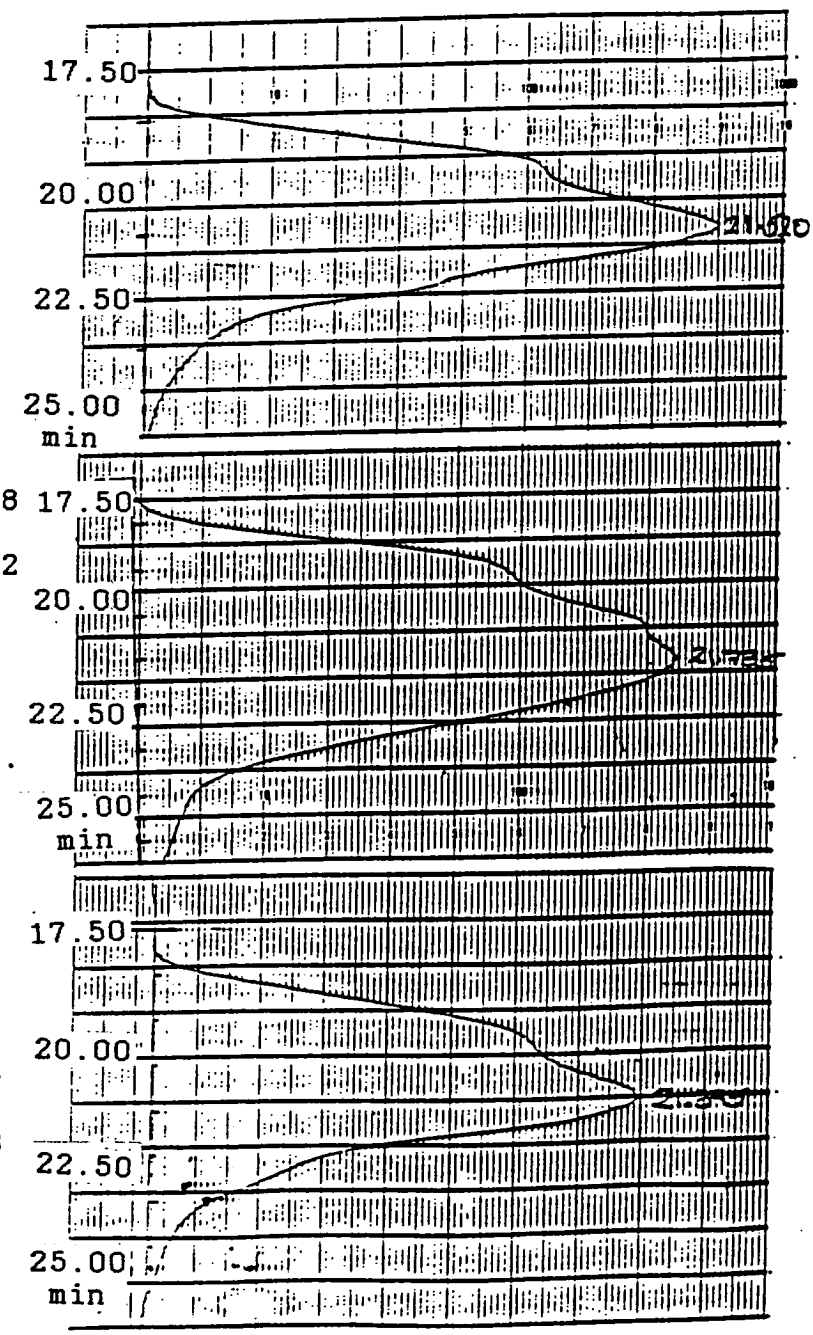
Gel Permeation Chromatograms were run on various guar samples, to investigate whether their molecular weight distributions were similar. Three guar gum samples which gave varying degrees of association when blended with acid washed CMC, but were all of

similar 1% Brookfield viscosities (4400-4600 cps) were run on a Waters GPC linear and 1000 column and their molecular weight distributions compared. It can be concluded from the similar retention times that it is the fine structural galactose distribution and not the molecular weight distributions of the guars that is the most important parameter influencing synergistic enhancement.

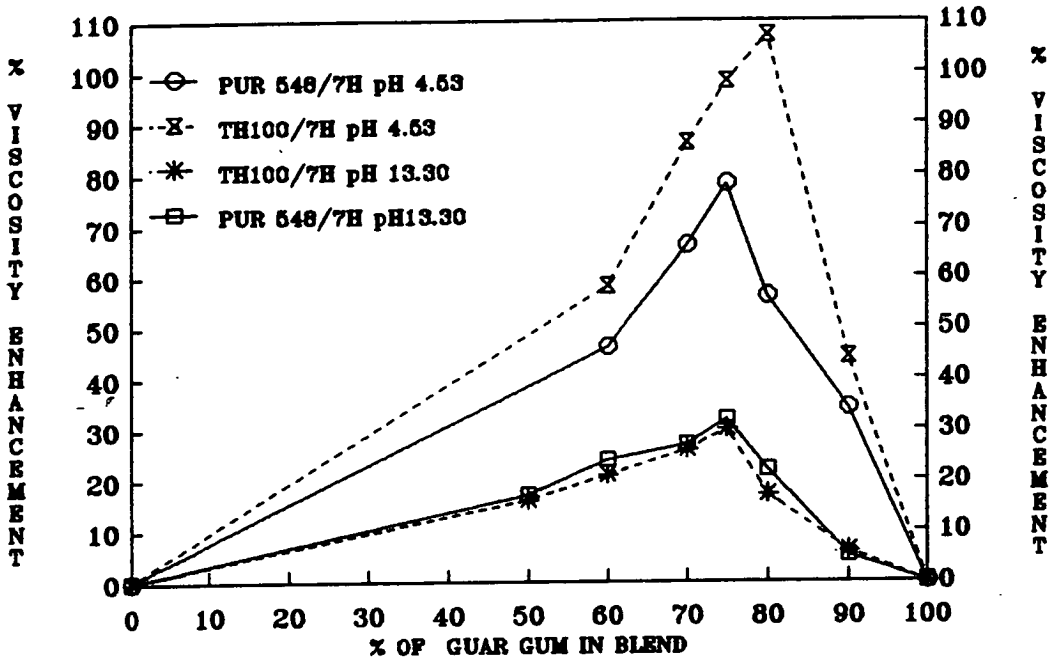
Trace 1. HF Guar TH 100.
0.1% polymer
0.1M $Na^+NO_3^-$
Buffer soln
Scale factor 8
Sensitivity 32

Trace 2. HF Guar Cerasol.

Trace 3. HF Guar Pur 548



VARIATION IN FREE CARBOXYL CONTENT
WITH VARIOUS GUAR FINE STRUCTURAL
DIFFERENCES HER 7H CMC/GUAR BLEND



GRAPH 7.29
1% TOTAL POLYMER CONCENTRATION

TABLE VII.1 13C N.M.R DATA OF GALACTOSE SUBSTITUTION ON TWO GUAR GUM SAMPLES OF VARYING FINE STRUCTURE.

SUGAR RATIO	GUAR TH 100	GUAR PUR 548
% GALACTOSE	37.9%	38.4%
% MANNOSE	62.1%	61.6%
G/M RATIO	0.61	0.62
DIAD FREQUENCY		
GG RATIO	0.40	0.41
GM + MG RATIO	0.39	0.43
MM RATIO	0.21	0.16

Some structural elucidation of the guar's galactose substitution pattern was undertaken at this stage using Nuclear Magnetic Resonance (NMR) spectroscopy to reinforce the suggestion that differences in fine structural distributions of various guar samples (of similar D.P) is responsible for varying interaction properties in polymer blends. NMR has been reported (75,76) to provide reliable imformation on galactose/mannose ratios and galactose sequence distributions in guar and other related galactomannans. The nature and rheological properties of the polymers being studied render NMR techniques difficult due to high intrinsic viscosities. To reduce the background signal/noise ratio it is necessary to use high concentrations of polymer solutions (77). However for this spectroscopic technique to function efficiently, molecules must tumble freely in solution. Due to the very high viscosities, at relatively low polymer concentrations of guar gum, molecular tumbling is inhibited.

To overcome this problem partially hydrolysed molecular fragments of the polymer are analysed by NMR. An enzyme digestion method, using a purified NOVO mannosidase enzyme solution was used to degrade the mannose backbone, but leave the galactose substituents unchanged. Carbon 13 NMR is reported to give more accurate galactose/mannose (g/m) ratios than proton NMR as well as allowing mannose sequencing at the diad level. The results from the proton and ^{13}C NMR

spectra of TH100 guar and PUR 548 guar are shown in Table VII.1.

The analysis shows that both samples have very similar g/m ratios, and that the carbon results are in good agreement with the proton results. The sequence distributions are slightly different at the diad level, however the slightly higher MM content of the TH100 sample is explainable in terms of a slightly "blockier" galactose distribution on the mannose backbone. This more "blocky" nature of the galactose substitution pattern would leave other sections of unsubstituted "smooth" mannose regions and hence explain the greater interactive properties of TH100 in a blend with CMC.

VII (vii) SOLID STATE N.M.R ON A SYNERGISTIC POLYMER BLEND.

Three samples were submitted, which had all been prepared from solutions by freeze drying by identical methods, for solid state NMR analysis. Solid state ^{13}C NMR enables the molecular associations observed in a LBG/CMC blend in solution to be investigated in the solid state. The samples were pure locust bean gum, acid washed carboxymethyl cellulose (Courlose P2500P), and a blend of the two in a ratio 75% LBG : 25% CMC. This polymer-polymer blend showed high synergistic characteristics in solution (graph

VII.1) which were believed to be retained in the solid state. The purpose of the investigation was to identify specific intermolecular interactions which might lead to further understanding of the proposed mechanism by which the synergistic association is occurring.

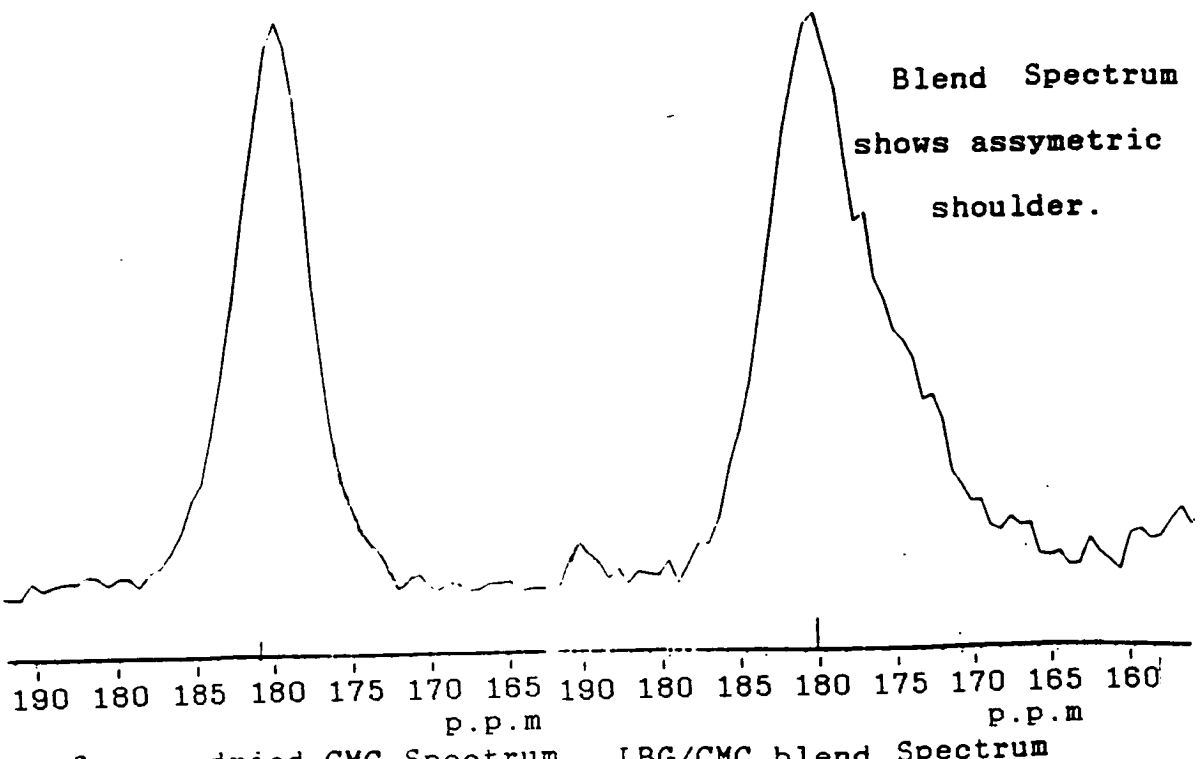
The three samples were each compacted in solid state rotors and the magic angle spinning/ crosspolarisation technique was used to aquire solid state carbon spectra (78,79). Proton Tle measurements were also carried out by indirect observation of the variation of carbon intensities with crosspolarisation delay. The most significant spectra to compare are CMC in isolation, and CMC in the presence of the interacting LBG. Only CMC has a substituent with a carbonyl (carbonyls from protein content in LBG is neglible), so any change in this must be due to an indirect effect of the LBG in the solid. The carbonyl resonance in the blend becomes a smaller proportion of the total because the mannose and galactose sugars of the LBG superimpose on those of the glucopyranosyl units of CMC. However the expansion of this region (160-190 p.p.m) clearly shows that in the LBG/CMC polymer blend there is an additional shoulder at a higher field, compared to the single symmetrical peak in pure CMC (Spectrum VII.1).

A further spectrum of the blend was acquired at a lower spin rate, so that it could be determined whether this observed assymetry on the blend carbonyl region could be a result of a second order

TABLE VII.2. RESULTS OF MATHEMATICAL CURVE FITTING PROGRAMMES TO DECONVOLUTE SHOULDER PEAK IN CARBONYL REGION OF BLEND SPECTRA.

LINESHAPE	GAUSSIAN MODEL	LORENTZIAN MODEL	GUASSIAN + LORENTZIAN
CHEMICAL SHIFT (ppm)	178 175	177 174	178 175
LINE WIDTH (Hz)	286 681	292 422	260 592
RELATIVE INTEGRAL	1.24 2.64	2.64 1.42	1.59 1.76

SPECTRUM 7.1 NMR SPECTRA OF CMC P2500P AND BLEND OF CMC/LBG IN CARBONYL REGION.



spinning side band from the main ring peak. It was concluded that the assymetry observed was a real effect, and not a second order spinning side band. The results suggest that the a proportion of the carbonyl groups in the blend are in a different chemical environment than those in the pure CMC sample. A NMR curve fitting program was adapted to the blend's carbonyl region to deconvolute the shoulder and the main peak. Several mathematical line shape functions were tried, the results of which are shown in Table VII.2. The table shows the chemical shift of the symmetric part of the peak at low field (178 p.p.m) and the shoulder peak at higher field (175 p.p.m). A simple subtraction of the CMC from the blend carbonyl suggested that the high ppm and low ppm blend peaks were in the ratio 1 to 2 respectively. This is consistent with the Gaussian model. The two spectra with specific carbonyl region of interest are shown in Spectra 7.1.

The proton Tle data also suggests that the CMC polymer is strongly interacting with the LBG. Pure CMC has a Tle of approximately 2.1ms and this increases to 2.9ms in the blend, measured through the carbonyl. The value for LBG is approximately 3.1ms. Since Tle is related to very local dynamic properties this influence of LBG on CMC indicates that the majority of the two types of polymer chains are within 20Å of each other. This reinforces previous evidence of the intermolecular association mechanism between an

anionic cellulosic and a non-ionic polymer.

VII (viii) RHEOLOGICAL PROPERTIES OF POLYMER BLENDS.

The effect of increasing shear rate on the rheological properties of various unlike polymer blends was investigated (37,80,81). An understanding of rheology (flow behavior), is of ultimate importance when examining the shear rate dependancy of polymer solutions. Basically four types of flow can be distinguished: Newtonian, plastic, pseudoplastic and dilitant. Plastic flow is characterised by the presence of a yield point above which the material starts to flow in a Newtonian manner. Thixotropy is a reversible, time dependant, shear thinning effect, which is caused by a temporary structure, which can break down under shear. Removing the shearing forces allows the structure to gradually rebuild. This study will not concern itself with dilitant flow behavior where viscosity increases with increase in shear rate. For simple "ideal" liquids like oils or solutions of small molecules (glucose), the shear rate increases linearly with shear stress and such materials ("Newtonian") have a single fixed viscosity (36). Concentrated polysaccharride solutions almost invariably exhibit non-Newtonian behavior. That is, doubling the shear stress produces more than twice the rate of flow.

The polysaccharides studied generally

undergo a shear thinning regime in which the relative viscosity decreases with increasing shear rate (82,83). The present studies were confined to flow behavior of polymers under lateral shear, since this is the most commonly utilised form of viscosity measurement. Shear viscosity is defined as the ratio of the applied shear stress to the resulting rate of shear. In this study the areas of interest are the shape of the non-Newtonian part of the apparent viscosity curve with increasing shear.

Solutions of guar gum have zero shear yield value at the most commonly used concentrations. They begin to flow as soon as the slightest shear is applied (33). The apparent viscosity of the solution decreases sharply as the rate of shear increases then levels off and approaches a minimum limiting value. CMC solutions can also exhibit pseudoplastic behavior but most derivatives having DS values below 0.8 are thixotropic (84). Guar and HPMC also exhibit some pseudoplastic shear thinning behavior. The pseudoplasticity of CMC and HPMC increases with increasing concentration and with increasing molecular weight, whereas Newtonian rheology is exhibited over relatively broad shear rates for low molecular weight polymers.

Pseudoplastic shear thinning properties are observed when the viscosity pathways of increasing and decreasing shear rates of these polymer solutions are traversed using a Haake viscometer and no

hysteresis loop is observed. This suggests that the relaxation of the polysaccharide solutions in the shear regime studied is rapid and occurs within a few seconds. The origin of pseudoplasticity in macromolecules has been suggested to be as a result of the following parameters, one or more of which might apply to a specific situation (32).

(1) increased orientation of asymmetric molecules with shear rate.
(2) change in the shape of flexible molecules with increased shear rate.
(3) effect of flow on intermolecular interactions.

The non-Newtonian behavior of dilute polysaccharide solutions may be explained by (1) and (2) whereas it is necessary to include (3) in discussions on concentrated solutions (above C*).

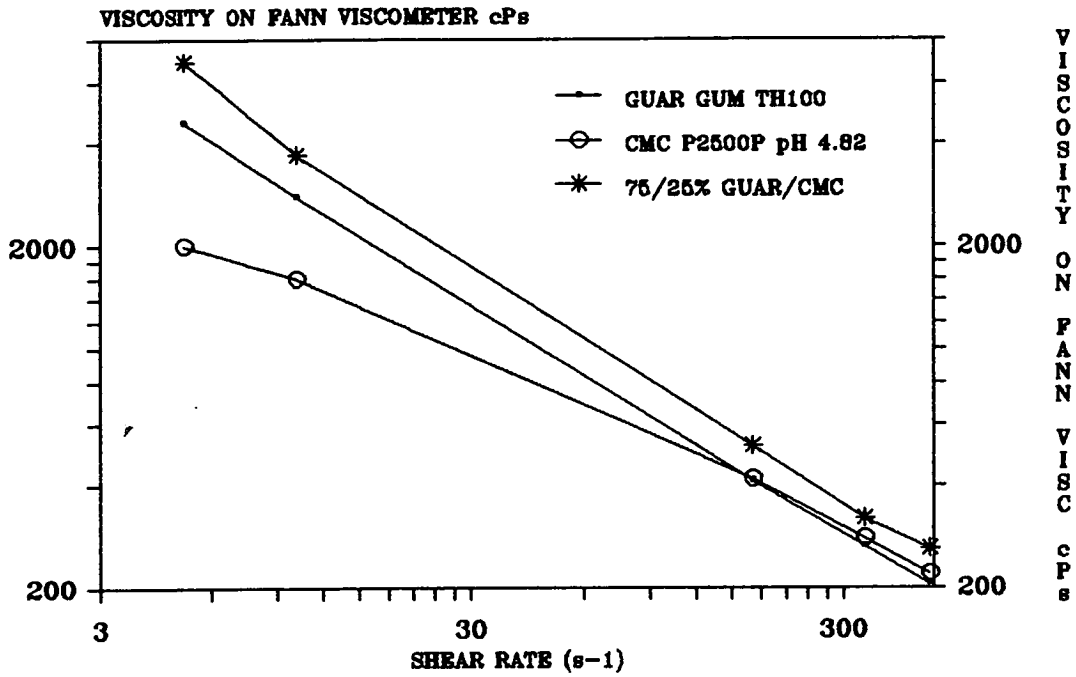
Asymmetric molecules such as rods and ellipsoids will tend to orientate themselves parallel to the direction of flow, when the viscosity will be a minimum. This orientation will be opposed by random movements caused by Brownian motion so at very low shear rates the molecules will be orientated randomly with a resultant viscosity maxima. Therefore reduction of viscosity from a maximum value with increasing shear is relatively minor for dilute solutions and arises from the alignment of transiently elongated coils in

the direction of flow (38). For more concentrated polymer solutions shear thinning properties are more marked and must be explained by mechanism (3) shown above.

Above C* the hydrodynamic volume of individual polymer chains exceeds the total volume of the solution, ie. polymer overlap occurs. Interpenetrations of polymer coils in more concentrated solutions give rise to a dynamic entangled network structure (37). At low shear rates, although the entanglements are disrupted, they are replaced by new similar interactions, thus very little apparent viscosity reduction occurs. At higher shear, the rate of formation of externally exposed movement disrupting the network exceeds the rate of formation of new entanglement, and viscosity reduction is more apparent (85). Shear thinning can also be understood in terms of timescale of relaxation for inter and intramolecular effects. At higher shear the timescale of relaxation is reduced and the entangled network is disrupted (81).

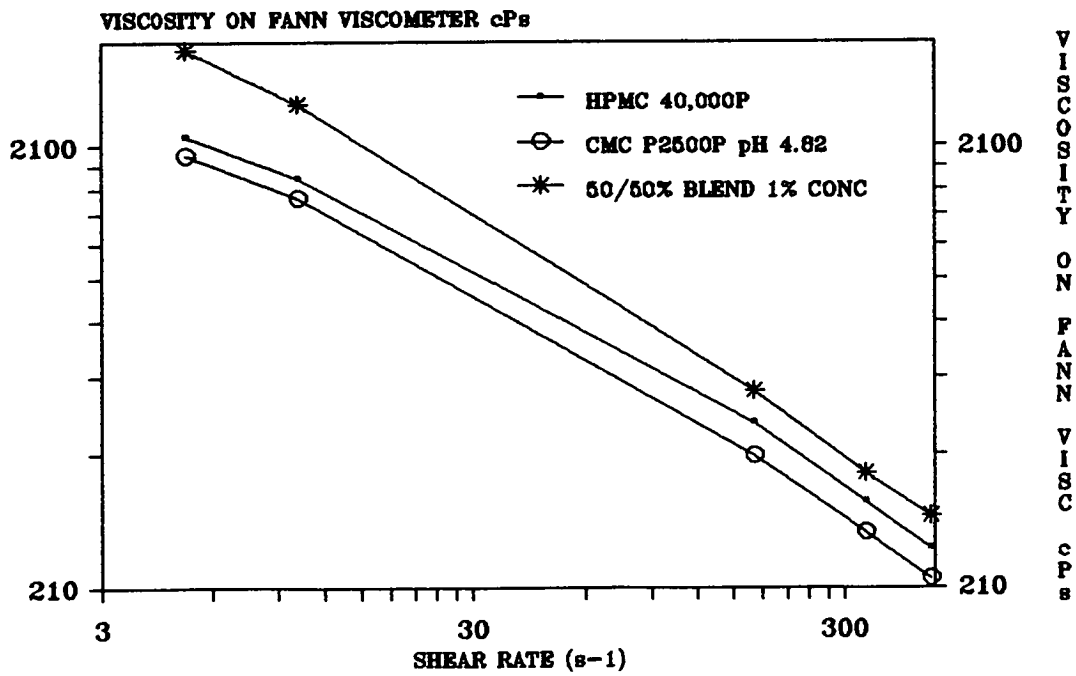
Graph VII.30 shows how the viscosities of an acid washed CMC, a guar gum solution and a 25/75% blend of the two components vary with increasing shear rate on a Fann viscometer. It appears that the viscosity of the blend is greater than either component viscosities even at higher shear rates. It is also interesting to observe that guar gum is more shear sensitive over this range of shear than CMC. A similar result is obtained in graph VII.31 with an acid washed

GUAR GUM TH100/CMC 2500P BLEND
EFFECT ON VISCOSITY OF INCREASING
THE SHEAR RATE



GRAPH 7.30
1% TOTAL POLYMER CONCENTRATION

CELACOL HPMC 40,000P/CMC 2500P BLEND
EFFECT ON VISCOSITY OF INCREASING
THE SHEAR RATE

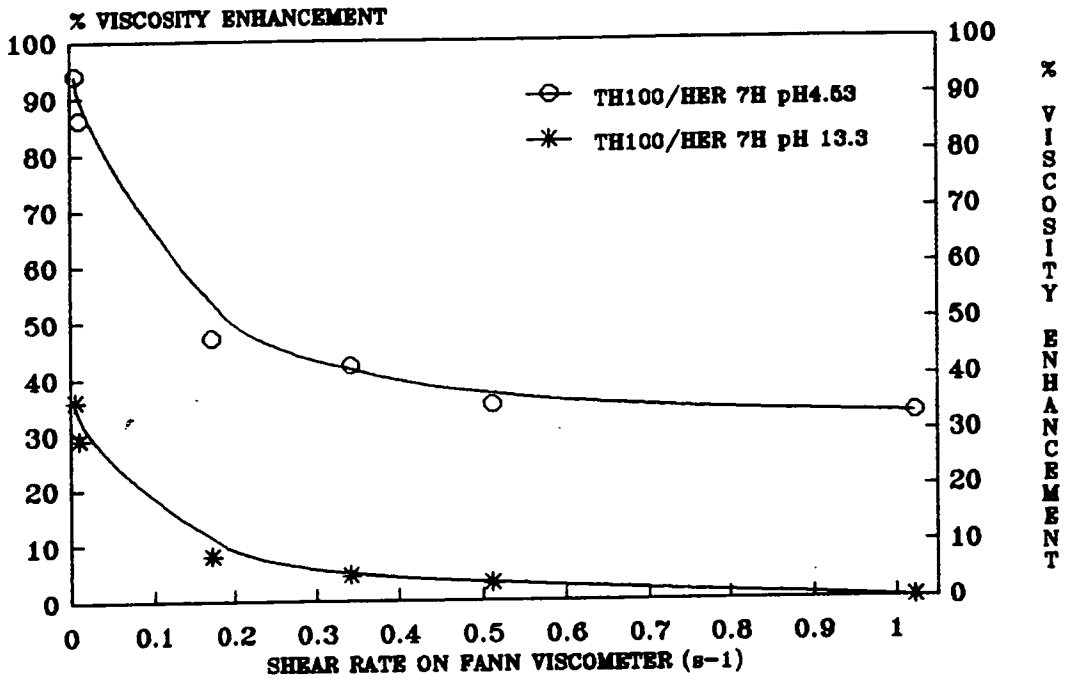


GRAPH 7.31
1% TOTAL POLYMER CONCENTRATION

CMC/HPMC blend (50/50% blend), where again the blend viscosity is greater than either component viscosities at all shear rates. This may indicate that some degree of molecular association is maintained at higher shear rates. However since two co-existing mechanisms have been proposed and competitive dehydration might also contribute to viscosity enhancement at high shear, similar blends using alkali washed CMC with guar and HPMC respectively were also examined.

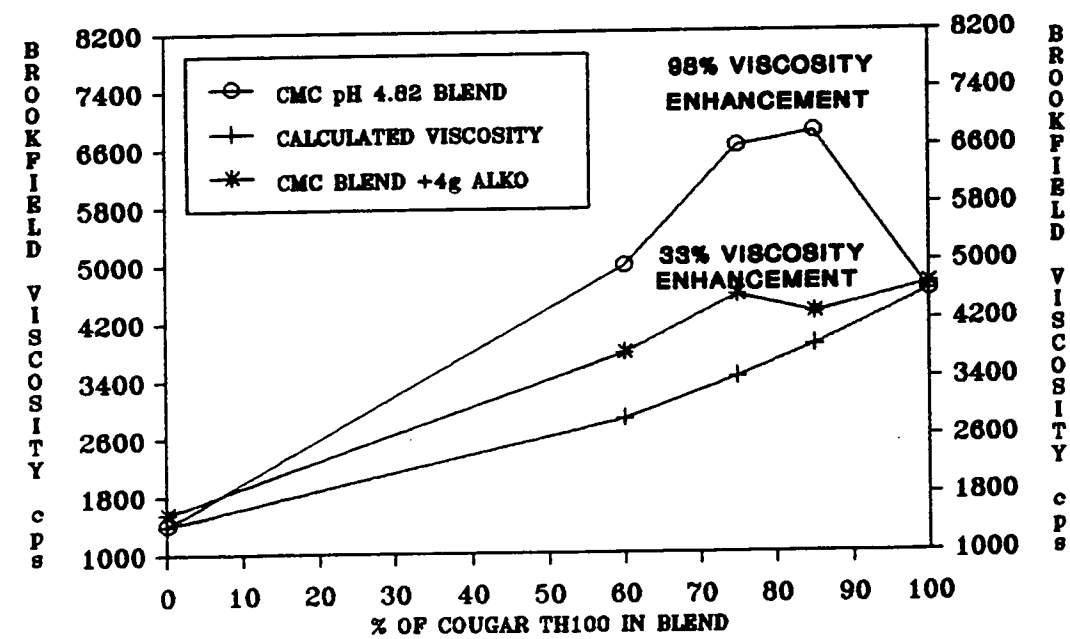
A plot of calculated viscosity enhancement compared to component polymers for the acid washed CMC/guar blend and the alkali washed CMC/guar blend were compared. The results are displayed on graph VII.32. It can be noted that the viscosity enhancement of the alkali washed CMC/guar blend falls from approximately 36% viscosity enhancement to almost zero with increasing shear rate, therefore the contribution from competitive dehydration to viscosity enhancement is almost eliminated at this higher shear rate. However in the acid washed CMC/guar blend the enhancement decreases as expected with increasing shear but then levels off. This indicates that the hydrogen bonds in the association mechanism with the acid washed, high free carboxyl CMC/guar blend are relatively strong. Enhancement might have been expected to fall off to almost zero with increasing shear due to reduction in relaxation time, i.e. once broken the interactions have insufficient time to reform.

EFFECT OF VARIATION IN FREE CARBOXYL
CONTENT ON SHEAR SENSITIVITY OF A
GUAR/HER 7H CMC 75/25% BLEND



GRAPH 7.32
1% TOTAL POLYMER CONCENTRATION

COMPETITIVE INHIBITION EFFECT ON
TH100 GUAR /CMC 2500P (75/25%) BLEND
ADDITION OF LOW MOLECULAR WEIGHT CMC



GRAPH 7.33
1% TOTAL BLENDED POLYMER CONCENTRATION
4g ALKO ADDED FOR COMPETITIVE INHIBITION

VII (ix) COMPETITIVE INHIBITION IN POLYMER BLENDS.

The next experiment was an attempt to block potential molecular interaction sites on one polymer chain deliberately, to inhibit the synergistic enhancement previously observed (graph VII.1) and thus reinforce the proposed mechanisms. Competitive inhibition has been explored to investigate specific intermolecular associations in several binary polysaccharide systems in recent years (86). For example, gel formation in a Xanthan/LBG polymer blend is significantly inhibited by the addition of a galactomannan with few unsubstituted chain sequences (ie a "hairy" backbone). It appears that this galactomannan associates with the xanthan without resultant gel formation. Competitive inhibition was used to probe the binding specificity in a guar/CMC polymer blend to attempt to reinforce the two previously proposed co-existing mechanisms.

It has been proposed that a proportion of the observed viscosity enhancement in an acid washed CMC/guar blend is a result of specific intermolecular hydrogen bonding between "free" carboxyl (COOH) groups on the CMC with hydroxyls on the non-ionic polymer. If no free carboxyls exist some viscosity enhancement is observed as a result of a competitive dehydration mechanism. Therefore if molecular association occurs it has been shown to give an increase in the overall hydrodynamic volume of the associated polymer chains

(graph VII.5). The hydrodynamic volume of two associated chains is greater than the sum of the two component hydrodynamic volumes.

A very low molecular weight acid washed sample of CMC (Alko CMC) was added to a dry premixed acid washed CMC P2500P/guar blend. This low D.P CMC has a Brookfield viscosity of approximately 10cps at 10% polymer concentration so does not effectively contribute to the overall viscosity of the polymer blend solution. The results are shown on graph VII.33. It can be seen that the viscosity enhancement in the control CMC/guar polymer blend solution where no very low molecular weight CMC has been added is 98% enhancement. This is a similar viscosity enhancement observed in previous experiments (graph VII.10). However the addition of 4 grammes of the low molecular weight CMC reduces the enhancement down to 33%. It is interesting to note that this is a similar magnitude of viscosity enhancement observed in a alkali washed CMC/guar blend where molecular association is not possible (graph VII.16).

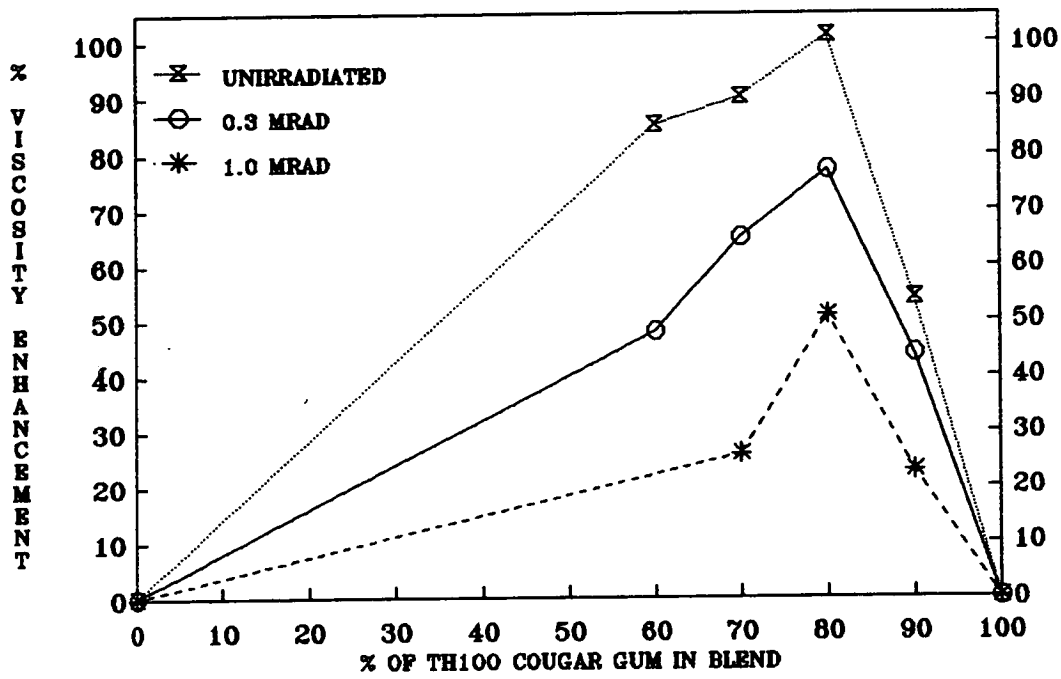
Thus the low molecular weight CMC appears to have blocked potential interaction sites on the guar chain and greatly reduced the chance of molecular association, with a resultant increase in hydrodynamic volume (87). Although the very low molecular weight CMC possibly may associate with the guar chains the increase in hydrodynamic volume of the associated molecules will be minimal. Hydrodynamic

volume increase of associated polymer molecules is maximised when the molecular weights of the non-ionic and anionic polymers are similar.

VII (x) EFFECT OF GAMMA IRRADIATION OF GUAR ON POLYMER-POLYMER INTERACTION.

Irradiation of guar gum by high energy gamma rays cleaves the mannose backbone and subsequently leads to a reduction in the molecular weight and Brookfield viscosity of the galactomannan (88,89). Various guar gum samples of differing molecular weights were blended with acid washed CMC P1500P. It has been shown (graph VII.14) that the contribution to viscosity enhancement from competitive dehydration is small, compared to that of molecular association, in the case when two high molecular weight species (CMC and guar) are blended. As the molecular weight of the guar decreases, the contribution from mechanism 2 (competitive dehydration) will decrease and and may eventually result in overall viscosity reduction. Irradiated guars (0 MRAD, 0.3MRAD and 1.0 MRAD) of varying molecular weight from the same parent (TH100) which had 1% Brookfield viscosities of; 4600, 2600 and 1800 cps respectively were each blended in similar mixing ratios with acid washed CMC P1500P. The results are shown in graph VII.34. This result suggests that synergistic viscosity enhancement when

EFFECT OF GUAR MOLECULAR WEIGHT
ON %ENHANCEMENT OF A TH100/CMC P1500P
SYNERGISTIC BLEND 1% POLYMER CONC.



GRAPH 7.34

MECHANISM 1

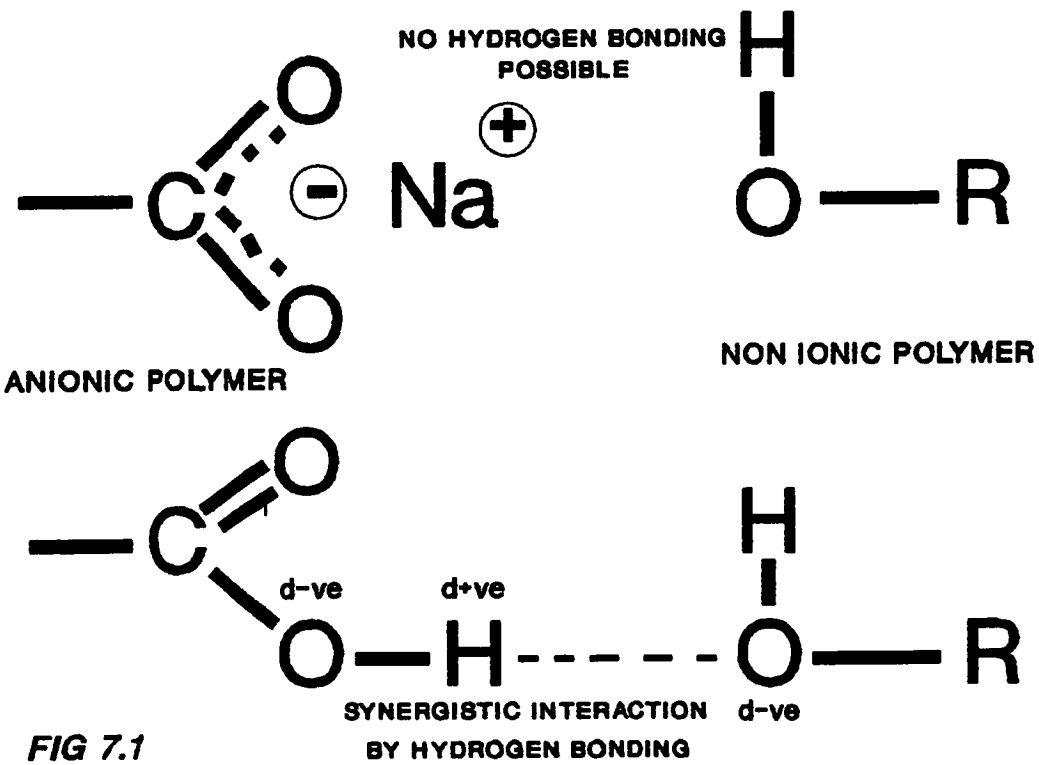


FIG 7.1

contributions from both mechanisms exist are greatest for the highest molecular weight guar. As stated above most of the viscosity enhancement in each case will arise from molecular association (Mechanism 1, refer to Fig 7.1). Care was taken to avoid very low molecular weight guars as these may have given a negative contribution to the competitive dehydration mechanism. It is feasible that the highest molecular weight guar is closest in size to the average CMC chain length and the chance of an increase in hydrodynamic volume by two polymers interacting is maximised.

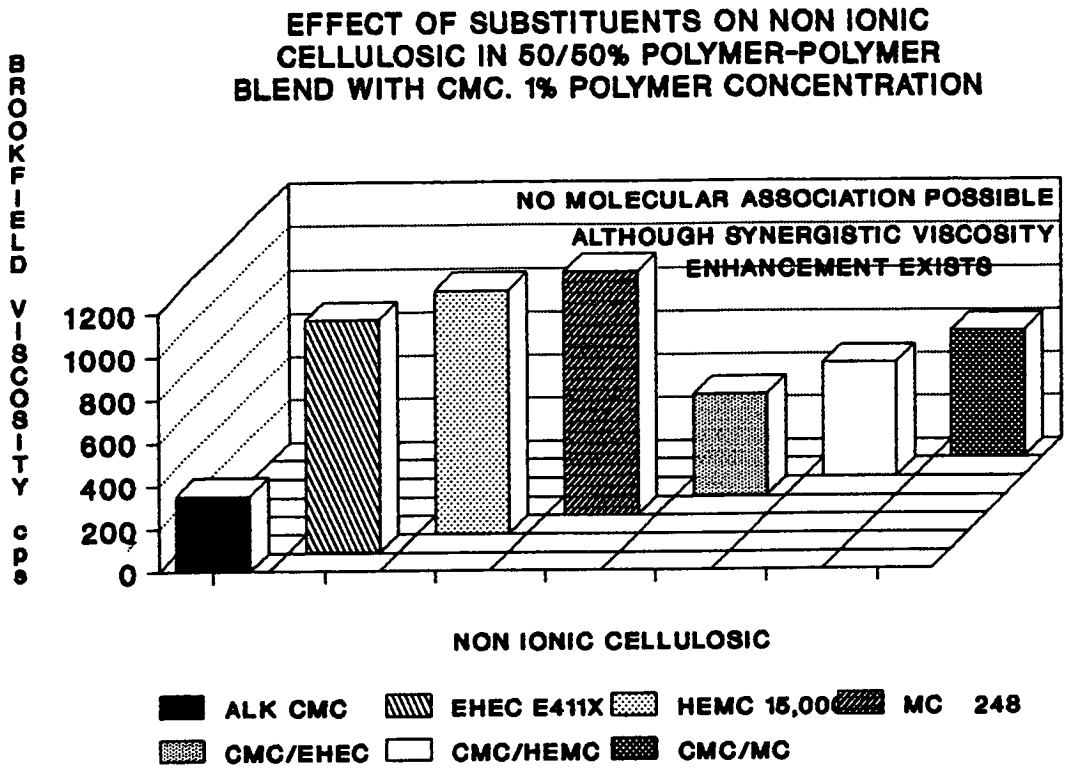
VII (xi) EFFECT OF SUBSTITUENT ON NON-IONIC CELLULOSIC POLYMER.

The commercially available water soluble cellulose ethers contain one or more of the following substituents: alkyl (methyl or ethyl), hydroxyalkyl (hydroxyethyl or hydroxypropyl) and carboxyalkyl (carboxymethyl). At least ten combinations of the above derivatives are commercially available and two or more substituents can exist in each type with corresponding degrees of substitution (33,90). For example in HPMC, variations in the methyl and hydroxypropyl contents of the derivative greatly affects the polymer's rheology and gelling characteristics (91).

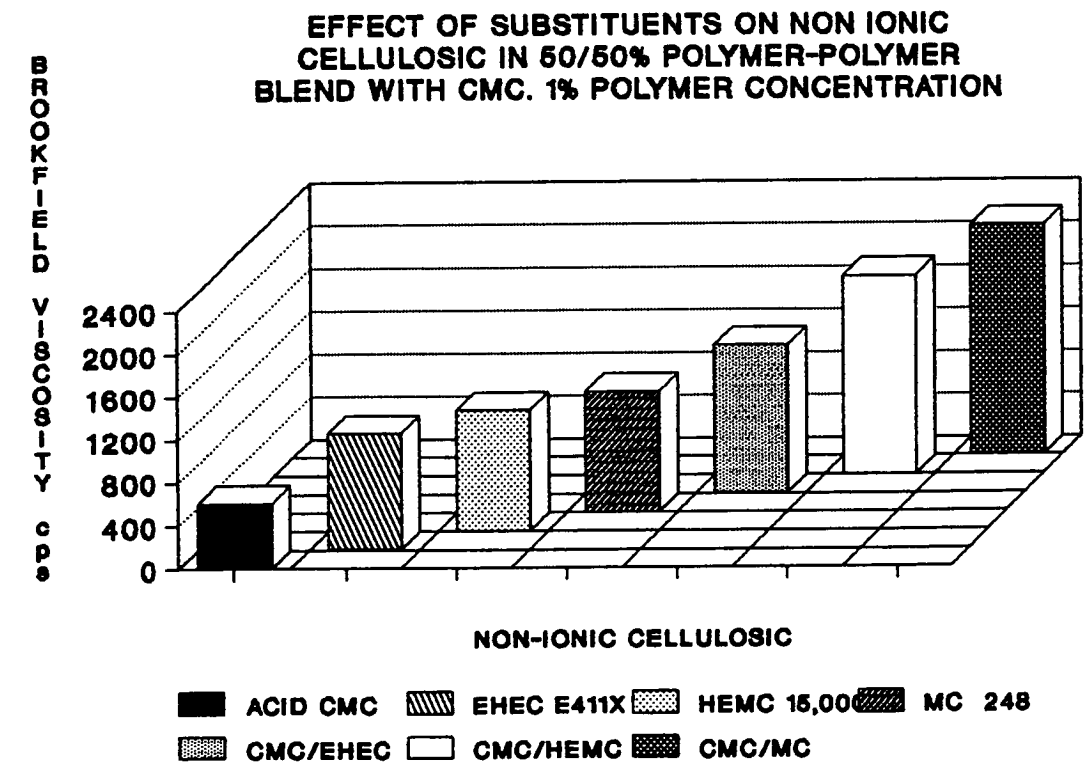
The relative hydrophilicity of these polymers has been related to the equilibrium moisture contents of the polymers (31), and is related to the

substituent type and the substituent level. Three non-ionic cellulosic polymers of very similar molecular weights and 2% Brookfield viscosities (15,000 cps) were selected and blended with the structurally modified acid and alkali washed CMC P400P. This experiment would attempt to demonstrate how different substituent levels on the non-ionic cellulosic affected the degree of synergistic interaction. The three non-ionic cellulosic polymers selected were: MC (methyl cellulose), HEMC (hydroxyethyl methyl cellulose) and EHEC (ethyl hydroxyethyl cellulose). Methyl cellulose (MC) is the most hydrophobic of the three non-ionic polymers and EHEC which has some of its hydroxyethyl substituents end capped with ethyl groups, is the most hydrophilic. CMC is however more hydrophilic than all three as a little amount of anionic character greatly increases a polymer's hydrophilicity (45,91).

When the three non-ionic cellulosics were blended with alkali washed CMC P400P, the mechanisms previously proposed (Fig 7.1) indicate that there should be no possibility of synergistic enhancement as a result of molecular association. All three non-ionics have almost identical 1% Brookfield viscosities and all show some degree of synergistic enhancement in the alkali washed CMC blend (graph 7.35). The competitive dehydration mechanism (Mechanism 2), as a result of the difference in the non-ionics hydrophilic/lypophilic balance in comparison to CMC is likely to be responsible for the observed viscosity



GRAPH 7.35
CMC- P400P. ALKALI WASHED



GRAPH 7.36
CMC- P400P. ACID WASHED

enhancement. The substituent levels on the three non-ionic cellulosics are shown below in Table VII.3.

All three non-ionics have similar viscosity concentration curves. The synergistic enhancement observed ranged from 44% for a EHEC/CMC blend to 59% for a MC/CMC blend. This correlates directly with the polymers hydrophilicity i.e. the more hydrophobic the non-ionic, the greater the synergistic interaction (assuming the D.P's of the non-ionics are similar).

This pattern should remain for the acid washed CMC blend also, but there should be additional synergy as a result of molecular association due to intermolecular hydrogen bonding between free carboxyl groups (COOH) on the CMC and accessible hydroxyls on the non-ionic.

Previously the accessibility of the hydroxyls on the non-ionic has been considered when looking at the comparative synergistic interaction of various guars with CMC. It was shown here that the fine structural distribution of galactose on the mannan backbone is important in estimating the overall enhancement. It has also been shown that a greater enhancement is observed in a LBG/CMC blend than in a guar/CMC blend (92,93). It has been suggested that the interaction is maximised by association of the anionic polymer with smooth regions of the mannose backbone.

Graph 7.36 displays the synergistic viscosity enhancement observed when acid washed CMC

TABLE VII.3 SUBSTITUENT LEVELS IN FOUR NON-IONIC CELLULOSE ETHERS.

NON-IONIC CELLULOSIC ETHER	HYDROXYALKYL SUBSTITUENT MS	ALKYL SUBSTITUENT DS
METHYL CELLULOSE 15,000	0.0	1.9
TYLOSE HEMC 15,000	0.2	1.8
BERMACOLL EHEC 15,000	1.4	0.8

TABLE VII.4 SYNERGISTIC VISCOSITY ENHANCEMENT OF TWO METHYL CELLULOSE SAMPLES IN A CMC P1500P POLYMER BLEND (2% TOTAL POLYMER CONCENTRATION).

CELLULOSE ETHER	METHYL D.S	BROOKFIELD VISCOSITY	
		50/50% ACID WASHED CMC	50/50% ALK WASHED CMC
CELACOL M5000 (MC) 4900 cps 2%	1.9	12,700 cps	7,900 cps
DOW A4M (MC) 4700 cps(2%)	1.9	15,100 cps	7,600 cps

P400P is blended with the same three non-ionics. If the number of hydroxyl groups on the non-ionic was the sole determining factor for viscosity enhancement, EHEC has the greatest availability and MC has the least (63,94). However the synergistic viscosity enhancement observed was: 78% for EHEC, 144% for HEMC and 184% for MC (95). Therefore an explanation for this apparently contradictory result may be based on the accessibility of the hydroxyl functional groups.

Many of the most accessible hydroxyls on EHEC exist on the side chain substituents and these may sterically hinder maximum association with an anionic CMC molecule. It has been shown that some molecular association still exists in a CMC/guar blend even at relatively high shear rates (graph 7.32), thus the association must be built on numerous hydrogen bonds between unlike polymer chains in a "zipper" type formation. When sheared, one bond may break, but it may be held in close spatial proximity by the other intermolecular hydrogen bonds. Therefore although hydroxyls on the side chain substituents would appear to promote maximum interaction, they appear actually to hinder the association. It is also possible that although the M.S (molar substitution) value of EHEC is 2.2 the D.S (degree of substitution) may be considerably lower as a result of substituent chain branching.

It appears that a greater interaction occurs if all the available hydroxyls are located on

underivitised hydroxyls on the glucopyranosyl backbone of cellulose. The methyl cellulose used has 30% of its hydroxyls capped with hydrophobic methyl substituents (63). However it has been shown by theoretical calculations (96), by the process conditions and the crystalline nature of cellulose that these methyl substituents are distributed in a "blocky" manner. As methylene chloride is introduced to the reaction pressure vessel and starts to react, subsequent derivatisation may occur on nearby derivatised units, where the closely bound crystalline regions of cellulose have been disrupted. In solution these hydrophobic methyl moieties will associate with each other and leave hydroxyl groups on unreacted glucopyranosyl units exposed for interaction with the free carboxyls on CMC.

Another study (48), has tried to correlate the interaction of various non-ionic cellulosics with CMC but the conclusions drawn were that it was difficult to equate the viscosity enhancement with substituent type. It is only possible if, as in the present study, the non-ionics have very similar D.P values and if the two co-existing synergistic enhancement mechanisms are studied in isolation ie. once one is calculated, its contribution to the other can be accurately estimated as shown above.

Table VII.4 shows the synergistic viscosity enhancement observed when two methyl

cellulose grades of very similar D.P's and 2% Brookfield viscosities are blended with alkali washed and acid washed CMC P1500P.

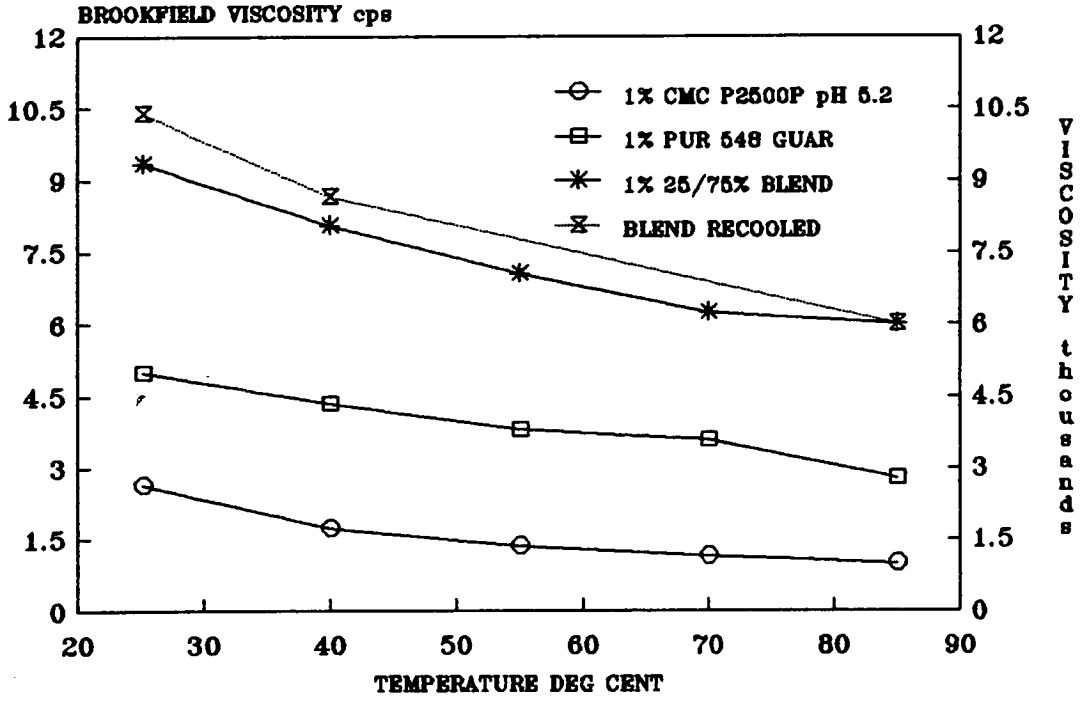
As these two methyl celluloses have the same degree of substitution and D.P, therefore similar hydrophilicities and viscosity/concentration curves, it would be expected that the viscosity enhancement observed when blended with the alkali washed CMC (no molecúlar association) would be similar. This appears to be the case and the viscosity enhancement in each case would be as a result of competitive dehydration. A similar result may be expected with the acid washed CMC P1500P blend. However the Dow A4M methyl cellulose appears to give greater viscosity enhancement than the Courtaulds Celacol M5000 grade. An possible explanation for this result is that the two non-ionic cellulose ethers are manufactured by different routes. Therefore although the average degree of substitution of methyl substituents along a polymer molecule is similar, the distribution of these methyl groups may be different. This would lead to the different distribution of accessible hydroxyls on the cellulose backbone of the two polymer grades. This result indicates that many factors must be considered when predicting possible synergistic viscosity enhancements between anionic and non-ionic cellulose ethers.

VII (xii) EFFECT OF TEMPERATURE AND ELECTROLYTES ON BLEND VISCOSITY.

The effect of increasing the temperature of a CMC/guar blend compared to its component polymers was investigated. Both CMC P2500P and guar gum (PUR 548) become less viscous as temperature increases, as the more energetic water molecules become less associated with the polymer's structure (97). The polymers usually return to their initial viscosity once recooled. Degradation may occur if the polymer is exposed to very high water temperatures for a prolonged time interval. Various reports have investigated the mechanism of thermal degradation of these polymers (98). These polymers are often exposed to high temperatures in the oil well drilling fields. Also in the food industry, especially in pet foods where the polymer jelly (which acts as a suspending agent for the meat pieces), is autoclaved, thus it is important to understand the polymers thermal stability.

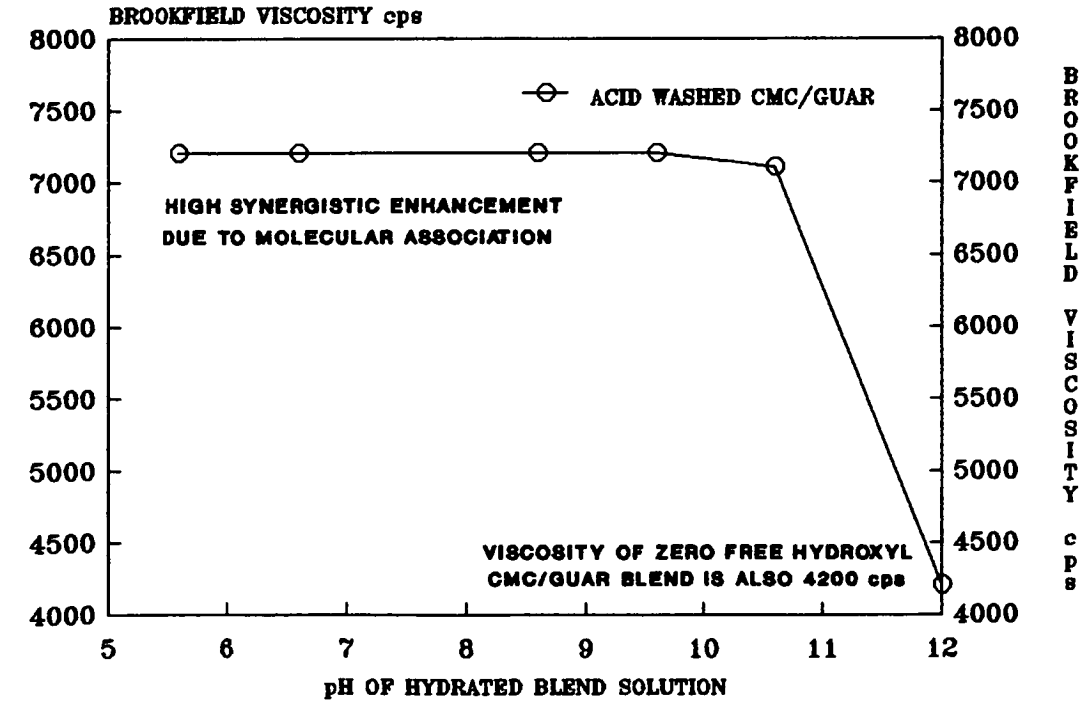
Graph VII.37 shows how the synergistic viscosity enhancement of an acid washed CMC/guar blend changes with temperature. It can be seen that the blend viscosity remains higher than either component's viscosity even up to 90° centigrade. If the intermolecular hydrogen bonding in the previously proposed interaction mechanism was weak, the blend's viscosity would fall to somewhere between the two component viscosities as the temperature was increased.

TEMPERATURE STABILITY OF POLYMER BLENDS
PLOT OF VISCOSITY AGAINST TEMP OF CMC/
GUAR 25/75% BLEND



GRAPH 7.37

EFFECT OF ADDITION OF SODIUM HYDROXIDE
ON % ENHANCEMENT OF SYNERGISTIC BLEND



GRAPH 7.38
1% TOTAL POLYMER CONCENTRATION

This result reinforces previous data on shear dependancy of the blends (graph VII.30 and 31) where it was concluded that the association mechanism is comprised of numerous relatively strong hydrogen bonds between adjacent unlike polymer chains.

Another interesting observation is that the final re-cooled Brookfield viscosity of the CMC/guar blend is higher than the starting viscosity (accounting for water loss due to evaporation), whilst the component viscosities remain unchanged. A possible explanation for this may be that both polymer's structures uncoil when heated exposing new potential interaction sites on both polymers (99). The two polymers may associate at these sites and remain associated when re-cooled. Therefore when re-cooled the polymers hold open their coiled structures by hydrogen bonding and an even larger viscosity enhancement is observed.

If the synergistic viscosity enhancements observed in an acid washed CMC blend with a non-ionic polymer, or any polymer blend, can be applied to real systems in the food industry an understanding of their tolerance to pH variation and addition of electrolytes is necessary (100). Graph VII.38 demonstrates the effect on viscosity enhancement of an acid washed CMC/guar blend when the pH of the solvent is increased. The initial pH of the polymer blend in solution is 5.8, synergistic viscosity enhancement appears unaffected up to pH 12, where it

reduces to the viscosity of the equivalent zero free carboxyl CMC/guar blend. This indicates potential drawbacks of the technology however it is rare for food formulations to be sold at this high pH range (101). The results also reinforce the proposed mechanism of synergistic enhancement and suggest that the observed molecular association is reversible.

In food systems low molecular weight compounds like citric acid are often added. This compound has three COOH groups and could associate with the non-ionic hydroxyl groups, therefore competitively inhibit the synergistic mechanism between CMC and HPMC (see graph VII.33). However an interesting observation was that when 1 gramme of citric acid was added to a 1% solution of HPMC 40,000P, the Brookfield viscosity increased from 4200cps in the control to 4500cps. A similar effect was not observed when 1 gramme of sodium citrate was added to a 1% HPMC 40,000P solution. Presumably the COOH groups on the citric acid can associate with hydroxyls on unlike non-ionic cellulosic polymer molecules but the COO^-Na^+ on the sodium citrate cannot. This result emphasises the importance of understanding the mechanism of association between unlike cellulose ethers in maximising the polymers performance in commercial application.

VII (xiii) AN EXAMPLE OF ANTAGONISM IN A BLEND OF AN ANIONIC AND A NON-IONIC CELLULOSIC POLYMER.

It has been suggested in the present discussion, that the synergistic interaction between acid washed CMC and non-ionic polymers involves two co-existing mechanisms, one of molecular association (Mechanism 1) and one of competitive dehydration (Mechanism 2). In all examples of polymer-polymer blends investigated so far, the contribution from Mechanism 1 has far exceeded the contribution from Mechanism 2. It has been proposed for a 50/50% polymer-polymer blend of acid washed CMC and non-ionic HPMC (hydroxypropyl methyl cellulose), assuming that association occurs, that the maximum potential for hydrodynamic volume increase and subsequent reduction in C*, is when the molecular weights of the two polymers are of similar magnitude.

The maximum possible positive contribution from the competitive dehydration mechanism must be attained when a high molecular weight HPMC (more hydrophobic) is blended with a low molecular weight CMC (more hydrophilic). The overall resultant viscosity enhancement in such a blend will be low, as the contribution from molecular association will be minimal. However if a blend of very low molecular weight HPMC and high molecular weight CMC are blended, although the contribution from molecular association

will remain minimal, the contribution from competitive dehydration will be significant, but in this case will be negative. Table VII.5 confirms these suggestions and overall viscosity antagonism is observed, when an acid washed CMC is blended with HPMC (P5/6 has a 2% Brookfield viscosity of 5 cps). This example emphasises the importance of considering both co-existing mechanisms of viscosity enhancement when blending unlike water-soluble polymers (102).

Structuraly modified alkali and acid washed CMC P480P were blended (50/50% at 1% total polymer concentration) with four Celacol HPMC's of varying molecular weight (2% Brookfield viscosities of 40,000 cps, 5,000 cps, 100 cps and 5 cps respectively). The results are shown in Table VII.6. It can be seen that the largest viscosity enhancement is observed for the acid washed P480P CMC with HPMC 40,000P. The previously proposed mechanisms (Mechanisms 1 and 2) for polymer-polymer interaction would predict in the alkali washed CMC/HPMC blends that the largest viscosity enhancement would be observed when the difference in molecular weights of the two polymers is greatest (no molecular association possible). Since HPMC is more hydrophobic than CMC, viscosity enhancement is expected in all the blends.

However it can be seen that overall viscosity antagonism is observed in the P5/6 HPMC blend. Here there is a negative contribution from the competitive dehydration mechanism. This suggests that

TABLE VII.5 RESULTANT VISCOSITY EFFECT OF BLENDING VERY LOW MOLECULAR WEIGHT HPMC WITH HIGH MOLECULAR WEIGHT CMC.

POLYMER IN BLEND	B/FLD VISC cps
CMC P2500P ACID WASHED 1% POLYMER CONC	3850
CMC P2500P ACID WASHED 0.75% POLYMER CONC	2120
0.75% CMC P2500P ACID WASHED 0.25% HPMC P5/6	1450
0.75% CMC P2500P ALK WASHED 0.25% HPMC P5/6	1260

TABLE VII.6. VISCOSITY ENHANCEMENT IN CELACOL HPMC AND COURLOSE CMC POLYMER BLENDS WITH VARIATION IN FREE CARBOXYL CONTENT ON THE CMC AND MOLECULAR WEIGHT OF THE HPMC.

% VISCOSITY ENHANCEMENT ALKALI BLEND	HPMC VISCOSITY GRADE	% VISCOSITY ENHANCEMENT ACID CMC BLEND
56%	40,000P	155%
29%	5,000P	128%
7%	100P	96%
-23%	P5/6	26%

the viscosity/concentration curve of P5/6 HPMC lies below that of CMC P480P which is indeed the case. In the other examples of alkali washed CMC/HPMC blends, the synergistic viscosity enhancement increases with increasing HPMC molecular weight as predicted (refer to graph VII.16 and 17 and discussion on Mechanism 2)). In the acid washed CMC/HPMC blends, similar contributions from competitive dehydration will exist but there is additional contribution to the overall viscosity enhancement as a result of molecular association. The situation is made more complicated by the fact that the acid washed CMC has a greater initial viscosity compared to the alkali washed CMC i.e. the two modified CMC's have different viscosity/concentration curves. Therefore it is difficult to compare directly the two acid and alkali washed blend's viscosity enhancements.

The contribution from the molecular association mechanism therefore, cannot be calculated by simply subtracting the percentage viscosity enhancements of the alkali and acid washed CMC/HPMC blends. It was previously proposed that molecular association is maximised when the molecular weights of both polymer components are similar. The results in table VII.6 suggest that this occurs in the CMC/HPMC 100P polymer blend i.e. the contribution from the competitive dehydration mechanism has the smallest deviation from zero). Therefore by utilising both co-existing mechanisms of polymer interaction, it may be possible to maximise viscosity enhancement in unlike

cellulosic ethers and galactomannan blends.

CONCLUSION.

Two co-existing mechanisms of polymer-polymer interaction between anionic cellulose ethers and non-ionic cellulose ethers (or galactomannans) has been proposed in this thesis. The first (Mechanism 1) is a molecular association mechanism between the free carboxyl groups on the anionic CMC, with the hydroxyls on the non-ionic polymer. Many parameters influence the magnitude of this association, and the observed synergistic viscosity enhancements in the blends' solutions, these include; polymer molecular weight, free carboxyl level on the CMC, polymer D.S on the anionic CMC, substituent type and levels on the non-ionic, distribution of substituents on non-ionic, and competitive inhibition factors.

The second co-existing mechanism which contributes to the synergy observed when an anionic and non-ionic cellulose ether solutions are blended (Mechanism 2), and which also explains the observed antagonism when two non-ionic polymers are blended together is based on a competitive dehydration mechanism. This mechanism depends on the differences in molecular weights and relative hydrophilicities of the polymers being blended.

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