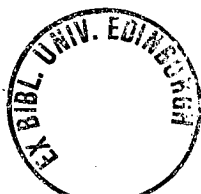


The **THERMAL**
STABILITY *of* **XANTHAN**

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Thesis presented for the degree of
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To my family and friends

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I also acknowledge the financial assistance from NTNF in Norway, without whose help this project would not have been possible.

ABSTRACT

The use of the bacterial polysaccharide xanthan in enhanced oil recovery is now well established. This is particularly so in the U.S., where reservoir temperatures are lower than those found in the North Sea.

The object of this study was to establish the polymer stability at higher temperatures. Two biopolymers, one commercial and one from a laboratory strain, were compared. These two polymers varied in their acyl content. Long-term stability tests occurred on sealed ampoules in either sea or distilled water in the absence of oxygen. The biopolymer breakdown was followed by measuring changes in viscosity, carbohydrate, pH etc. From the results, it seems that chain scission is the reason for rapid viscosity loss, and where an increase in viscosity is observed, microgel dissolution is the most probable explanation.

ABBREVIATIONS

EM	electron microscopy
Glc	glucose
HPLC	high performance liquid chromatography
Man	mannose
MW	molecular weight

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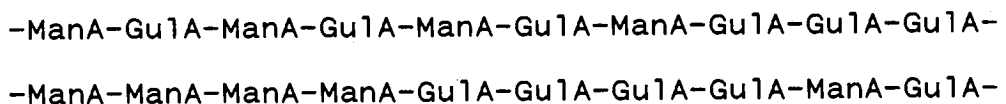
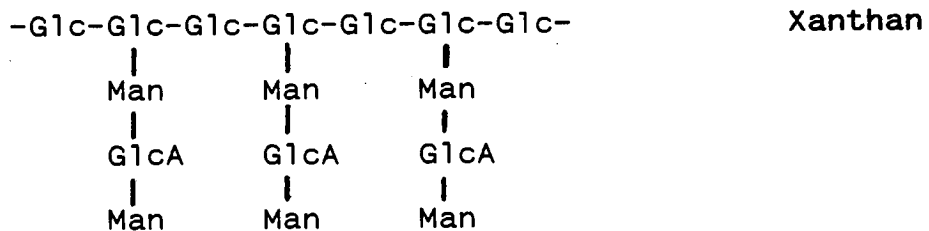
I. CHAPTER ONE: INTRODUCTION

A. EXTRACELLULAR POLYSACCHARIDES

1. Bacteria producing extracellular polysaccharides

Extracellular polysaccharides, also called exopolysaccharides, are slimes and capsules secreted by bacteria (Kenne and Lindberg, 1983). The exopolysaccharides are not necessarily bound to the cell surface and can vary greatly in their chemical composition. Xanthan, for example, has a glucan backbone with trisaccharide side-chains and is very regular. Bacterial alginates (made of an alternating sequence and blocks of β -D-mannuronic acid and α -L-guluronic acid as one single chain), produced by *Azotobacter vinlandii* and *Pseudomonas aeruginosa*, have a similar chemical composition to that of alginate synthesised by brown algae, but also carry O-acetyl groups on some mannuronosyl residues (Painter, 1983). The polysaccharide allows the bacteria to build up a protective shield against dehydration and immunological attack

Fig.1.1. The chemical structure of xanthan (for full structure see fig. 1.2) and alginate. (Glc=glucose; GlcA=glucuronic acid; Man=mannose; ManA=mannuronic acid; GulA=guluronic acid)



Alginate

round its cell. The exopolysaccharide probably also serves the bacteria to recognise plant hosts (Morris *et al.*, 1977), and eases the penetration of the bacteria into either plant or animal cells. The polymer xanthan is synthesised by a plant pathogen *Xanthomonas campestris*, whereas the alginate produced by *Pseudomonas aeruginosa* is associated with bacterial isolates obtained during the

treatment of cystic fibrosis patients. *A. vinlandii* is a saprophytic bacterium, widely found in soil.

2. The Biosynthesis of Xanthan

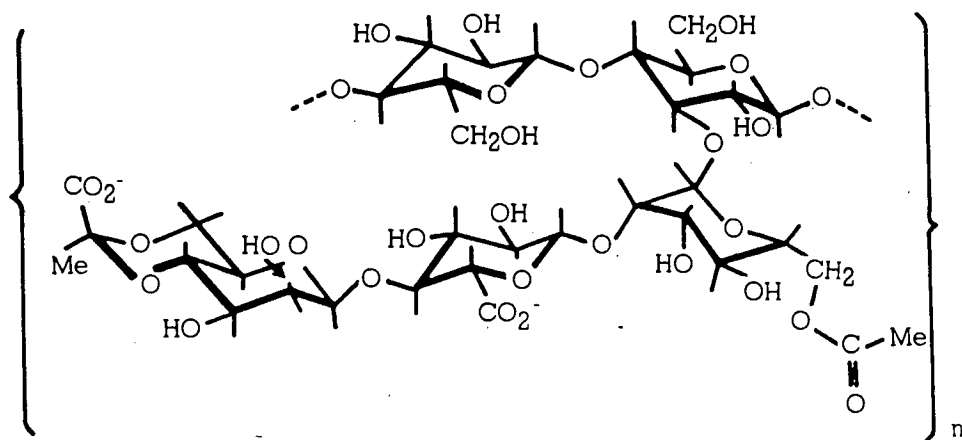
The *Xanthomonas* species producing xanthan was originally isolated from a *Rutabaga* species (ie swede/turnip), and was found to have a wide range of potential industrial uses (Jeanes *et al.*, 1961). A more up to date review has been prepared by Yalpani and Sandford (1987). This bacterial strain produced a large quantity of slime, ie an exopolysaccharide that is exuded outside the cell and is not normally attached to the cell surface. The biosynthetic pathway has been elaborated by Ielpi *et al.* (1981 a, b), and recently a cluster of genes that are essential for xanthan biosynthesis have been analysed (Harding *et al.*, 1987). Ielpi *et al.* found that UDP-glucose, GDP-mannose and UDP-glucuronic acid were all precursors for xanthan synthesis. The sugars were transferred sequentially to an isopentenyl pyrophosphate lipid. The lipid-bound pentasaccharide was then pyruvylated and acetylated by transferase enzymes utilising phosphoenol-pyruvate and acetyl CoA respectively, before polymerisation. The method by which the polysaccharide is transported from its intracellular site of synthesis through the cell membrane and wall has still not been elucidated. The molecular weight is thought to be at least 2 million Daltons (Dintzis *et al.* (1970), but

various workers using different techniques have estimated values in the range $1-15 \times 10^6$.

B. THE CHEMICAL STRUCTURE OF XANTHAN

Xanthan was originally thought to have 9 sugar residues per repeat unit (8 in the backbone, 1 in a sidechain, Jeanes *et al.* 1961). It was also known to contain glucose, mannose, glucuronic acid and pyruvate (Sloneker and Orentas, 1962). The correct structure was however first elucidated by Jansson *et al.* (1975) and Melton *et al.* (1976). The backbone was made of a β -(1 \rightarrow 4) linked glucan chain, which is similar to that of cellulose. Cellulose has however a 2/1 helical twist, whereas xanthan has a 5/1 helical twist as found by Moorhouse *et al.* (1977). This is due to the backbone of xanthan carrying a trisaccharide sidechain on every second glucose residue. The sidechain is linked to the backbone by an α -(1 \rightarrow 3) linkage, and consists of a β -D-mannopyranosyl-(1 \rightarrow 4)- β -D-glucuronopyranosyl-(1 \rightarrow 2)- α -D-mannopyranoside (6-O-acetate). The terminal mannose residue of the sidechain frequently carries a 4,6-O-linked pyruvyl-ketal group. Whereas the backbone and sidechain sugars of xanthan appear to remain unchanged, xanthans vary in their acyl content (Sutherland, 1981), and different xanthan preparations show variable physical properties. I.e. xanthan from *Xanthomonas campestris* is high in both pyruvate and acetate substitution, whereas

Fig.1.2. The pentasaccharide repeat unit of xanthan
(from Dea, 1987).



xanthans from *X. phaseoli* has been found to be high in pyruvate, low in acetate or vice versa, depending on the strain analysed. Although not every sidechain was thought to contain a pyruvyl and acetyl group, Rinaudo et al. (1983) found by ^1H NMR and ^{13}C NMR on a commercial xanthan preparation (Rhodopol), that every sidechain was substituted by both the pyruvyl and acetyl group. Previously, chemical methods had been used. It is possible that these methods might not be as accurate as those of NMR. On the other hand, the different *Xanthomonas* strains produce xanthans with varying acyl contents (Sutherland, 1981). Also, Tait (1984), by using N-methyl-N'-nitro-N-nitrosoguanidine as a mutagenic agent, produced different *Xanthomonas* mutant strains that gave

variations in their acyl substitutions and, in one case, in the sidechain sugars. Variation in acyl content of xanthan can also be obtained through the use of different cultural conditions. More recently, a xanthan-type polymer with one sidechain sugar has been genetically engineered (Betlach *et al.*, 1987).

C. THE PHYSICAL PROPERTIES OF XANTHAN

1. The Molecular Weight of Xanthan

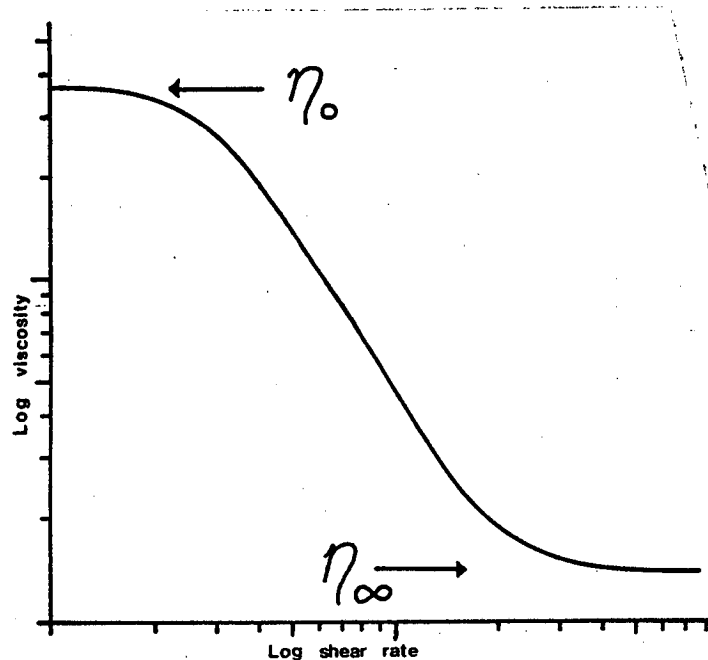
There has been a lot of discussion and variable results obtained when looking at the molecular weight (MW) of xanthan. Values varying from 1.4 to 60×10^6 daltons have been mentioned. All agree to that in its native form, xanthan has a very high molecular weight of at least 1 million daltons. Dintzis *et al.* (1970), found when measuring the MW by classical light scattering techniques, that the MW was varying from 1.4 to 3.6×10^6 daltons. Holzwarth (1978), reported xanthan from fermentation broth to have a MW of 60×10^6 using a fluorescing molecule attached to the xanthan in sedimentation studies. This high value was probably observed due to microgels being present in the solution. Rinaudo and Milas (1978), reported the MW to be between 2 and 3×10^6 daltons, and this was found to be similar by Southwick *et*

a7. (1982). In their publications, the Rinaudo laboratory have generally quoted 2.8×10^6 as the MW of xanthan. This is probably quite an average value.

2. Rheology and Pseudoplasticity

The viscosity of a Newtonian solution is given as $\eta = T/\dot{\gamma}$, where T is the shearing stress and $\dot{\gamma}$ the rate of shear. This means that the viscosity is unaffected by the shear rate. For a pseudoplastic (shear thinning) solution however, the viscosity decreases as the shear stress increases. However, even pseudoplastic fluids have their Newtonian regions as well. This is so at both very low and very high shear rates, and a typical graph of a pseudoplastic solution is given in fig. 1.3. The viscosity of a fluid can be explained as its resistance to flow. Thus it is given in the units Pa.s which is the same as $N \cdot s/m^2$. For our purposes, the viscosity is given as mPa.s, which is the same as the older units centipoise. To quote viscosities of xanthan solutions, or the viscosity of any pseudoplastic fluid for that matter, one has to give its intrinsic viscosity, ie. when the shear rate $\dot{\gamma}$ goes toward zero, and the measurement is thus in the lower

Fig.1.3. Viscosity plotted against shear rate for a typical pseudoplastic fluid. Newtonian regions are found at very high ($n \rightarrow \infty$) and very low ($n \rightarrow 0$) shear rates.



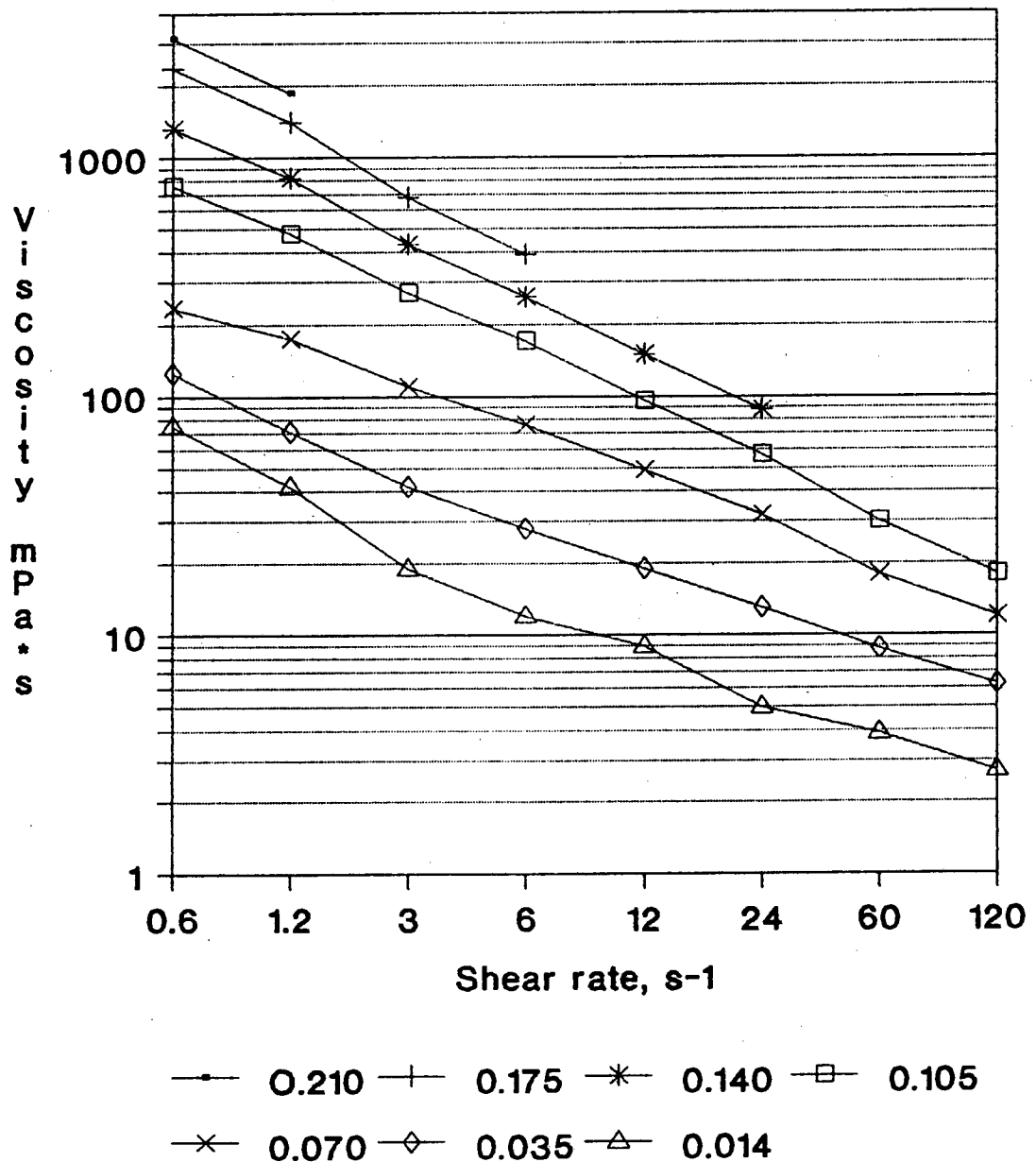
Newtonian region. The intrinsic viscosity of a solution is given as:

Fig.1.4. The intrinsic viscosity of a solution is defined as (from Rees *et al.*, 1982):

$$[\eta] = \lim_{c \rightarrow 0} ((\eta_{\text{solution}} - \eta_{\text{solvent}}) / \eta_{\text{solvent}} c)$$

At room temperature, water will have a viscosity of 1 mPa.s whereas lubricating oil will lie in the region of 300-800 mPa.s. Bitumen shows a viscosity of 10^5 Pa.s!

Fig.1.5. The viscosity plotted against shear rate for xanthan solutions at various concentrations (in %) in seawater. Measurements were made in the non-Newtonian region.



Xanthan has values in between these limits depending very much on the polysaccharide concentration (fig. 1.5.).

Sandford et al. (1977), concluded from experimental results that an increasing pyruvyl-substitution gave the polymer an increased viscosity. Bradshaw et al. (1983) thought that this was rather due to comparing the xanthan viscosity obtained from varying strains rather than due to increased intermolecular action and this was recently confirmed by Callet et al. (1987a). This latter group found that the intrinsic viscosity of any xanthan solution was independent of acyl-substitution as long as salt and temperature conditions and xanthans of similar molecular weights were compared. So although the T_m might vary for the different xanthans (again confirmed by Callet et al., 1987a), it seems the pyruvyl and acetyl substitutions have little real influence on the viscosity performance of the polymer. One particular pyruvate rich xanthan has been proposed for enhanced oil recovery use due to its allegedly superior properties (Philips et al., 1982), but the validity of this claim may now be questioned. The improved properties found in this polymer are probably due to excellent fermentation conditions causing some subtle changes in the chemical and physical attributes of the polysaccharide, rather than solely a high pyruvate content.

3. The Order/Disorder Conformations of Xanthan

It seems from various experiments that the xanthan can exist in two different conformations in solution. One is

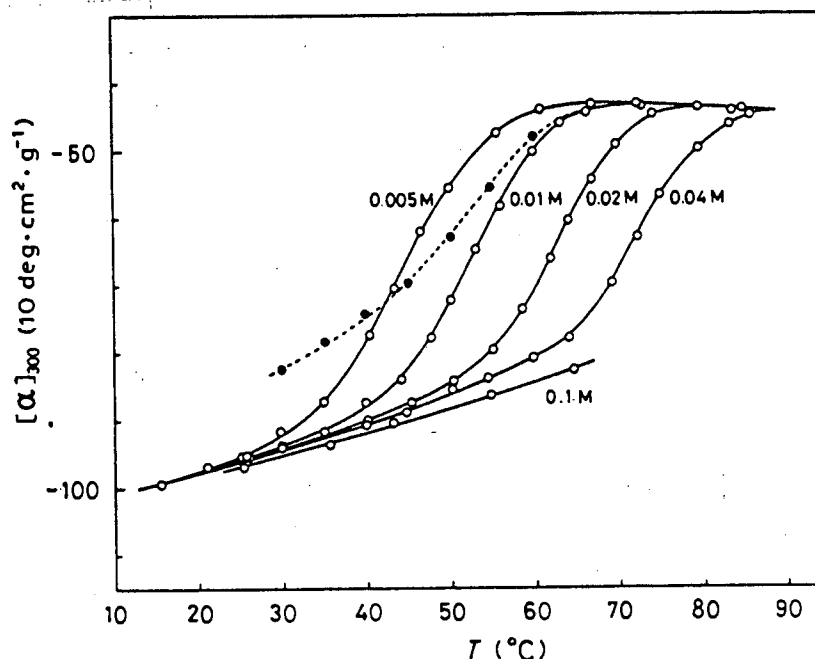
the ordered form where the xanthan is in the helix conformation as found by Moorhouse *et al.* (1977). These workers found by X-ray crystallography that the most likely conformation would be a 5/1 helix. I.e. there would be five repeat units for every turn in the main chain. The pitch of the helix was found to be 4.7 nm per turn or 0.47 nm per glucose residue. These values were also confirmed by Sato *et al.* (1984a). However, this ordered conformation can be converted into a disordered conformation, which is thought to be a stretched or random coil, by increasing the solution temperature. This change in conformation may primarily be due to sidechains moving away from the close association with the backbone. This may be accompanied by or occur before conformational changes in the backbone (Moorhouse *et al.*, 1977). The change from one to the other and the behaviour of the xanthan molecule in solution can to some extent be followed by the change in optical rotation of the solution upon change in temperature.

4. The Transition Temperature of Xanthan Solutions

Many polysaccharides go from one conformation to another through a very abrupt temperature transition, often noted over only 2-3 degrees change. This can be observed by the change in optical rotation of a solution upon changing the solution temperature. In this respect, the transition for xanthan was unusual in that it changed

slowly over 20-30°C or more (Jeanes *et al.*, 1961). The transition temperature (T_m) was found to be very much dependent on salt concentration (Milas and Rinaudo, 1979, Liu *et al.*, 1987), and also pyruvate substitution (Holz-

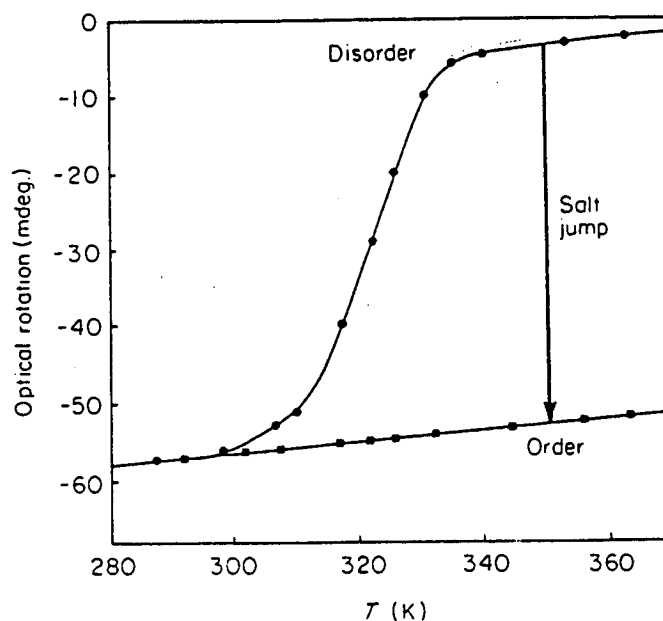
Fig.1.6. Temperature dependence of the specific rotation, $[\alpha]_{300}$, for a xanthan sample at various salt concentrations (from Liu *et al.*, 1987).



warth and Ogletree (1979); Smith *et al.* (1981)). The transition temperature (T_m) is taken as the temperature of the polysaccharide solution at which it is at the midpoint between being in the ordered and disordered conformation.

On the addition of salt to xanthan in the disordered form, Norton *et al.* (1980) found that the solution went

Fig.1.7. The "salt-jump" observed by Norton *et al.* (1980), when mixing a salt solution with xanthan in the disordered form.



through a "salt-jump" back to the ordered form (fig.1.7). The change from the ordered into the disordered conformation is dependent on the temperature of the solution and the salt concentration (fig.1.6). Thus the disordered form can be found at low temperatures when the polymer is in solution in the Na^+ salt form (Milas & Rinaudo, 1979). The salt concentration at which this occurs, is also dependent on the cation used. Lambert *et al.* (1985) found that the sodium equivalent concentration had to be greater than 10^{-2} to obtain the xanthan in an ordered form. For the divalent calcium ion, an equivalent concentration of 10^{-3} was given to obtain the same degree of order, i.e. a tenfold reduction in ion concentration. Also, if the xanthan is in the Na^+ form,

then the Ca^{2+} will displace these ions (Lambert et al., 1985).

Circular dichroism (C.D.) results obtained by Dentini et al. (1984) also indicate that if Ca^{2+} was present in a ratio of 0.2 on a molar basis to xanthan, then the xanthan would be in an ordered form at 25°C. An increase in the molar ratio of calcium ions from 0.2 to 1, showed no great change in the spectrum. The C.D. spectrum of native xanthan in the sodium form in water was however quite different from that found when the Ca^{2+} ions were present in a molar ratio of 0.2. From the slight shifts of peak positions and especially peak height differences, it was deduced that this native xanthan in the sodium form was in the disordered conformation at 25°C in solution.

Smith et al. (1981) examined the influence of the pyruvate group on the solution properties of xanthan. It was found that an increasing pyruvate substitution gave a fall in transition temperature. This was thus attributed to the intramolecular electrostatic repulsion between the pyruvate groups. Some stabilisation on the other hand was found to be due to apolar interactions of the acetyl groups. The T_m was thus found to be -66°C when the ratio of pyruvyl/ acetyl substitution was 0.9, whilst it was -74°C when the ratio was 0.4. Xanthans from different strains of *Xanthomonas* were used for these experiments.

Dentini et al. (1984), found a corresponding relationship between pyruvyl and acetyl substitution and transition temperature when they used xanthan from one source and then modified it chemically to give acetate and pyruvate free xanthans, or xanthan from which both acyl groups had been removed. The native xanthan (NX) thus gave pyruvate-free xanthan (PFX), acetate-free xanthan (AFX) and acetate and pyruvate-free xanthan (APFX). The acetate group was found to have a smaller stabilising effect on the ordered conformation of the molecule than the pyruvate had a destabilising effect. Thus the order of decreasing thermal stability was given as PFX >NX >APFX >AFX, both when comparing the xanthans in distilled water or with Ca^{2+} ions present (0.2 mol / mol xanthan). For acetate-free xanthan, the same authors found that a very much larger Ca^{2+} ratio than 0.2 was necessary to convert this polymer into the ordered form. This was in agreement with results obtained by Rinaudo et al. (1983) and Holzwarth and Ogletree (1979). Dentini et al. (1984) also inferred from circular dichroism experiments that the above substitutions of pyruvate and acetate gave xanthan the different transition temperatures.

5. The Thermal Stability of Xanthan

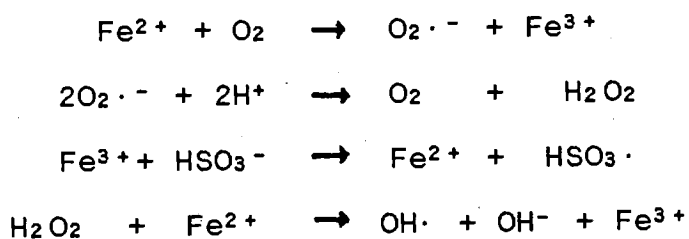
The stability of xanthan is greater in the ordered form than in the disordered form. This is true both of biological stability (Rinaudo and Milas, 1980), and of physical stability against thermal degradation (Rinaudo *et al.*, 1983; Milas *et al.*, 1988; Kierulf and Sutherland 1988). The disordered form obviously opens the chain up to some degree which makes it more susceptible to attack by enzymes or by chemical or physical factors. Therefore, it would be expected that the higher the T_m , the higher the temperature at which degradation could be prevented. With the transition temperature being about 100°C under optimal salt concentrations, then it is possible that polymer degradation would be prolonged at this temperature. It seems however, that thermal degradation becomes very important when the temperatures reach 90°C and above. At these temperatures degradation is noted whatever the salt concentration.

Polysaccharides can be degraded by radiation, biological, chemical, mechanical and thermal means. In respect to the various degradation mechanisms, radiation is of low priority to enhanced oil recovery (EOR). As mentioned before, the xanthan solutions are very stable towards shear stress and thus not liable to mechanical degradation (Hsia-Chen *et al.*, 1980). Also biological de-

gradation can be avoided by the addition of a biocide like formaldehyde to the xanthan solutions. This is already standard practice in the oil industry. So the main degradation procedures that will form a threat to xanthan stability is from chemical and thermal (Seright and Henrici, 1986) attack.

Chemical degradation is likely to occur mainly through the formation of free radicals in the polymer flood water. It has been found in laboratory experiments, that the formation of free radicals is mainly due to two chemical components in the flood water, oxygen and iron (Wellington, 1980). These two chemicals form the initiation step of free radical formation given below.

Fig.1.8. Some of the major reactions forming free radicals that can attack the polysaccharide in solution (Ash *et al.*, 1983).



There are many radicals produced in this process, but according to Parsons *et al.* (1985), the most reactive

species is the $\cdot\text{OH}$ radical. This radical has the ability to abstract hydrogen atoms from many positions in the polymer, and can also lead to glycosidic bond breakage. When these main chain bonds are broken, a drastic loss in viscosity will be observed. Thus exclusion of oxygen and ferrous ions is absolutely essential when considering xanthan use for EOR purposes.

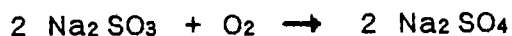
Ferrous ions will always be present in the seawater used for EOR purposes. They need not be from the seawater itself, but some will always be dissolved as the solutions pass through the steel pipes used in drilling etc. Citric acid chelates ferric iron, but it is a fairly impractical proposition as other ions stabilising the xanthan will be affected as well.

The easiest method by which to avoid free radical attack is therefore to remove all oxygen from the solution. That means that the seawater should be degassed before use, and mixed with the xanthan concentrate under inert conditions before being pumped down into the oil-well. Even so, trace amounts of oxygen are sure to remain in the solution. To avoid chemical attack on xanthan, Wellington (1980), suggested a synergistic formulation as given below to act as a protection "package" for the

xanthan. It should contain:

- a radical transfer agent
- a sacrificial chemical that is easily oxidised
- an oxygen scavenger
- a biocide
- adequate brine concentration

The way to avoid oxygen is to add some sodium sulphite, acting as an oxygen scavenger (Judson, 1986). The reaction



may remove most of the oxygen in the solution. Whatever free radicals are still produced, can be made to react with an easily oxidisable chemical. In figure 1.9. on the following page, is given a list of compounds which react with hydroxyl free radicals. The rate constants are compared with that of xanthan.

P-cresol and phenol have the highest reaction rates with hydroxyl free radicals, but are rather dangerous chemicals to work with. Thus thiourea and iso-propanol were more acceptable for EOR use.

Fig.1.9. Rate constants for hydroxyl radical reaction with various compounds compared with xanthan (Ash et al., 1983)

	K (M ⁻¹ s ⁻¹ x 10 ⁻⁸)
p-cresol	100
thiourea	50
phenol	20-100
iso-propanol	10-30
ethanol	10
ethylene glycol	7
xanthan	2.5

The protection package for xanthan solutions used in EOR thus contains sodium sulphite as an oxygen scavenger, thiourea and iso-propanol as radical transfer reagents and easily oxidisable compounds, formaldehyde as a biocide and seawater contains sufficient ions to ensure good stability. This package will give the polymer solution sufficient protection up to fairly high temperatures. Above 90°C, the effect of thermal energy on the polymer solution is observed, as rapid degradation of the polysaccharide occurs whatever protection package is used.

6. Is Xanthan Single or Double Stranded?

Much physical evidence has been brought forward to support the finding that the xanthan is single-stranded when in the ordered conformation. This single-stranded conformation has been supported by Moorhouse *et al.* (1977), Morris *et al.* (1977), Milas & Rinaudo (1979, 1986), Norton *et al.* (1980, 1984) using varying techniques. The latter group found by stopped flow polarimetry that the reaction rates, when mixing deionised (and disordered) xanthan and a KCl solution, followed first order kinetics (see fig. 1.7). Others assumed from the T_m dependence on salt-concentration that the xanthan was in a single-helix conformation. However, other interpretations are possible.

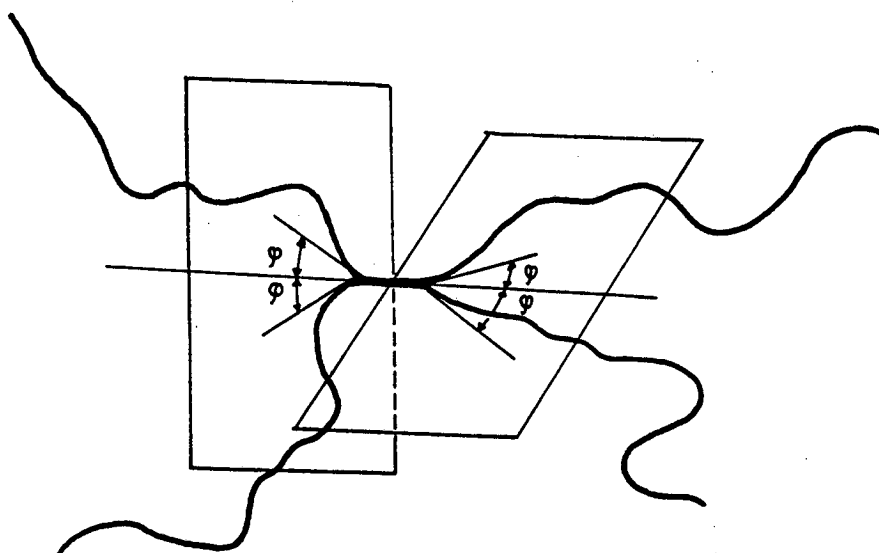
Holzwarth and Prestridge (1977) produced an EM micrograph which gave an early indication that xanthan might be in a multistranded conformation. Holzwarth (1978) also found that the mass per unit length was 1900 daltons/nm, ie corresponding to the double-stranded conformation. Paradossi and Brant (1982) found a similar relationship when looking at the light-scattering properties of the xanthan in solution. Results obtained by Sato *et al.* (1984a, b), Zhang *et al.* (1987) and the EM micrographs by Stokke *et al.* (1986, 1987) also support this ordered structure.

Xanthan can probably exist both in a single- and double-stranded conformation in solution. This would depend on the solute. Dintzis *et al.* (1970), showed that urea dissolved xanthan in the single-stranded form. Urea was thus used by Southwick *et al.* (1980), to suggest that xanthan undergoes self-association in aqueous solutions. Cadoxen, (tris-(ethylene-diamine)-cadmium dihydroxide) has been shown to dissolve the xanthan in a similar conformation (Sato *et al.*, 1984a; Kitagawa, 1985). The results obtained by this latter group have however been disputed by Callet *et al.* (1987b), who think the alkaline cadoxen solution degraded the xanthan into smaller molecular weight species.

It is of interest to find the true xanthan conformation in solution. If the xanthan was in the double-stranded form, then backbone linkages broken could be supported by the adjacent chain. On the other hand, had xanthan been in the single-stranded form in solution, then a hydrolysed backbone linkage should lead to a degradation in molecular weight and thus a fall in viscosity. Milas and Rinaudo (1986) performed an experiment that showed this last statement need not be true. The relative viscosity of partially hydrolysed xanthan was measured. It was found that if this xanthan was heated above the transition temperature, then a loss in relative viscosity was observed and this was not reversible. They concluded that these results showed a very strong interaction

between backbone and sidechain that masked the backbone breakages until heated above the transition temperature. Obviously, further studies are required. Liu *et al.* (1987), have come up with another novel picture of xanthan in solution (fig. 1.10). This picture of xanthan as a worm-like coil joined in the centre with four single

Fig.1.10. The worm-like dimer model of xanthan as given by Liu *et al.* (1987) in a 0.01M aqueous NaCl solution at 80°C, with four subchains.



strands forming the ends, could to a certain degree explain the factors concerning single-double-stranded characteristics found experimentally.

7. The Flexibility of the Xanthan Polymer in Solution

The flexibility of polymers in solution can be determined through their various physical properties. Whether

xanthan is a rigid rod or a worm-like chain has been much disputed (Holzwarth, 1981). For a single-stranded worm like model, Muller *et al.* (1984) found that the persistence length (q , see fig. 1.12 for definition) would be about 50nm when determined by intrinsic viscosity measurements. When the xanthan is however in the double-stranded configuration, the persistence length q becomes 100-150 nm. These latter values of q have been found by the molecular weight dependence of the radius of gyration (Sato *et al.*, 1984a) and from the intrinsic viscosity (Sato *et al.*, 1984b). Stokke *et al.* (1987) found good agreement with these values by measurements on EM micrographs. These had been prepared by the method of Tyler & Branton (1980) and Elgsaeter (1978). Although the polymer on the micrographs is in two dimensions rather than the three dimensions as in solution, Stokke *et al.* (1987), showed that the results were compatible with those found by physical measurements when using the method of Frontali *et al.* (1979). Further evidence that the persistence length of xanthan in solution is 50 nm has been obtained recently by Callet *et al.* (1987b).

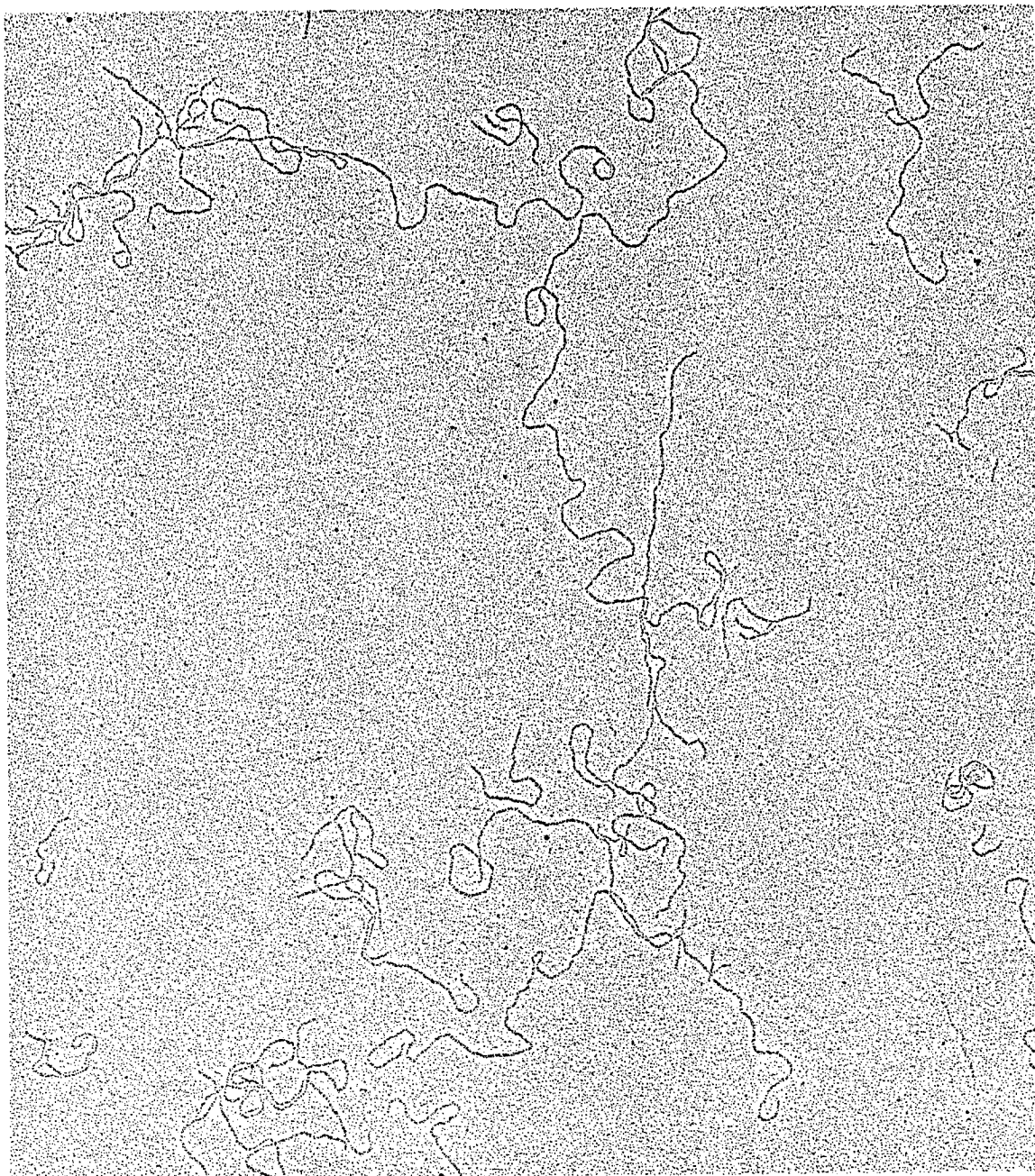
8. Electron Microscopy

Holzwarth and Prestridge (1977), first produced electron micrographs of xanthan. These were prepared by drying a drop of a xanthan solution (6ppm) on an EM grid covered with a carbon film. The drying and attachment of the

polysaccharide to the film was facilitated by using a drying hood. Thus the drying process caused the polymer to stretch out and the resulting electron micrographs show that the polymer is very stiff. From the solution properties of xanthan, the same authors conclude that the xanthan is most probably in a semi-rigid conformation in solution when salts are present. The EMs also show that denatured xanthan can have a split chain (obtained by heating in deionised water for 15 minutes, then quenching in ice water) and that the polymer is a double-helix (righthanded).

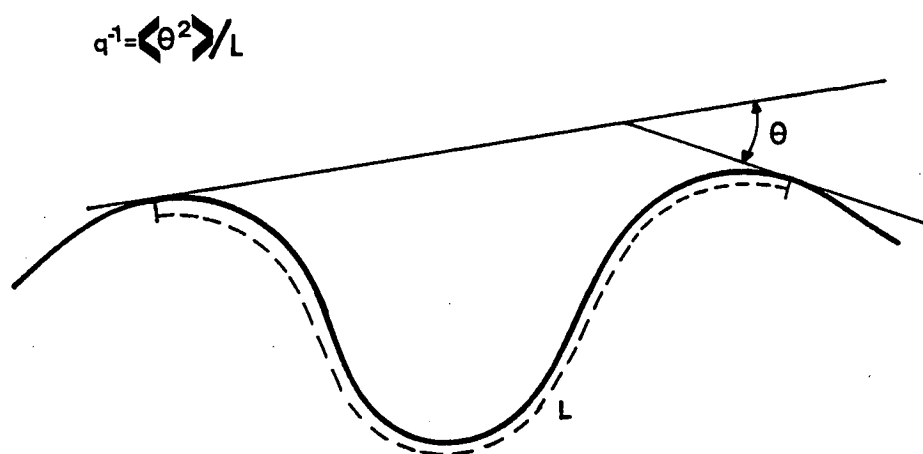
Stokke *et al.* (1986,1987), show EM micrographs taken under different conditions. The biopolymer solutions were mixed with 100% glycerol and molar ammonium acetate before spraying on to freshly cleaved mica discs and then drying. The pictures taken of xanthan and other polymers

Fig.1.11. Xanthan (Flocon, not heated), photographed after being prepared by B.T.Stokke (personal communication). X 50'000.



by this technique show them to be semi rigid and flexible (fig. 1.11). The authors also calculated the flexibility of these polymers in the two dimensional state by the method of Frontali and coworkers, and others (see Stokke *et al.*, 1987).

Fig.1.12. Method of calculating the persistence length (q) of xanthan used by Stokke *et al.* (1987).



The results show that the persistence lengths of xanthan calculated by this method correspond to the values obtained by others from measurements of solution properties. The values for single and double stranded xanthan are given as 60 and 150 nm respectively by Stokke *et al.* (1987).

There are clearly differing views about the validity of seeking xanthan conformational properties from electron microscopy. This is especially true of single and double-stranded xanthan. Callet *et al.* (1987b), mention

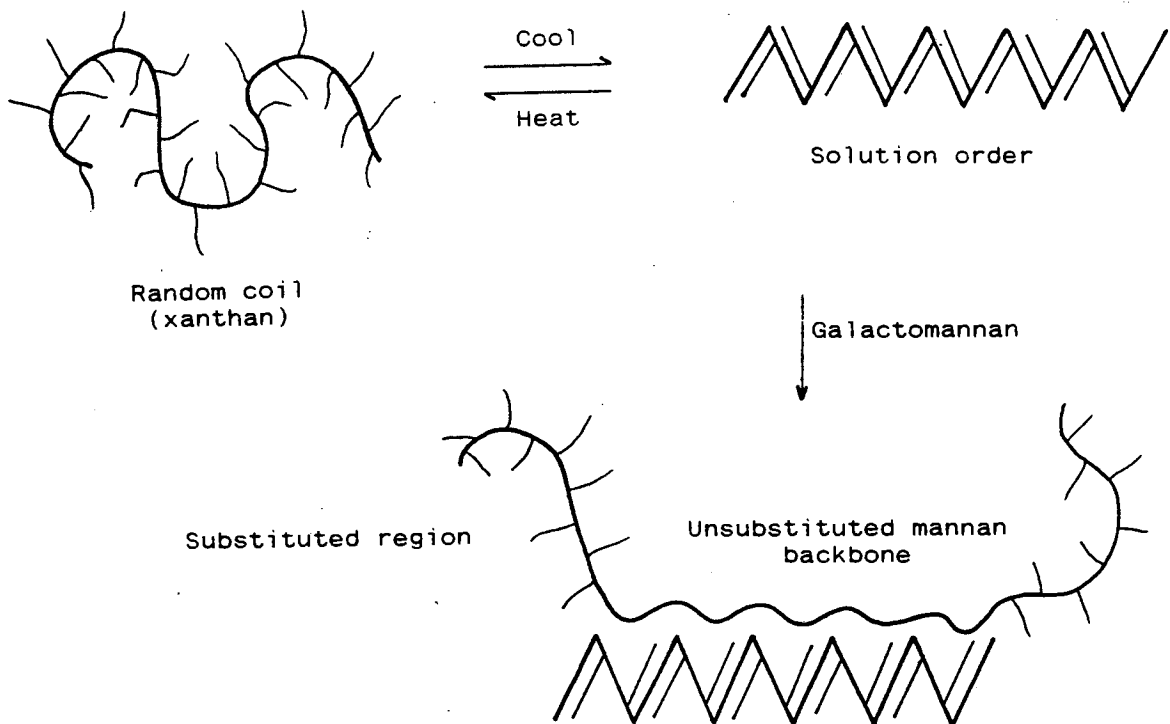
that with unpublished results from De Murcia, EM was not valid for following changes in conformation under any circumstances. They claim that the results mainly point out aggregates, like side by side intrachain associations, whatever the experimental conditions.

9. Gelation Studies of Xanthan

Various polysaccharides gel by various means. Alginate has generally an alternating sequence of β -D-mannuronic acid and α -L-guluronic acid. It can however also have blocks of guluronic or mannuronic acid sequences, and has the ability to form gels in the presence of divalent cations such as Ca^{2+} and Sr^{2+} . This is mainly due to the blocks of guluronic acid, and thus dimerisation of two chains may occur with the cations building the "bridges" between the two chains. At very low ionic concentrations, the xanthan molecule is in the disordered conformation, and an addition of divalent ions will only transform the polymer into the ordered conformation. Xanthan does however gel in the presence of trivalent chromium ions, and is being used in this form for profile modifications of oil reservoirs (Burkholder, 1984; Chang, 1985). The xanthan needs to be in a 0.2% concentration and the chromium ions at a strength of 100 ppm. The usefulness of this gelling property to the oil industry will be discussed further under oil recovery.

Xanthan has another unique property when it comes to reacting with other polysaccharides. Together with galactomannan, a seed reserve polysaccharide, which has a β -(1 \rightarrow 4)-mannan backbone with galactose sidechains, in a mixture of 0.1% of the two polysaccharides the solution showed a higher viscosity than either of the two polymers alone would show, ie. synergism occurs. Xanthan and galactomannans can also form "true gels" at concentrations where neither on its own would gel (Dea *et al.*, 1977; Dea, 1987).

Fig.1.13. A schematic representation of the interaction of xanthan and galactomannan (adapted from Morris *et al.* (1977)).



At a concentration of 0.3%, these two polymers form thermoreversible gels under conditions in which neither of them alone would form a gel. The various galactomannans have often galactose substitutions in a block structure. These gels become firmer the lower the galactose content of the galactomannan and it is due to the interaction between the ordered xanthan and the unsubstituted mannan backbone that the gels get their strength. The ordered xanthan thus interacts with the mannan backbone that is without sidechains (Cairns *et al.*, 1986). Guarana, which is a galactomannan that has sidechains on every backbone residue, does not form a gel with xanthan. The reactions with the galactomannans have partly formed the concept that the exopolysaccharides are produced to recognise sites of penetration in plant hosts as these galactomannans are also found on the surfaces of plants (Morris *et al.*, 1977).

D. THE USE OF XANTHAN IN INDUSTRY

1. Industry in General

The biopolymer xanthan yields a high viscosity when it is added in low concentration to aqueous fluids. A highly viscous and pseudoplastic solution is reached (~2000 mPa·s at a shear rate of 1s^{-1} in seawater) when adding only 0.2% of the polymer. At the same time it is very

stable towards heat, and also stable against shear degradation to a larger extent than many other biopolymers. In contrast to these biopolymers, the addition of salt does not cause the structure in solution to collapse, but rather stabilises the viscosity achieved against heat-degradation. All these aspects of the biopolymer make it very useful to industry. The worldwide production of xanthan is today estimated to be 20,000 tonnes, at a cost of £ 7,500 per tonne (Yalpani and Sandford, 1987).

Of critical importance to the use of xanthan, is its pseudoplasticity, ie. shear thinning properties. On increasing the force on the liquid, the liquid can change from being a thick fluid to flow very easily. This shear-thinning behaviour allows a thick suspension of xanthan to flow even through small pores when under pressure, but once being relieved of the force, it returns to its original state and behaves like a thick suspension. Thus it is easily handled, and very readily utilised in many industrial applications. It is used as a suspending agent in agriculture, mining and oilfield applications. In more recent years it has been also used in jet and foam printing. Xanthan was also the first bacterial polymer to be licensed for use in foods. This was granted by the U.S "Food and Drug Administration" (FDA) in 1969 and a few years later by the EEC. It is now found in dried and canned foods, bakery and dairy

products, confectionery etc and is given the number E415 under EEC food additive regulations. A list of its uses is given in table 1.1.

TABLE 1.1.

Industrial uses of xanthan

agriculture	flowable pesticides
	liquid fertilisers
	liquid feed supplements
cleaners, polishes	suspending agent
oilfields	drilling muds
	enhanced oil recovery
polymerisation	suspensions
textiles	jet- and foam- printing
foods etc.	dried, canned, dairy,
	baking, salad dressings
	frozen foods, confectionery,
	etc.
other uses	suspending agent in
	coal slurries
	thermal heat storage

The two latter uses are of a quite recent nature. Coal is probably the fossil fuel that we will have the most use of in the coming centuries. One of the techniques to make the use of coal more attractive, is to mix the fine-

ly crushed coal with water to make it fluid. To keep the coal-dust in suspension, the solution is stabilised by using xanthan as a viscosifier (Ito et al., 1988). Mixing coal-dust with water also enhances the heat efficiency of the burning process.

The second process is a rather ingenious process where xanthan is mixed with a nucleating agent like sodium acetate. This solution is heated to 80°C until all solid particles had melted. The sample was stored at 10°C for 150 days and seeded with sodium acetate (trihydrate). A rise in temperature to 55°C was observed. The solution can be reheated to 70°C, cooled and reused (Matthews and Thomas, 1982). Thus it is used in life-saving jackets in cold weather climates, ie. in the North Sea.

2. The industrial production of xanthan

Xanthan can be produced by *Xanthomonas* species cultured on glucose and other nutrients by batch or continuous fermentation procedures. The isolation of microbial strains producing exopolysaccharides and other procedures are well documented by Sutherland (1983). Due to the viscous nature of the product, batch fermentations in various stages are the most common (Vincent, 1985). The conversion rate from glucose to xanthan varies, but high yields of 40-75% have been achieved (Slodki and Cadmus, 1979). The solutions can become so viscous that they are

difficult to handle. Because xanthan is a biopolymer produced by bacteria, ie. produced by living cells, the solution produced does not consist of the polymer alone. Cells rapidly multiply under ideal growth conditions, and for enhanced oil recovery cells are not the desired product. Cells may plug the small pores, and thus prevent further oil production from the reservoir.

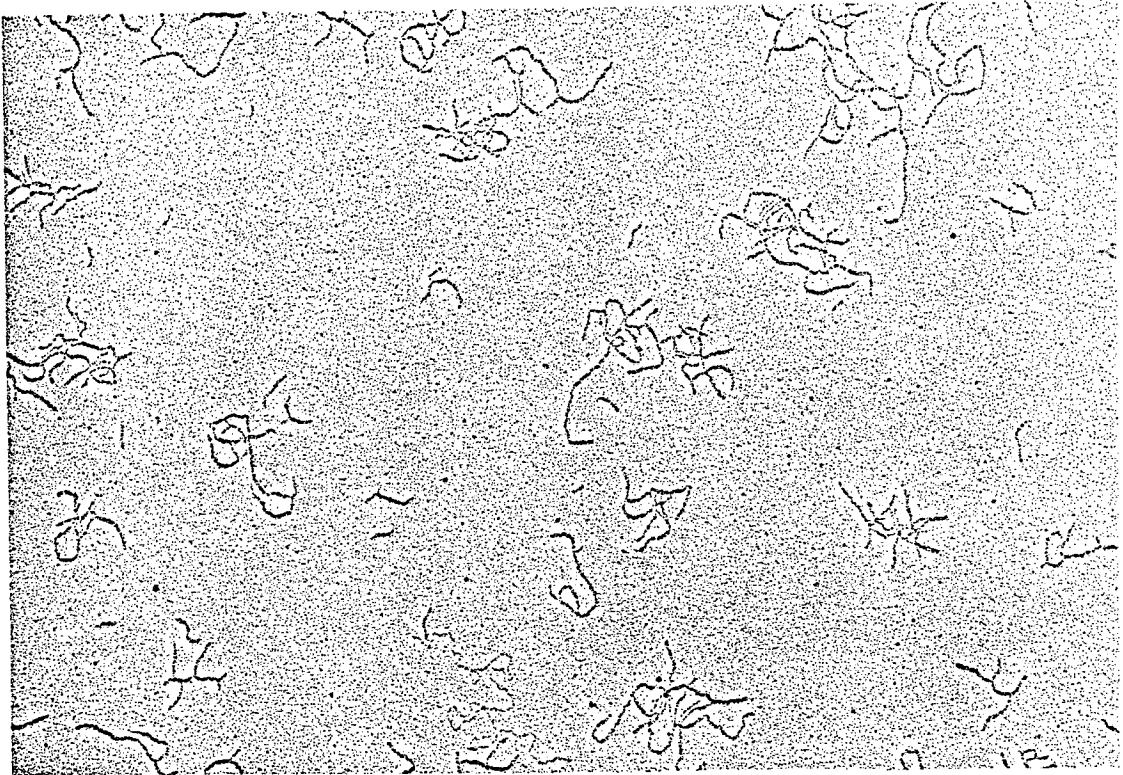
These cells can however be removed from the solution or at least reduced to very low levels by high speed centrifugation or enzymic degradation respectively. These methods are however too costly for the large quantities of polymer involved in EOR. Rather than remove the cells altogether, xanthan is produced commercially in large fermentors under ideal growth conditions for the production of xanthan alone (Vincent, 1985). These media have a low nitrogen content, thus slowing down further cell production and rather enhancing the output of xanthan. The cells thus produced are small and few compared to other production methods. Commercial preparations therefore contain some cellular material as well as xanthan.

3. Microgel formation and dissolution

It has been known for quite some time , that broths made up from dried xanthan polymer have a tendency to plug small pores and solutions to these problems have been sought (Patton, 1973, Abdo, 1973). On the other hand,

fermentation broths do not contain microgels (Kohler & Chauveteau, 1978). More recently, Kolodziej (1987) has given some background information as to how they are formed. Microgels were found to be produced during the drying process of the fermentation broth when proteins and salts, or only one of the two, were present. It did not seem that cellular material was involved in the formation of microgels. The drying process was thought to involve denatured proteins, which exposed hydrophobic

Fig.1.14. An EM micrograph of an unheated, freeze-dried xanthan 646 sample solubilised in seawater. Photograph prepared by B.T. Stokke. X 50'000.



groups, which in turn led to salt induced associations between these protein molecules and the biopolymer. The addition of small amounts of a surfactant alleviated this

problem. Microgels are assumed to be pictured in the electron micrograph shown on the previous page (fig. 1.14). It is taken of a freeze-dried xanthan that has been dissolved in seawater at room temperature. When compared with the picture in figure 1.11, the large difference between these preparations can be seen.

As mentioned earlier, small pores can be blocked by large aggregates and microgels. This is not so crucial for normal industrial use, but in EOR it could lead to problems. The oil is found mostly in sandstone formations where the pores may be small. Therefore, Kohler & Chauveteau (1978), devised a method for comparing the filterability of a xanthan solution in the laboratory. They used an assembly of two stacks of 3 μ m Millipore filters on line. The length of a xanthan molecule should be 1-2 μ m long and also be deformable under stress. The bacterial cells should be smaller than 3 μ m as well, so the filters should not be clogged by these on their own. Upon passing fermentation broth through the filters, no clogging was experienced. If the xanthan broth was however prepared from a dried preparation, then clogging occurred.

Numerous improvements in xanthan filterability and other solution properties have been sought. The methods patented vary from enzymic treatment, to heat treatment, treatment by basic solutions etc. (Abdo, 1973;

Wellington, 1978; Rinaudo et al., 1982; Holzwarth, 1984). The problems of pore plugging and solving these problems have also been taken up in this laboratory (Schroeder et al., 1985), as well as by Kohler et al. (1985). The indications are that these problems can be solved, but on an individual basis. That is, that the varying strains of *Xanthomonas* used in production of xanthan, require different approaches. For example, proteases all have the function of breaking down proteins, but they vary a lot in their ability to alleviate the filter clogging problem (Schroeder et al., 1985). This treatment adds however substantially to the cost of producing a xanthan solution useful to industry.

Thus, for EOR purposes, the preferred method of shipping the xanthan from the production place to place of use, is to concentrate the polymer to a very thick suspension (5-6% polymer), to which a bacteriocide like formaldehyde is added. This concentration by filtration allows the large volumes produced to be shipped in comparatively small volumes, then to be diluted at the site of use to the appropriate concentration.

The aim of this project was however to look at the potential for the use of xanthan in enhanced oil recovery (EOR). It is not a new application for xanthan. It has

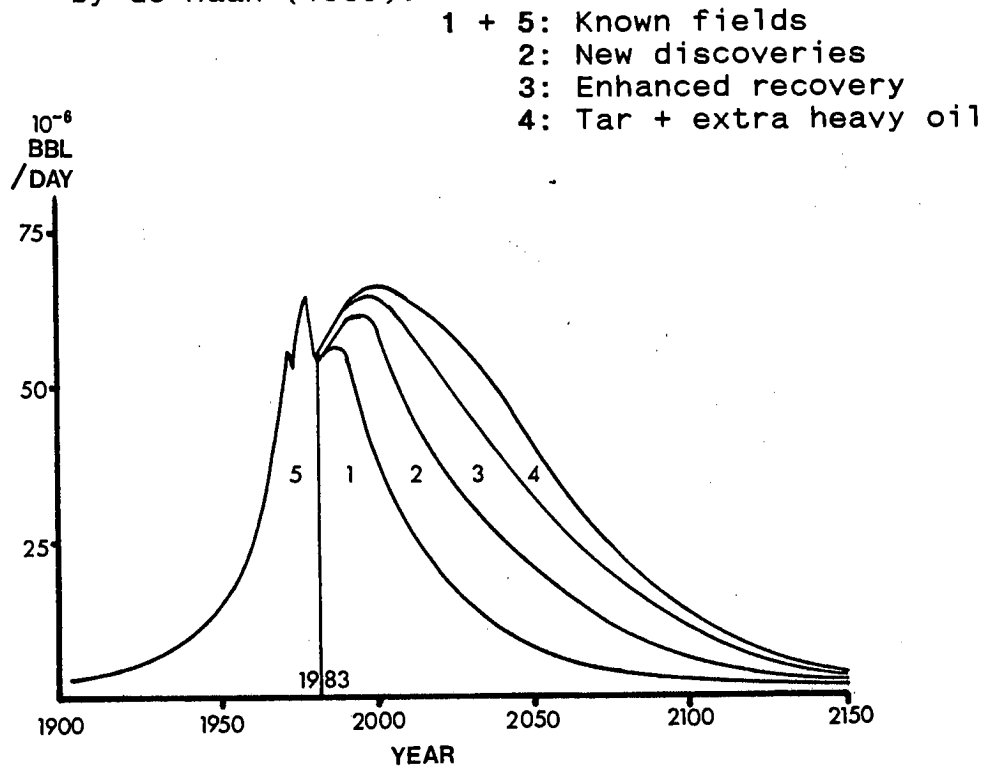
been used in EOR for quite some time, but mainly in the US. However, the temperature at which it has been used is much lower than expected in the North Sea reservoirs. Thus in this project the xanthan is mainly tested for its heat stability at the high temperatures at which the North Sea reservoirs are found.

4. Xanthan in the Oil Industry

Although other energy sources are available, none are so convenient to use as products from oil. They are liquid, and give a high energy output in a low volume. It is therefore very important for the transportation industry, as well as being a base chemical for the synthetic chemical industry.

The world's oil production reached a peak at the end of the seventies, then experienced a small drop to 1983, but is set to climb to a second peak about the year 2000 (see fig. 1.15, from de Haan, 1985).

Fig.1.15. The production of oil from 1900 to 2150 as seen by de Haan (1985).

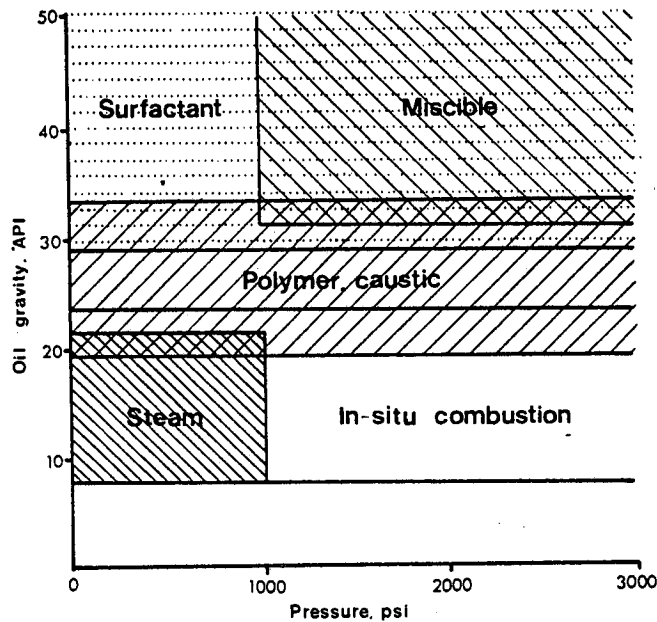


By the year 2100, oil production could be as low as 20% of that in the year 2000. The best picture of this is perhaps shown by the oil recovery efforts in the USA. They have had a decline in oil production from 1970 onwards, when 3,517 million barrels of crude oil was produced. The enhanced oil recovery (EOR) processes are therefore especially well developed there. Stosur et al. (1985), calculate that of the 33% of the original oil in place (OOIP) recoverable by conventional methods, 27% have already been produced whilst 6% is remaining. Thus there is 67% of OOIP still remaining in the reservoir. The predictions are that these large, presently

unrecoverable resources can be tapped to some extent by EOR. Therefore development of enhanced oil techniques and the testing of other energy sources are extremely important. Although the estimates given by de Haan (1985) are hypothetical, they seem realistic in today's economical and political climate.

Oil recovery is not a straightforward process. The methods used differ depending on the crude oils being heavy or light (see fig. 1.16), the type of geological formation in which they have accumulated, reservoir

Fig.1.16. The oil gravity ($^{\circ}$ API), versus the pressure at which the various EOR processes are feasible.



temperatures etc.. The suitability of a reservoir for EOR processes is often evaluated by mathematical modelling (Hughes *et al.*, 1988). Normally, the reservoirs are under pressure and oil will flood out naturally

if a suitable exit well is provided. To maintain the pressure, water or gas is pumped into the oil well (secondary recovery). Water has very different rheological properties to that of oil, which might mean that the water is found mixed with oil at an early stage in the oil producing well, ie. an early "water breakthrough" (ie. fingering) occurs. Some of the enhanced oil recovery processes are thus concerned in trying to avoid fingering and increase the efficiency of the water sweep.

TABLE 1.2

The four main oil recovery processes.

1	miscible and immiscible processes	CO ₂ and hydrocarbon gas
2	thermal processes	a) steam drive b) in situ combustion c) hot water
3	mining	heavy oil and tar sands
4	chemical processes	a) surfactant flooding b) polymer flooding c) caustic flooding

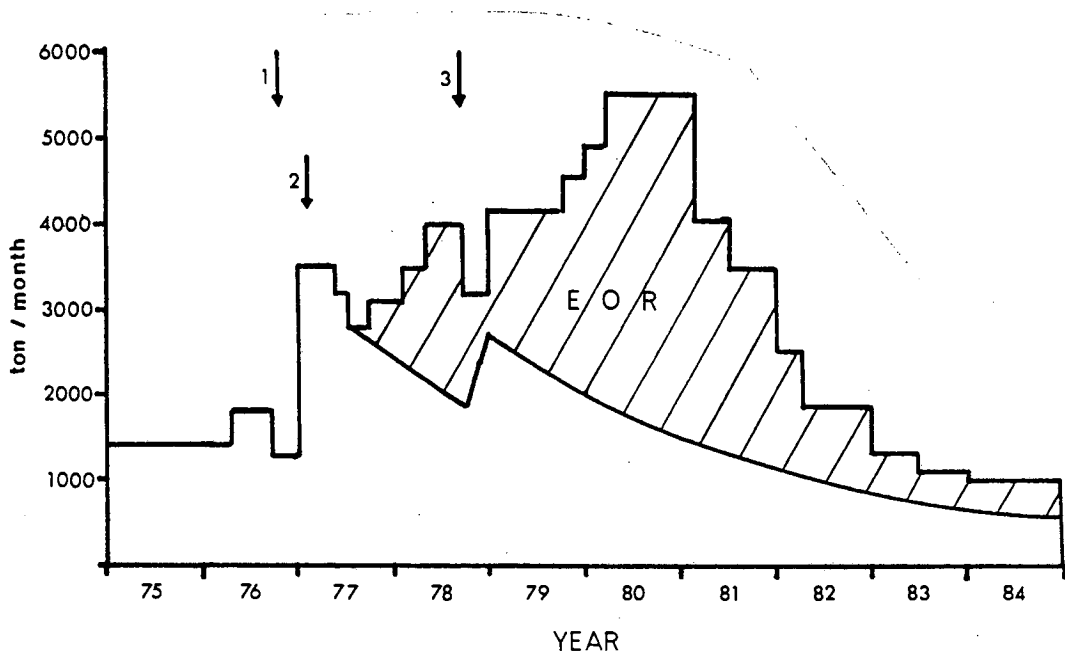
Globally, of all the methods mentioned above, the thermal and miscible methods are the most prominent. Similarly, of the chemical methods, use of surfactants will probably

be most important. Thus polymer flooding will only play a minor role in the overall picture of EOR although it may have considerable importance locally. How successful it can be, has been described by Maitin (1985). In the Hankensbuettel field (West Germany), a polyacrylamide flood increased the oil output from 48% of OOIP to 65% of

Fig.1.17. A simplified graph of oil production development at the Hankensbuettel field before and after polymer injection (from Maitin, 1985).

1 and 3=New wells

2= Start of polymer injection



OOIP. This was an increase of 35% on the original output. This increase came about by injecting 500,000m³ of the polymer solution over a period of 2 years. The field involved was small, but it shows how successful polymer EOR can be when the conditions are right.

Of the chemical methods, surfactant flooding is the most important. The surfactants reduce the surface tension, thus making the oil more fluid. Caustic flooding acts by forming surfactants with the oil in the reservoir. It is the least used and has yet to overcome many potential problems. However, the chemicals for this method are cheap. Polymer flooding is the EOR process in which we are interested. Both synthetic and natural polymers can be used and new polymers are still continuously being tested (Doe *et al.*, 1985). Polyacrylamide is the most common of the former, whereas xanthan is the most common biopolymer.

Xanthan as a polymer for EOR, is in direct competition with polyacrylamide. The biopolymer xanthan and the synthetic polymer polyacrylamide, show large differences in their tolerations for salt etc. as well as in price. Polyacrylamide is cheap to produce, contains no cells or microgels, but is not as shear and salt tolerant as xanthan. So in reservoirs where conditions are appropriate, polyacrylamide would be the polymer to use. If the reservoir has a high salinity or the polymer will be liable to shear degradation, then xanthan is the obvious choice. Some of their varying qualities are given table 1.3 on the next page.

With the differences in suitability for the EOR processes

TABLE 1.3.

A comparison of xanthan and polyacrylamide with respect to their usefulness in EOR.

	XANTHAN	POLYACRYLAMIDE
Viscosity	good at low concentration	high concentration needed
Salt tolerance	good up to 20%	good up to 2%
Shear tolerance	no breakdown	breakdown
Filterability	good	average
Costs	high	low
Particulate material	cells and microgels	none
Temperature stability	good	good

noted in table 1.3., the main disadvantages of using the xanthan instead of polyacrylamide is the particulate matter in xanthan solutions and the high cost of producing it. Xanthan however has the benefit of tolerating high salt (Akstinat, 1980; Auerbach, 1985) and shear conditions, so the cost has to be balanced against its usefulness.

The area of EOR in which most of the polymer will be used in the future is however in micellar/polymer floods, such

as the Loudon project (Bragg 1982). The surfactants in the flood lower the tension between the surface of the rock and the oil, whereas the preceding polymer slug shifts the high saline solution in the reservoir in front of it. Thus no low saline preflush is required. These micellar/ polymer floods are much more efficient than either flood on their own. Surfactants have however high production costs, thus giving a rise in overall production costs.

In the USA, where xanthan has been used most frequently so far, the reservoir temperatures have been relatively low, ie around 60°C. At this temperature, xanthan degrading bacteria (Cadmus *et al.*, 1982; Cadmus and Slodki, 1987) might survive, so a bacteriocide has to be added to the water that is to be pumped into the oilwell. However, there will be no thermal degradation of the polysaccharide. With North Sea reservoirs, it is rather different (Davison and Mentzer, 1980). Here temperatures are often found to be over 90°C and xanthan has to be tested at this temperature to see if it is stable against viscosity loss. Precautions against biological degradation of the xanthan still have to be taken until it attains the reservoir temperature.

II. CHAPTER TWO: MATERIALS AND METHODS

A. XANTHAN PREPARATION IN THE LABORATORY

Strains which had been previously selected were grown in a Bioengineering 1.5 l fermentor. A 100 ml inoculum was used. The medium used for growing the *Xanthomonas campestris* strains was a "synthetic yeast extract medium with added amino acids". It's contents are given below and it's final pH was set to 7.2.

<u>Chemical ingredients</u>	<u>g/l-1</u>
Na ₂ HPO ₄	10.0
KH ₂ PO ₄	3.0
K ₂ SO ₄	1.0
NaCl	1.0
MgSO ₄ · 7H ₂ O	0.2
CaCl ₂ , 1.0 ml of 1% solution	0.01
FeSO ₄ , 0.1 -----	0.001
Casamino acids	1.0
Yeast extract	1.0
Glucose	20.0

The viscous broth produced was concentrated in a Millipore Pellicon cassette system (cat. no. XX4200080) using a 100,000 daltons cutoff filter made of polysulfone (a 1 million dalton cellulosic filter is available, but was found to be too weak for these viscous fluids). The concentrate was then frozen, and if need be, centrifuged and lyophilised.

B. XANTHANS USED IN PREPARATIONS

Various xanthan preparations were used in setting up the solutions for testing thermal stability. The molar acyl substitutions are given in parentheses.

Flocon 4800C: a commercial xanthan preparation produced by Pfizer Inc. (New York). This xanthan is high in pyruvate (0.75) and low in acetate (0.16). It is shipped as a concentrate of 5-6% strength with respect to xanthan. The concentrate contains formaldehyde as a preservative.

The two following strains were originally obtained from the National Collection of Plant Pathogenic Bacteria, Harpenden, UK.

Xanthan 1128: A laboratory preparation of xanthan from strain 1128 . This xanthan is low in pyruvate (0.22) and high in acetate (0.62).

Xanthan 646: Another laboratory preparation which is high in pyruvate (0.57) and high in acetate (0.79).

C. HEAT-STABILITY EXPERIMENTS:

Xanthan samples were either prepared from a broth (FLOCON, from Pfizer) or from a freeze-dried polymer preparation (1128). Both were dissolved in distilled water first, then dialysed before use. 0.45 μ m filtered seawater from the North Sea was degassed before addition of citric acid (100 ppm), thiourea (1000 ppm), iso-propanol (2000 ppm) and sodium sulphite (2000 ppm), according to the method of Wellington (1980), although a higher sodium sulphite concentration was used. The solution was then purged with oxygen-free nitrogen before adding the polymer, which had been dialysed against distilled H₂O. The polymer was dissolved in the solution with a magnetic stirrer whilst still flushing with N₂.

Ampoules (10 ml freezedrying ampoules, taking ~30 ml in volume), were rinsed with distilled water and dried.

Ampoules were flushed with N₂, before about 25 ml polymer solution as prepared above was added. The air space left was flushed with N₂ continuously whilst sealing the ampoule. Sealed ampoules were then put in the ovens for variable amounts of time at varying temperatures (60, 91, 96 and 100°C were used in these experiments). Oven temperatures varied slightly during the incubation period, especially at the highest temperatures where +/- 3-4°C was measured.

On taking out ampoules, the solutions were 3.0 or 5.0 µm filtered to remove broken glass and some particulate material. An Amicon 8010 ultrafiltration cell was used in the procedure.

D. VISCOSITY

The viscosity of a solution was determined using a Brookfield LVTDCP cone-plate viscometer with a digital read-out. Shear rates were variable from 0.6 to 120 s⁻¹.

E. TOTAL CARBOHYDRATE

The total amount of carbohydrate in solution was determined by the method of Dubois *et al.* (1956), the method being adapted to a microscale. To 200µl of solution containing less than 15µg carbohydrate, 200µl phenol (5%), was added and mixed, before adding 1ml concentrated H₂SO₄ and mixing quickly (CARE!). The solution was left

to cool before reading at 490nm. Standards were prepared from a 1µg/µl glucose solution.

F. REDUCING SUGAR

To a solution of 500µl, containing carbohydrate, 500µl of a mixture of solutions A/B (ratio 25/1) was added before heating at 100°C for 20 minutes according to the method of Ashwell (1957). After cooling, 0.5 ml of solution C was added and mixed.

Solution A: 25 g anhydrous Na₂CO₃
 25 g Rochelle salts (sodium potassium
 tartrate)
 20 g NaHCO₃
 200 g anhydrous Na₂SO₄
 dissolve in 800 ml, make up to 1000 ml

Solution B: 15 % CuSO₄ * 5H₂O
 1-2 drops of conc. AR H₂SO₄ / 100 ml

Solution C: 25 h NH_4 -molybdate, dilute to 450 ml
with H_2O
21 ml conc. AR H_2SO_4
3 g $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ in 25 ml H_2O
Store in brown bottle

The result was read at 510nm. Standards were prepared from a $1\mu\text{g}/\mu\text{l}$ glucose solution

G. PYRUVATE

The amount of pyruvate in solution was determined by the method of Sloneker & Orentas (1962). The solution to be assayed was heated at 100°C for 3 hours in a 1 molar HCl solution in a sealed glass ampoule. Of this solution, 200 μl was mixed with 100 μl 0.5% 2,4 dinitrophenylhydrazine in 2 molar HCl , left at room-temperature for 5 minutes before extracting it with 500 μl ethyl acetate and discarding the lower aqueous layer. The ethyl acetate solution was removed. The reacted pyruvic acid was extracted twice with 500 μl 10% Na_2CO_3 , removing the aqueous layer into a clean tube. The solution was then diluted with 1.5 ml distilled H_2O .

Samples were read at 375 nm. The standard was pyruvate (0-20 μg) in 1 molar HCl .



H. ACETATE

The amount of acetate in solution was determined by the method of Hestrin (1949). A volume of 200 μ l, containing 1-10 μ g acetyl groups, was mixed with 400 μ l of a 1:1 mixture of solutions A & B, left for 2 minutes at room temperature, then 200 μ l each of solutions C & D were added and mixed consecutively.

The readings were made at 540 nm.

- Solution A: Hydroxylamine HCl (hydroxy-ammoniumchloride), 2M, stored at 0°C
- Solution B: 3.5 M NaOH
- Solution C: conc. HCl, specific gravity 1.18, dilute in H₂O 1:2
- Solution D: FeCl₃*6H₂O, 0.37 M in 0.1 M HCl
- Standard: 0.04 M acetylcholine chloride in 0.001 M sodium acetate, pH 4.5.

I. GLUCURONIC ACID

The uronic acid content of the solution was determined by the method of Blumenkrantz & Asboe-Hansen (1973). To a 100 μ l sample containing 0-20 μ g uronic acid, 600 μ l icecold acid tetraborate was added and mixed CAREFULLY.

The solution was heated for 5 minutes at 100°C, then cooled. After adding and mixing 10 µl mHDP reagent the solution was read at 520 nm.

Solutions: 0.15 % m-hydroxy-diphenyl in 0.5%
NaOH (obtained as m-phenyl-phenol
from Kodak).

0.0125 M sodium tetraborate
solution in conc. H₂SO₄.

Standard: 1µg/µl glucuronic acid

J. HPLC: SAMPLE PREPARATION

Neutral sugars: Hydrolysis: The polymer solution was hydrolysed in 0.1 M H₂SO₄ for 18 hours at 100°C in a sealed ampoule. A sample of the hydrolysed solution was neutralised with regenerated Amberlite IR410 HCO₃⁻ resin (BDH, Sigma). The liquid was removed and the resin washed with more deionised water before microfuging and filtering the solution (0.45 µm). The solution was then frozen until used.

Analysis: Neutral sugars were analysed by injecting the hydrolysed products into a lead cartridge column (30 cm microbore column from Brown-

lee Laboratories Inc). Flowrate was 0.2 ml/min (in deionised water), standards were glucose and mannose.

K. MOLECULAR WEIGHT DETERMINATION:

Gel-filtration: Samples of the polymer were run on a LKBAcA 34 column (60*1 cm) at a flowrate of 3.5 ml/hour. The total amount of carbohydrate in the various fractions was determined by the method for total carbohydrates as explained earlier.

HPLC: Samples of the polymer (dialysed and 0.45 μ m filtered), were run on TSK G4000PW and TSK G6000PW columns. The standards were pullulan of various MWs, varying from 5800 to 853,000 Daltons (from Polymer Laboratories Ltd, UK)

L. AUTOHYDROLYSIS:

The xanthan 1128 for this preparation had been exhaustively dialysed against distilled water and ultracentrifuged (100,000g for 2 hours). The solution was put through mixed bed resin (BDH, Sigma), giving it a pH of 3.5 or lower. Glass ampoules were filled and sealed. Autohydrolysis was achieved by heating the ampoules in

the 100°C oven for 30 hours. The different molecular weight sizes were separated by Millipore cutoff filters using the Amicon ultrafiltration cell.

M. PAPER CHROMATOGRAPHY:

Hydrolysed polysaccharide fractions (as for HPLC) were spotted on Whatman chromatography paper and eluted with butanol-pyridine-water (6:4:3 v/v) [neutral sugars, solvent A] or with ethylacetate-acetic acid-formic acid-water (18:3:1:4) [for oligosaccharides, solvent B].

III. CHAPTER THREE: RESULTS AND DISCUSSION

A polysaccharide is useful to the oil industry (and especially for EOR purposes) only if it maintains stability with respect to viscosity after prolonged storage at higher temperatures. It also has to have the physical properties mentioned in table 1.3. That table compares the biopolymer xanthan with the synthetic polymer polyacrylamide. The high temperatures found in North Sea reservoirs restrict the use of xanthan for EOR purposes. As cooler water is used for water injection in secondary oil recovery, some cooling of the reservoirs will occur. This cooling of the reservoirs might make more reservoirs available for future polysaccharide treatment.

To find the temperature at which there is no degradation of the polysaccharide, is therefore most important. It varies with the type of xanthan used, but the extent of degradation depends most probably on the conditions under which the biopolymer has been produced. The pseudoplastic and viscosifying effect of xanthan is most impor-

tant, and following the fall or increase in viscosity of a solution during heat storage, gives the first indications on how well the integrity of the polysaccharide has survived at higher temperatures. Following the amount of carbohydrate and the substitutions of the acyl groups quantitatively, might also show how the degradation of the polysaccharide occurs. Additives can be added to the solutions which might improve the stability of the polysaccharide markedly.

A. CHANGES IN VISCOSITY

The changes in viscosity of various preparations were followed closely during storage at higher temperatures. These changes are especially important when considering polysaccharides for EOR purposes. The viscosity measurements were however not done at the actual temperatures at which the experiments were performed. They were made on heated solutions which had been allowed to cool for at least some hours before the viscosity was measured at a constant temperature of 25°C. It is assumed that measurements made at 25°C correlate with the viscosities at the higher temperatures.

1. Xanthan stored in seawater

The Flocon solution (0.2%) prepared for long-term stability experiments at elevated temperatures were prepared from a liquid concentrate. Previous tests have shown it to be stable for at least 800 days at 80°C (fig. 3.1) after an initial viscosity loss of 25%. In the experiments shown in fig. 3.2, continuous viscosity loss was already showing at 60°C in seawater, with a protection package similar to that used previously. This discrepancy might be due to oxygen being present in the ampoules, although every possible action was taken to avoid this happening and is not thought to be present. Alternatively, the difference could be due to the fact that the earlier polysaccharide solution was not dialysed before being set up, as was done with this preparation. It is however presumed to be due to use of a solution prepared from a concentrate which had been stored for some time at room temperature. Formaldehyde was present as a preservative in the concentrate, but it might not have been sufficient to avoid subtle changes in the Flocon polymer. Thus the Flocon did not perform as well as expected, and this has to be taken into account when considering the results given.

In the samples stored at 60°C in seawater, the solution loses 30% of its original viscosity during the first 50 days. This increases to a loss of 40% after 440 days and

Fig. 3.1 The viscosity of the Flocon solution that showed stability up to a 800 day period. This was after an initial drop in viscosity of about 25%.

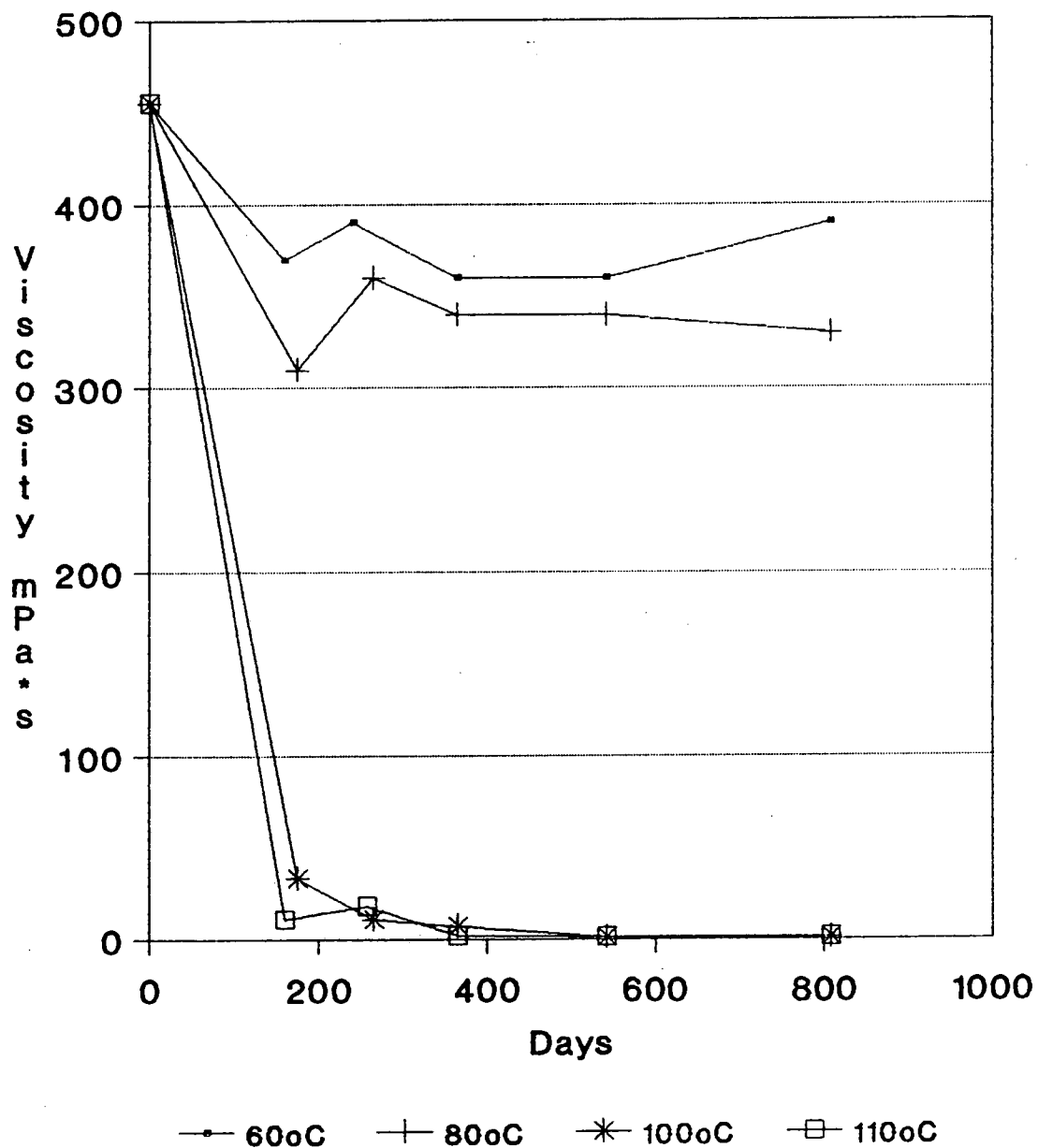
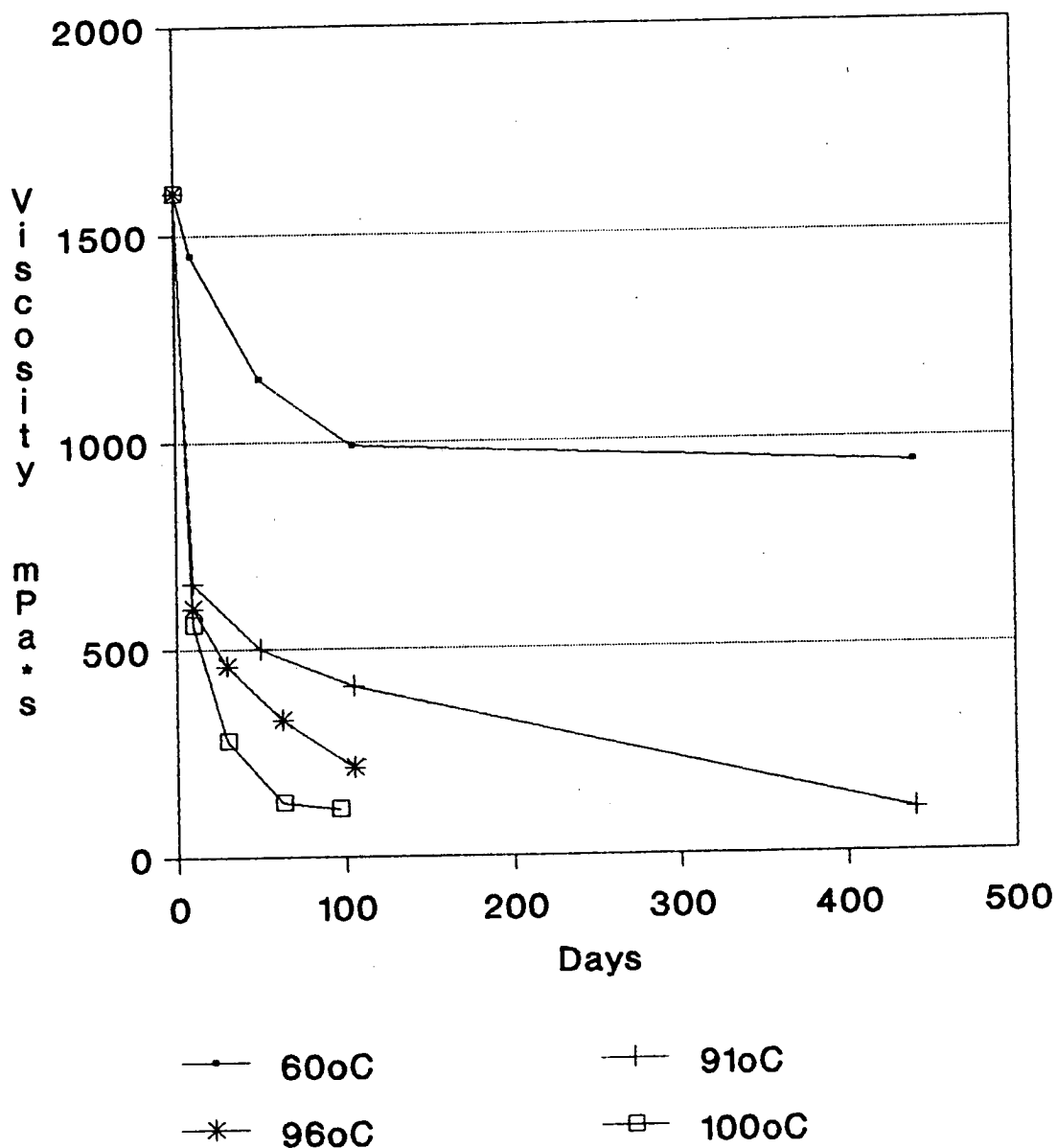


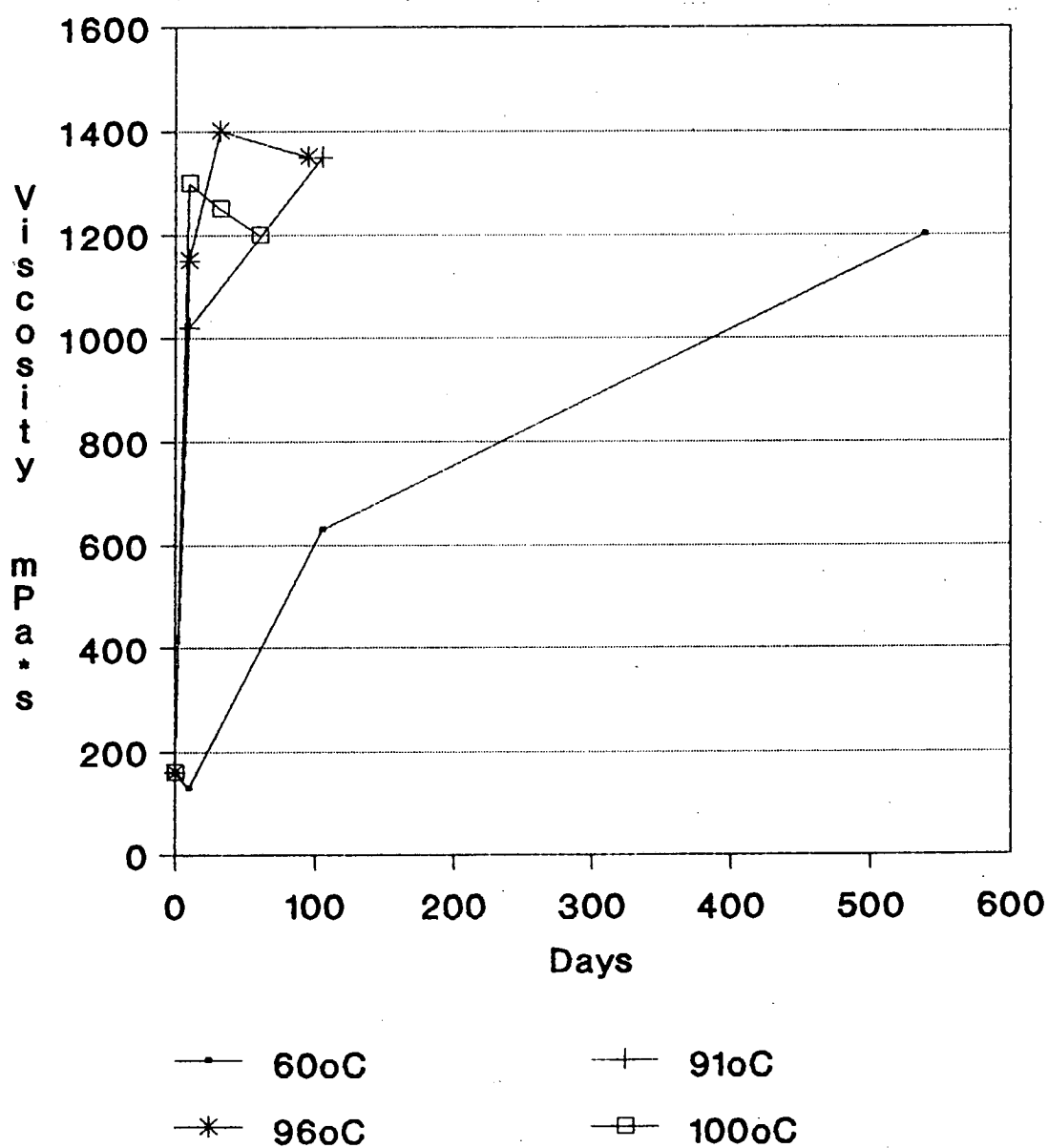
Fig. 3.2. The viscosity of the Flocon solution which showed a large initial loss in viscosity, even at 60°C. At the higher temperatures, an even more marked viscosity loss is noted.



the viscosity of the xanthan seems to have stabilised at this value. In the previous preparation stored at the same temperature, the viscosity fell 20% from its original level, and stabilised at this value. Thus there is a marked difference in stability at this temperature. The corresponding viscosity losses after 50 days of storage at 91, 96 and 100°C was 70, 75 and 90% respectively. Thus the degradation rate at 91°C and above are much too high for consideration in EOR.

Viscosity changes for the xanthan 1128 are quite different from those of Flocon due to the polymer solutions being initially prepared from a freeze-dried preparation. Total solubilisation of the polymer does not occur immediately, and needs heating to be fulfilled. This is clearly shown in fig. 3.3, where during the first 10 days the viscosity increases by 800% when stored at 100°C. The increases are slightly lower for samples stored at 91 and 96°C at 10 days, but at 600 and 700% respectively they are still impressive. Both these latter samples increase their viscosity upon further heat storage and the 96°C sample reached a maximum viscosity increase of 900% after 30 days. The probable explanation for this increase in viscosity is that the microgels formed during freeze-drying (Kolodziej, 1987), dissolve upon heating.

Fig. 3.3. The viscosity of the xanthan 1128 solution upon storing at elevated temperatures. The large initial increase in viscosity is assumed to be due to the dissolution of microgels upon heating. At 60°C, this is much slower than at 91°C and above.



It is interesting to note that once the maximum viscosity is obtained, the viscosity falls somewhat at the higher temperatures. A slight fall is noticed for the 96°C sample between 30 and 100 days, whereas it is more pronounced for the 100°C sample. The 91°C sample had an increasing viscosity even up to the final measurement at 100 days. Sufficient samples were not available for measurements after this period. For the 100°C samples, some samples were saved for measurements at a later stage, and the solution was visibly very viscous even after 100 days.

At 60°C, which is well below the T_m of the polymer in seawater, there is a slight fall in viscosity measured at 10 days before a steady climb to the values comparable to those obtained at the higher temperatures after a shorter time period. Even at much lower temperatures than the T_m , the microgels therefore seem to be able to dissolve and form a more viscous solution. This change takes place over a prolonged period, needing over 100 days for a 400% increase in viscosity. Viscosity measured after 540 days showed an increase of more than 700%, so the polymer was possibly not fully hydrated even after this amount of time.

Comparing the breakdown models of the two polymers in seawater, there is a marked difference between them. For

xanthan 1128, an initial loss in viscosity can be masked by the solubilisation effects of the polymer. Ie. the dissolution of the polymer could increase the viscosity to 1000% over the first few days, and is then reduced by degradation to ~800% after 10 days. Taking the viscosity measured after 10 days, it seems from then on to remain largely unchanged up to 100 days when stored at 91°C (up 23%) and 96°C (up 8%). The 100°C sample experiences an 8% loss up to 60 days and will probably continue to lose its viscosity fairly rapidly. Compare this with Flocon. Samples stored at 91°C experience a further 36% loss, at 96°C a 63% loss and at 100°C a 80% loss in viscosity between 10 and 100 days. Whereas some further viscosity is lost for the 60°C sample of Flocon after 10 days (a further drop of 33% to 440 days), the 60°C sample of 1128 increases in viscosity to a level similar to that of 1128 stored at the higher temperatures. This increase in viscosity is slow, and it means that although the 1128 polymer is not fully dissolved to begin with, viscosity is not lost at any one time even though the polymer might not be in its ordered conformation. The action of hydrolysis on the polymer, must therefore be inhibited by the salts present, and of the two polymers compared here, xanthan 1128 seems to be the more stable.

2. Xanthan stored in distilled water.

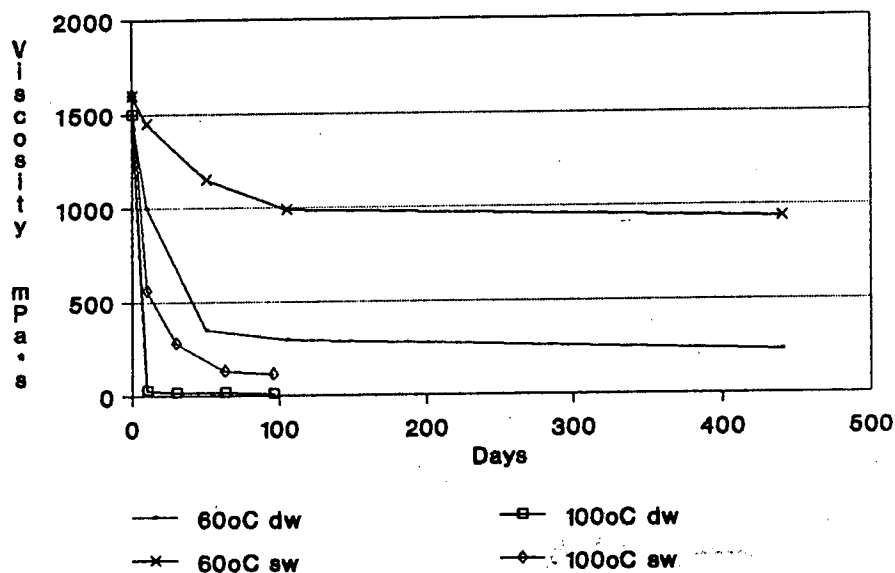
For both polymers, ^{(loss of attainable} viscosity was very severe in distilled water, even at a low temperature of 60°C with a protection package. This is seen from fig. 3.4 a and b, and shows that only 25% of the original viscosity is left after 50 days for the Flocon sample, whereas at all temperatures in excess of 90°C only 2% was left after 10 days. The stabilisation by salts (ie. in seawater), thus has a very marked effect on the viscosity retention of Flocon at higher temperatures.

Unlike Flocon, the viscosity of the 60°C sample of xanthan 1128 remained more or less unchanged from the time of preparation. Over the 540 day period, the increase on initial viscosity is only 30%, which can be compared to that of 700% when solubilised in seawater. For xanthan 1128, the loss in viscosity of samples stored at 91, 96 and 100°C in distilled water are as high and abrupt as for Flocon.

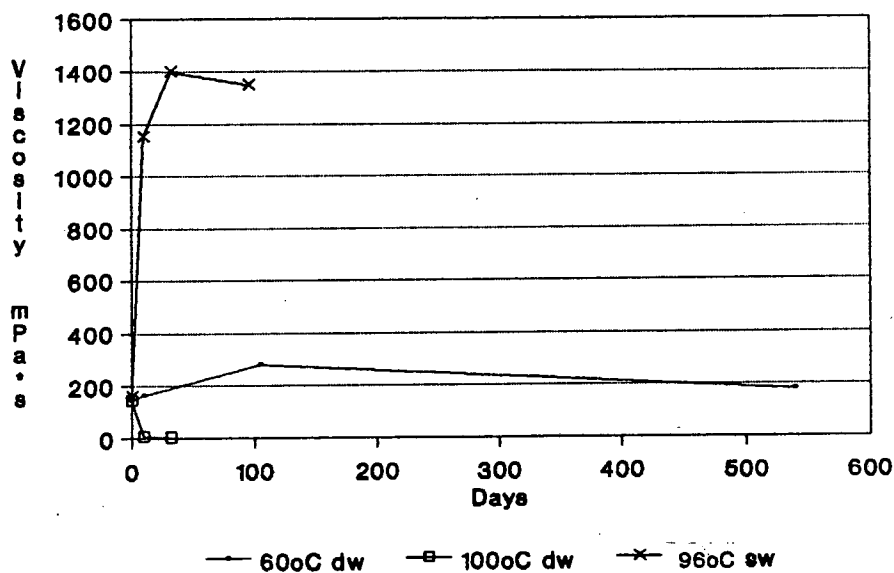
In some way, the stabilisation of the polymer structure by the salts must enhance its ability to withstand thermal degradation. This is irrespective of the sample of xanthan polymer used. Distilled water has thus a very detrimental effect on storage of both polymers at ele-

Fig. 3.4. The viscosity of Flocon (fig 3.4a) and xanthan 1128 (fig 3.4b) when stored in distilled water. For comparison, some seawater samples have been added to the graphs. At 91°C and above, both samples show an immediate loss in viscosity and follow the 100°C curve. sw=seawater/dw=distilled water.

a)



b)



vated temperatures. The salt concentration necessary to achieve this stabilisation has not been tested, but is presumed to be rather lower than that found in seawater. For instance, following the analysis of the CD spectrum, xanthan was found to be in the ordered conformation after the addition of a molar fraction of 0.2 of calcium ions w.r.t. that of the polymer (Dentini *et al.*, 1984). These measurements were however made at 25°C, and this molar fraction would certainly have to be increased to maintain the ordered structure at higher temperatures.

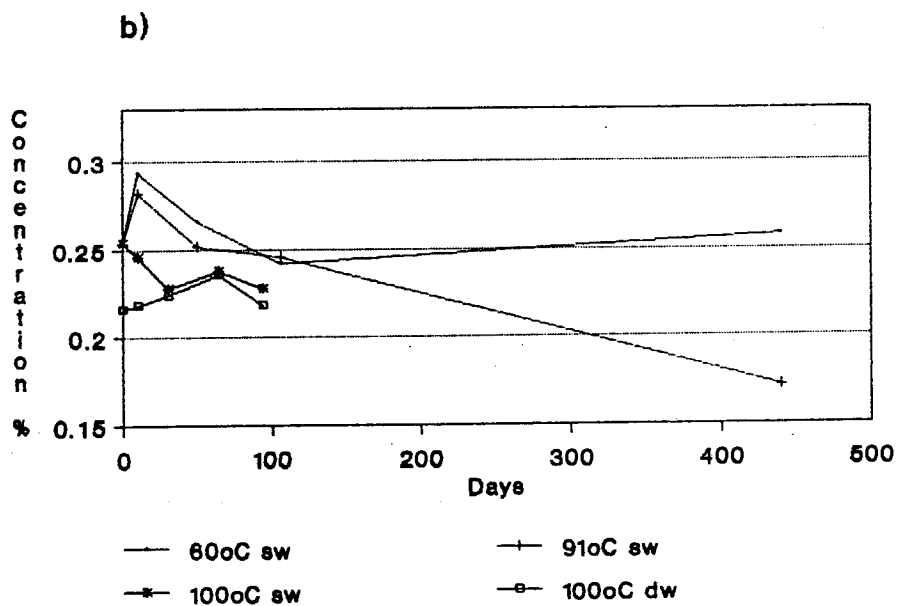
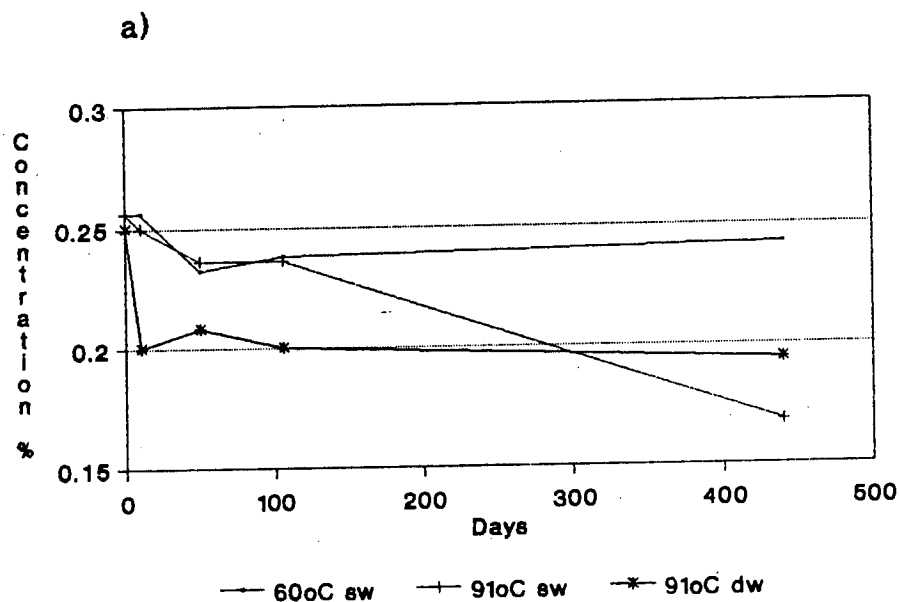
B. TOTAL CARBOHYDRATE

Total carbohydrate was determined in all samples prepared, both before and after filling ampoules for heat storage. Loss in carbohydrate would to some extent show in what manner the xanthan was broken down. The carbohydrate content was determined both when taking the solution straight from the ampoule (ie. 3.0 or 5.0 μm filtered only), and upon dialysed samples. These results are shown in figures 3.5 (Flocon) and 3.6 (Xanthan 1128).

1. Storage in seawater

It was found that the amount of carbohydrate did not decrease as dramatically as did the viscosity for the

Fig. 3.5. The loss of carbohydrate from the Flocon solution. Fig. 3.5a shows the carbohydrate content taken straight from ampoule, fig.3.5b the residual carbohydrate. Some samples are omitted, but the samples stored at higher temperatures tend to show a similar pattern and also if its sea- or distilled water samples.



Flocon sample stored in seawater.

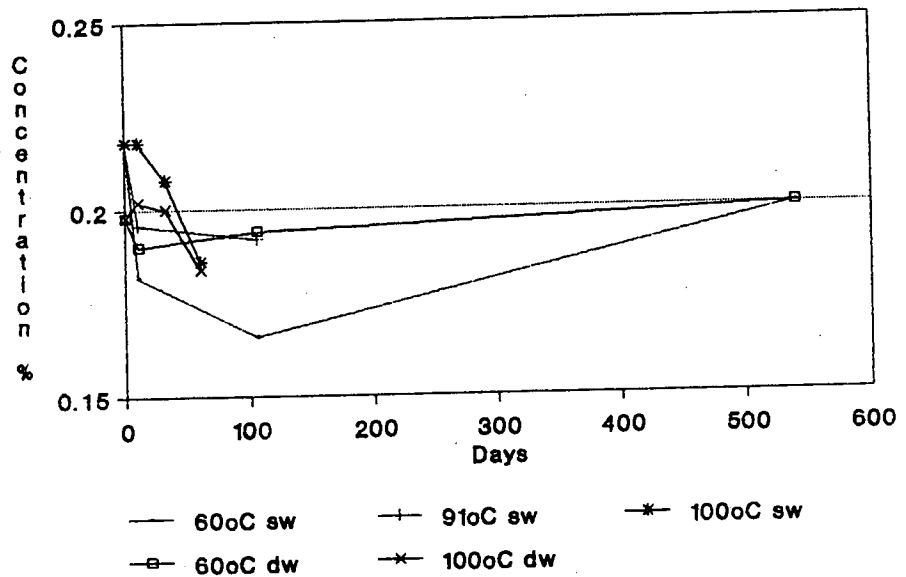
The corresponding carbohydrate loss was 9%. Similarly, at 91°C and above, a large viscosity loss was observed after only fifty days, and that corresponding losses in total carbohydrate were 8, 3, and 10% for the 91, 96 and 100°C samples respectively. Although a larger drop in carbohydrate was observed when measuring the residual carbohydrate content after dialysis, there was not any comparable viscosity loss. After 440 days at 60 and 91°C in seawater, the carbohydrate content had fallen by 4 and 30% respectively. The corresponding viscosity losses were 40 and 93% respectively.

When comparing the curves in figures 3.5 a and b, it is seen that upon dialysis there is not a large change in concentration for any of the samples, and the 100°C sample stored in distilled water follows more or less the pattern of the seawater sample.

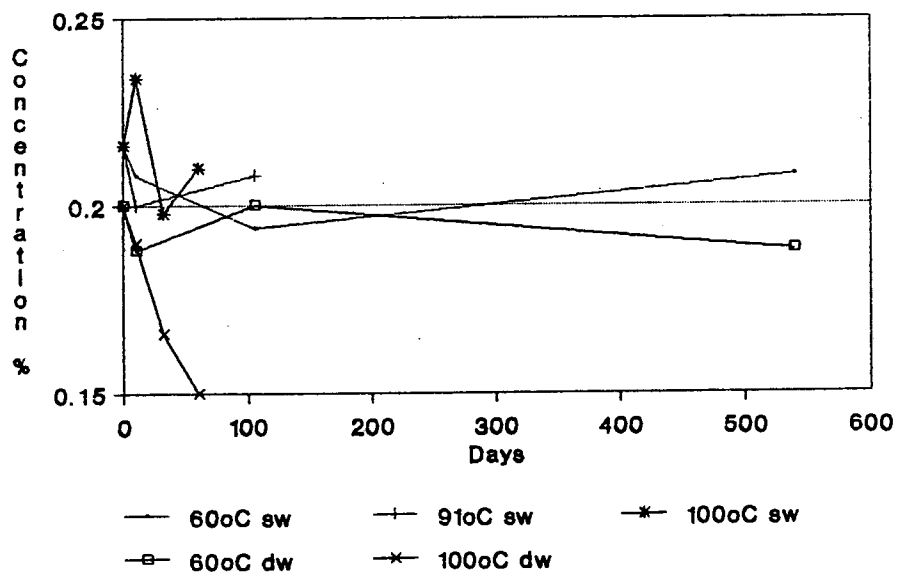
For 1128, the results with respect to the carbohydrate contents are different. No large viscosity losses were noted on these samples stored in seawater. The concentration of carbohydrate in solution does not change markedly either (fig. 3.6 a). For the 91°C sample, taken out after 105 days, the total carbohydrate when measured

Fig. 3.6 The carbohydrate content of xanthan 1128 samples upon storage. Except for the 60°C sample taken straight from the ampoule (fig. 3.5a) and the 100°C sample which has been dialysed (fig.3.5b), no large difference in carbohydrate content was noticed in any of the samples.

a)



b)



straight from the ampoule was 12% below the original value. The 100°C sample was down 15% after 60 days whereas the 60°C sample only fell 8% over 540 days. The 60°C sample had shown a greater loss before this time (down 24%), but it is thought that this is probably due to an inaccurate assay with microgels still being present.

Upon dialysis of the same samples, the residual carbohydrate content of the xanthan 1128 samples remain more or less consistent with the results given above for total carbohydrate. Some initial increase in carbohydrate concentration is noted in the 96 and 100°C curves given in fig. 3.6 b., but on this dialysed material, only a 7% loss or less was recorded over the whole period (ie. 94 and 60 days respectively). In seawater, 1128 thus does not seem to degrade to any great extent when looking at the carbohydrate concentrations.

Storage in distilled water

A similar breakdown can be found when Flocon is stored in distilled water (fig. 3.5). Surprisingly, even though the viscosity loss was very great (98% within 10 days at 91°C and above), the greatest loss in carbohydrate of samples measured was after 94 days at 100°C, when only 31% was lost.

In distilled water, the total carbohydrate concentration of xanthan 1128, when measured in samples taken straight from ampoules remains more or less unchanged as well (fig. 3.6). Similar results were obtained when testing the carbohydrate content in the dialysed samples, except for storage at 100°C. In these 100°C samples, the amount of non-dialysable material falls by 25% over a 60 day period.

Although there was no residual viscosity in the high temperature samples of Flocon, why more carbohydrate is not lost on dialysis is surprising. The xanthan appears to be broken down into smaller MW material that must be greater than the exclusion limit of the dialysis membrane. These molecules can not however contribute much to the viscosity of the solution. This is also true for both xanthan preparations stored in distilled water.

C. REDUCING SUGAR

Measurements of reducing sugar were sought to find the amount of carbohydrate broken down into single sugar residues. This would show that smaller sugar units had been released, and possibly also that the backbone had been broken to give free reducing termini. Reducing sugars of both polymers have been measured on samples taken straight from the ampoules, not dialysed. In some preparations the amount of reducing sugar found upon

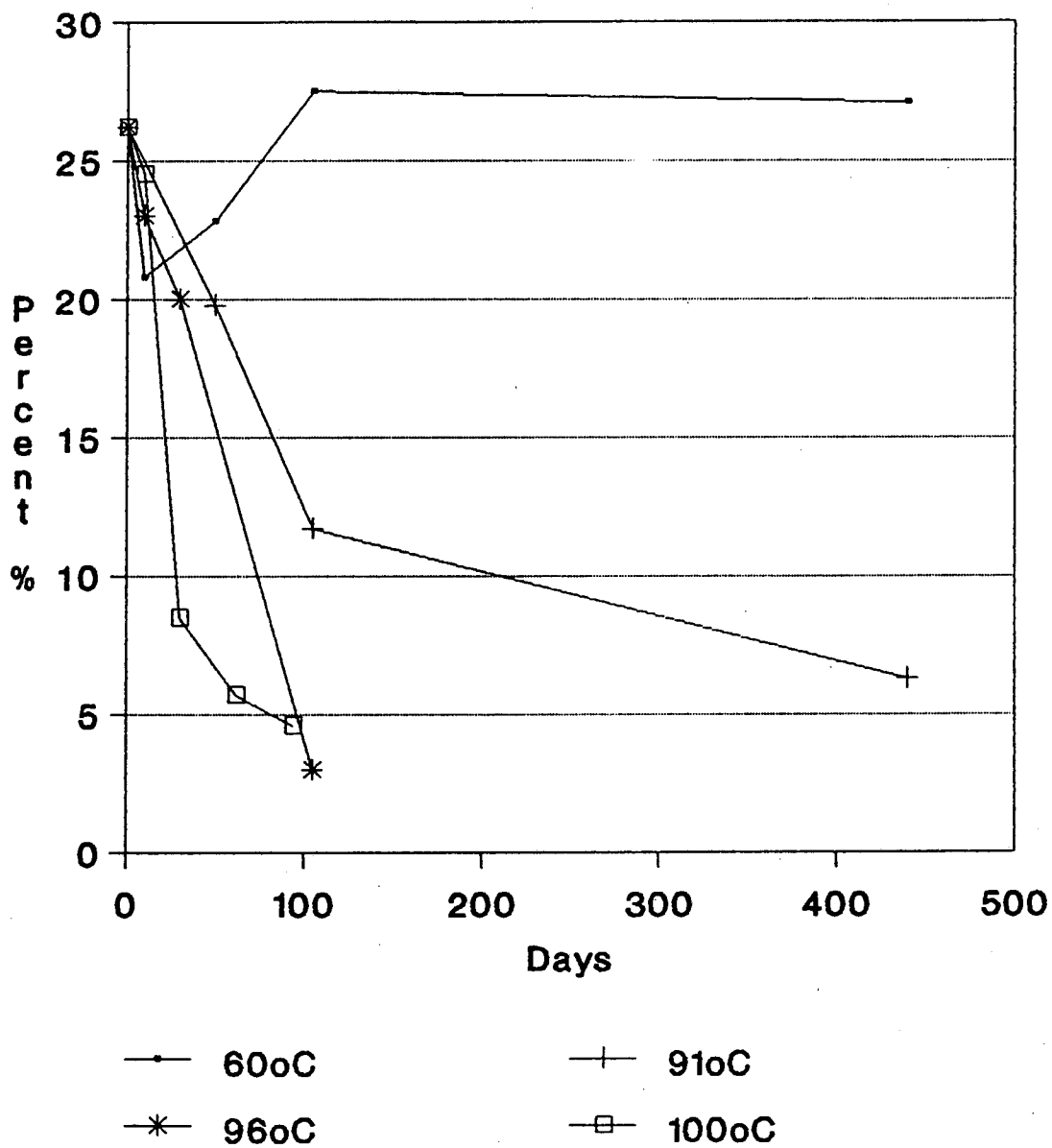
analysing the various samples mirror the measurements found for the total carbohydrate, in others not.

1. Xanthan stored in seawater

The Flocon, although dialysed before putting into ampoules, has a fairly high content of low MW carbohydrate to begin with (fig. 3.7.). At 60°C, where no carbohydrate degradation occurs in seawater (but a 40% loss in viscosity!), the amount of reducing sugar remains unchanged during the whole period under which the solutions were tested. So at this temperature, further degradation of carbohydrate to chemicals that do react with the Ashwell method did not occur. On increasing the temperature to 91°C and above, an increasing degradation rate is again noticed. On only a 9°C elevation in temperature, from 91 to 100°C, the reducing sugar degradation is 6 times as fast during the first 30 days, and about twice as fast when compared at 94 days.

For both the 91°C and 100°C samples, the degradation rate of the reducing sugar seems to slow down after about 100 days and 30 days respectively. This might be due to the fact that insufficient smaller molecules could be broken down into molecules detectable by the reducing sugar assay, whereas to begin with there had been an adequate amount.

Fig. 3.7. Flocon stored in seawater. Loss of reducing sugar is seen at 91°C and above. At 60°C there seems to be no degradation of the reducing sugar material. Samples are not dialysed.

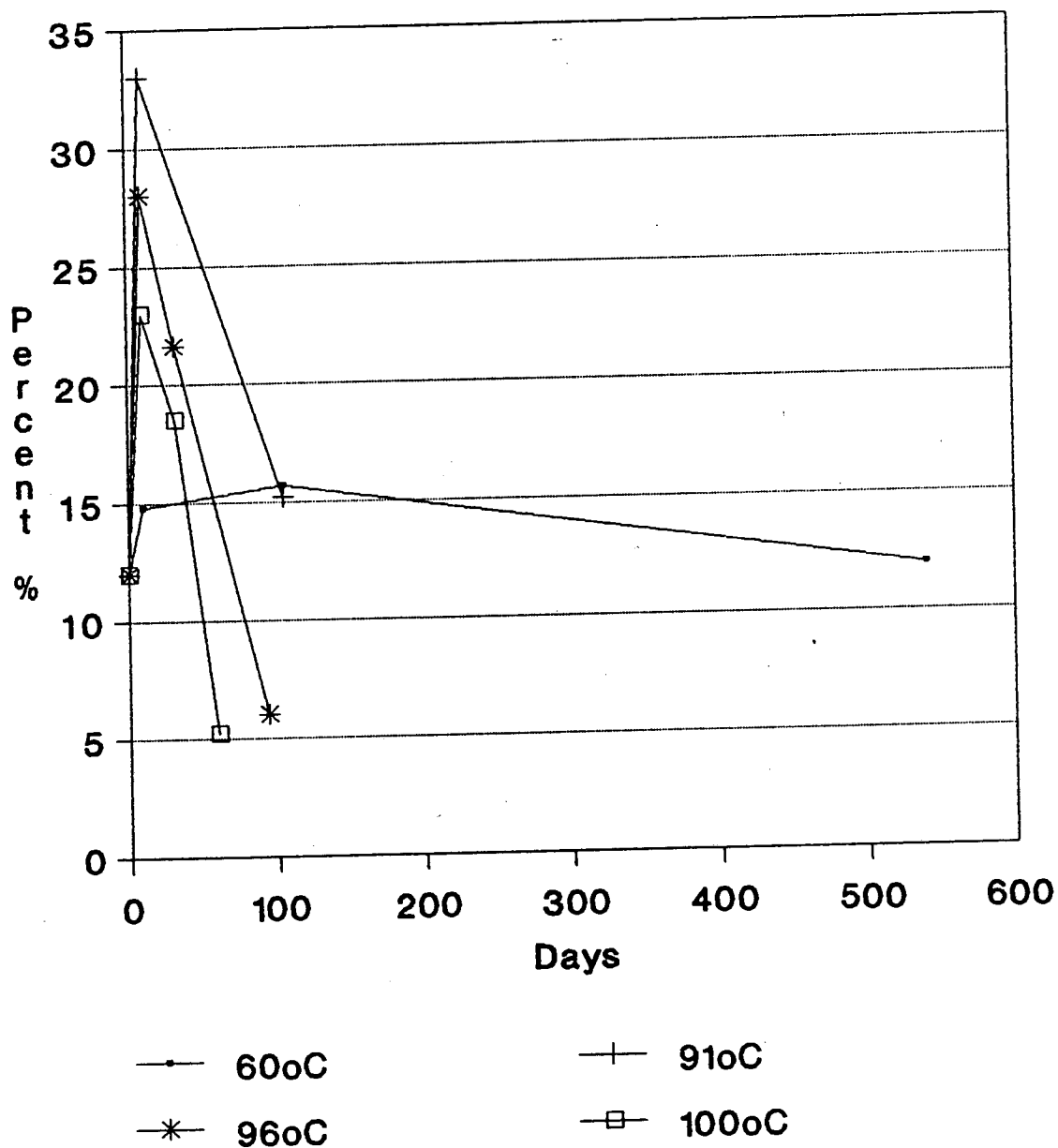


Compare this with the picture of 1128 (fig. 3.8). Again, at 60°C the amounts of reducing sugar are more or less unchanged during the whole period of 540 days. Although there is a substantial viscosity increase during this period, ie. due to a change in the physical appearance of the polymer, it does not seem that the reducing sugar is produced in any way during this process.

At 91, 96 and 100°C the reducing sugar produced during the first ten days is in marked contrast to that of Flocon. Whether this is due to the fact that 1128 was prepared from freeze-dried material rather than a concentrate is not known. The reducing sugar produced falls rapidly towards the end of the 100 day period.

The formation of breakdown products at 100°C is assumed to be quicker than at 91°C. The same goes for the degradation of these breakdown products to a chemical composition that does not react with the reducing sugar assay. It is also thought that these increases in reaction rate would be proportionately the same. The graph shows clearly that the amount of reducing sugar measured at 10 days is greater in the 91°C than the 100°C ampoule and remains higher throughout the period. An explanation to this might be given if it is assumed that a limited proportion of the polymer strands are susceptible to degradation. At 100°C, both the formation of reducing sugar material, and degradation of it, is assumed to be quicker

Fig. 3.8. Xanthan 1128 stored in seawater. Production and loss of reducing sugar at higher temperatures (above 91°C), whereas at 60°C there is no great change.



than at 91°C. So, by the time of the first measurements at 10 days, the 100°C sample will have already degraded much of the reducing sugar material. As the 91°C samples contain more reducing sugar than the 100°C samples throughout the period measured, the relative production rate of the reducing sugars has to increase more on this 9°C increase in temperature than its corresponding degradation rate.

It would be interesting to follow this formation of reducing sugar more closely. It was attempted to use the glucose oxidase method for the detection of any glucose produced. This was however not successful due to the disappearance of the ABTS colour as will be explained later.

Obviously, at 91°C and above, there is some degradation of the polysaccharides. Although no large loss in total carbohydrate is observed, loss in reducing sugar points to some degradation. Possibly, for xanthan 1128 the increase in viscosity would have been greater if no degradation had occurred at 91°C and above.

Xanthan stored in distilled water

For both Flocon and xanthan 1128 stored in distilled water, the formation of reducing sugar followed exactly the same trend as for the respective xanthans when stored in seawater. This is therefore not shown.

D. ACYL SUBSTITUTION

According to various authors (Okuyama, 1980; Dentini, 1984), the acetyl groups stabilise the ordered conformation of xanthan, whereas the pyruvyl groups destabilise the same conformation. It was of interest to determine if this showed in any way on the degradation procedure of the polymer when stored at higher temperatures? All measurements were done on dialysed material.

1. Xanthan stored in seawater

Flocon has a more or less unchanged pyruvate substitution when stored at 60°C, even after 440 days (fig. 3.9a). Pyruvate is lost at increased temperatures. Thus half the pyruvate content is lost after 100 days at 91°C, whereas the same amount is lost after only 50 and 30 days when stored at 96°C and 100°C respectively. The loss of pyruvate groups is clearly slower than that of the acetyl groups.

The initial molar ratio of acetate in Flocon in seawater was found to be 0.16. Some reduction in the acetyl content is even found after storage at only 60°C. On Flocon, some 40% of the original amount, ie. molar ratio

0.06, still seems to be substituted on the polymer after 440 days (fig. 3.9 b). This ratio is however so low that it could be due to inconsistencies with the assay. At higher temperatures, 91°C and above, the acetyl groups were lost rapidly from the Flocon. In a different set of experiments, where the acyl contents were measured by both chemical methods and NMR, the acyl substitutions found by NMR were much lower than those found by using chemical methods on samples stored even for a short period at 80°C (Stokke, personal communication).

The initial pyruvate substitution in xanthan 1128 is much lower than for Flocon, 0.22 compared to 0.75. At 60°C, the degree of substitution remains again, as for Flocon, more or less unchanged (fig. 3.10). Again in seawater, at the higher temperatures of 91°C and above, rapid loss of pyruvate is experienced, and only 50% of the original substitution levels are left after 80 days (91°C) and 20 days (96°C and 100°C) respectively.

The substitutions of acetyl groups on xanthan 1128 was initially 0.62 on a molar ratio. At the temperatures above 90°C, no acetyl substitution was measured after 10 days. Even the 60°C sample showed a sharp fall in acetyl content after 10 days. At 100 days, none was measured, but it is assumed that the acetyl groups were lost much earlier than this last measurement at 60°C might indicate.

Fig. 3.9. The pyruvate (fig. 3.9a) and acetate (fig. 3.9b) substitutions of Flocon. (sw=seawater/dw=distilled water).

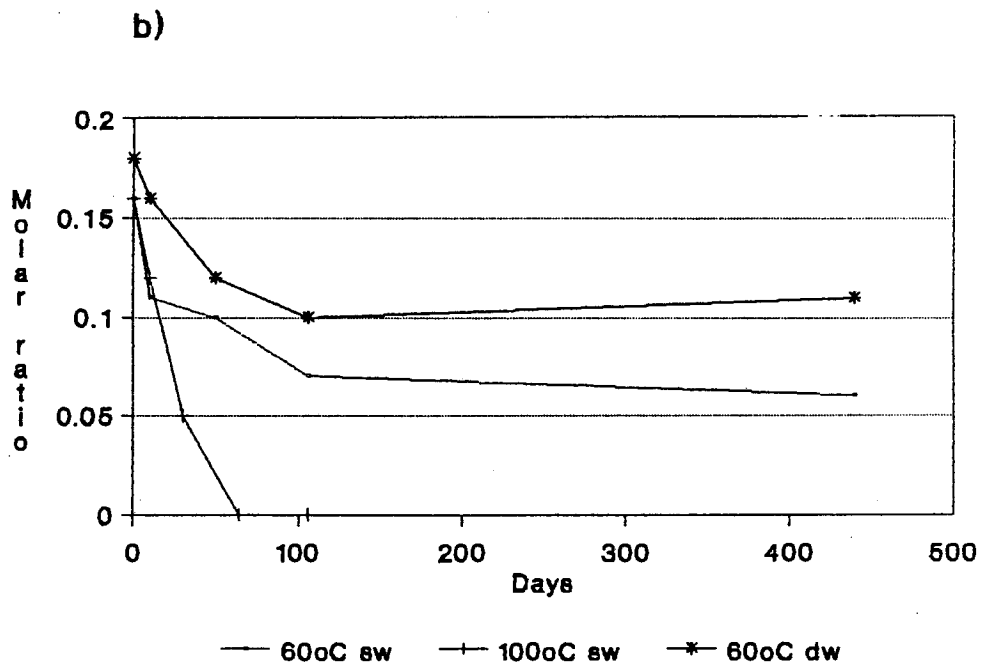
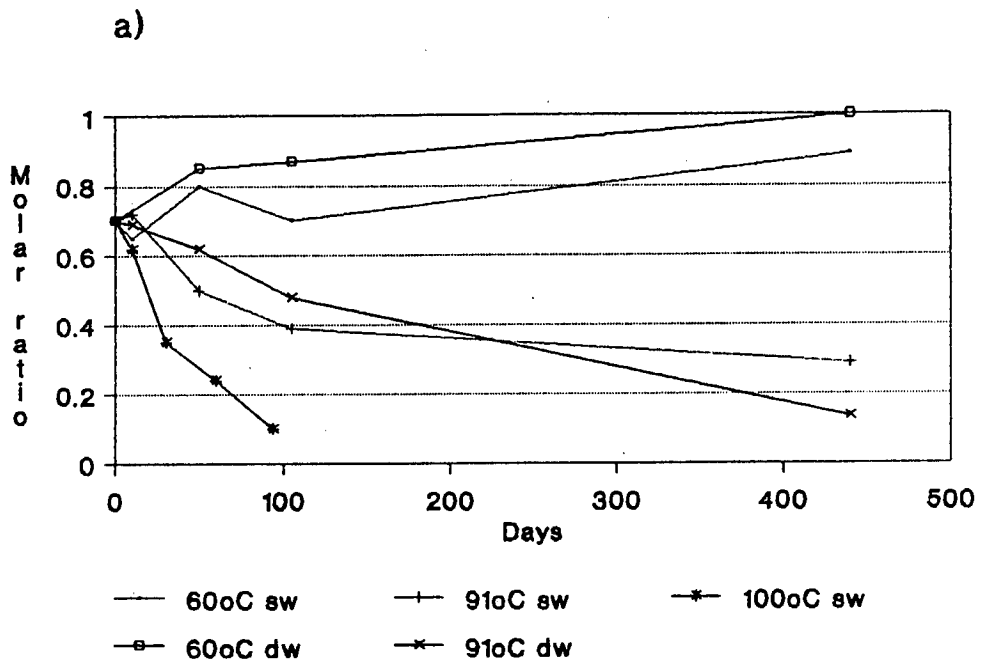
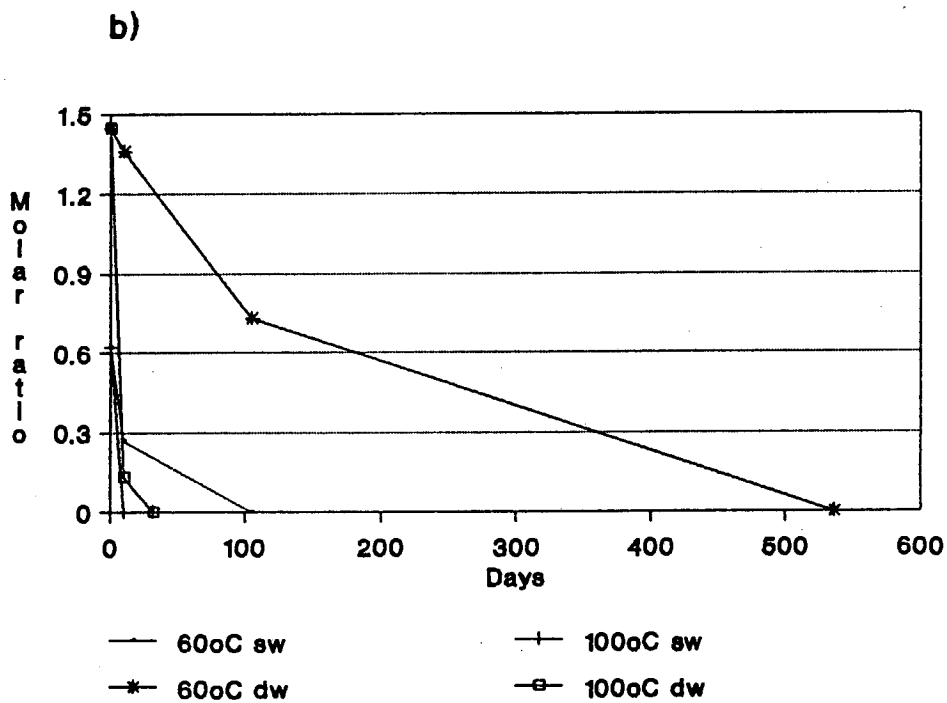
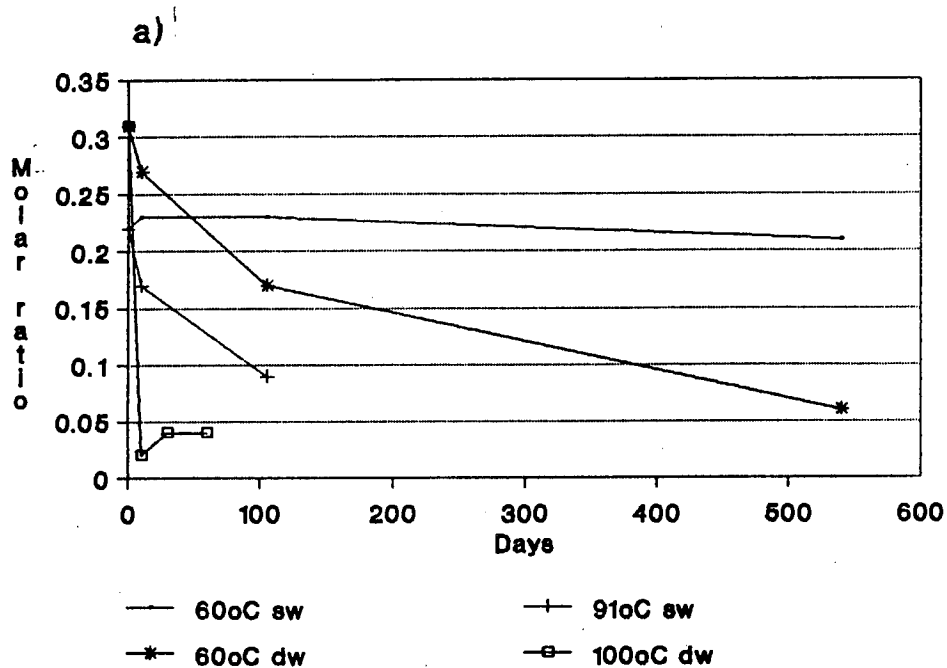


Fig. 3.10. The pyruvate (a) and acetate substitutions of xanthan 1128. (sw=seawater/dw=distilled water)



In seawater, it seems therefore that acyl groups are lost irrespective of any viscosity loss. Flocon loses some viscosity at all temperatures concerned, whereas no pyruvate is lost at 60°C. For 1128, no large viscosity losses are observed, but at temperatures above 91°C pyruvate is again lost. Also, there is no correlation between acetyl loss and viscosity for either polymer, though it seems that the acetyl groups are lost more easily than the pyruvate groups (opposite to the finding of Lambert and Rinaudo, 1985).

2. Xanthan stored in distilled water

Similar experiments were performed on the polymers when stored in distilled water. On Flocon, pyruvate seems to be present for a similar time-period as when prepared in seawater. At 60°C, the pyruvate substitution remains more or less unchanged, although the viscosity drops as mentioned previously. At the higher temperatures, pyruvate still seems to be present for a long time. Again, for xanthan 1128, the acyl groups are lost rapidly. This occurs even at 60°C, though the speed of acyl-group loss again increases the higher the temperature at which the samples were stored. Xanthan 1128 also seems to lose the acyl groups more rapidly than Flocon.

There is a large discrepancy between the amount of acyl groups determined in xanthan 1128 in seawater and distilled water. The ratio of substitution upon setting up the solutions were 0.62 and 1.45 in seawater and distilled water respectively. This difference is not understood. The second acetyl residue per repeat unit found in distilled water could have been protected in seawater, ie. have higher affinity with the molecule, to avoid reaction in the basic medium of the reagent used. Where this second acetyl group is placed on the polymer is not known, but that there is one is supported by Milas (personal communication).

Whereas there have been numerous papers produced on xanthan stability with or without pyruvate and/or acetate, it seems that for enhanced oil recovery purposes, these acyl groups are of relatively minor importance. The xanthan 1128 achieved stability at 91°C and above, and viscosity was maintained even after the acyl groups were missing. Thus it must be the biopolymer backbone and sidechain structure which are most likely to give the solution its viscosity and stability.

E. GLUCURONIC ACID

The amount of glucuronic acid in solution was determined by the method of Blumenkrantz and Asboe-Hansen. This

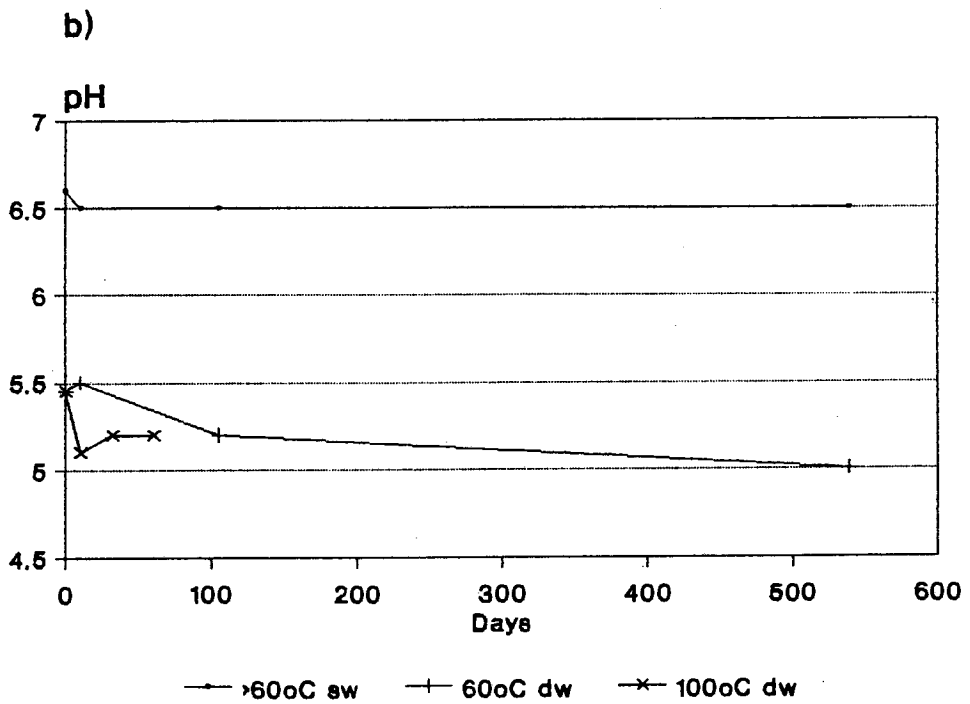
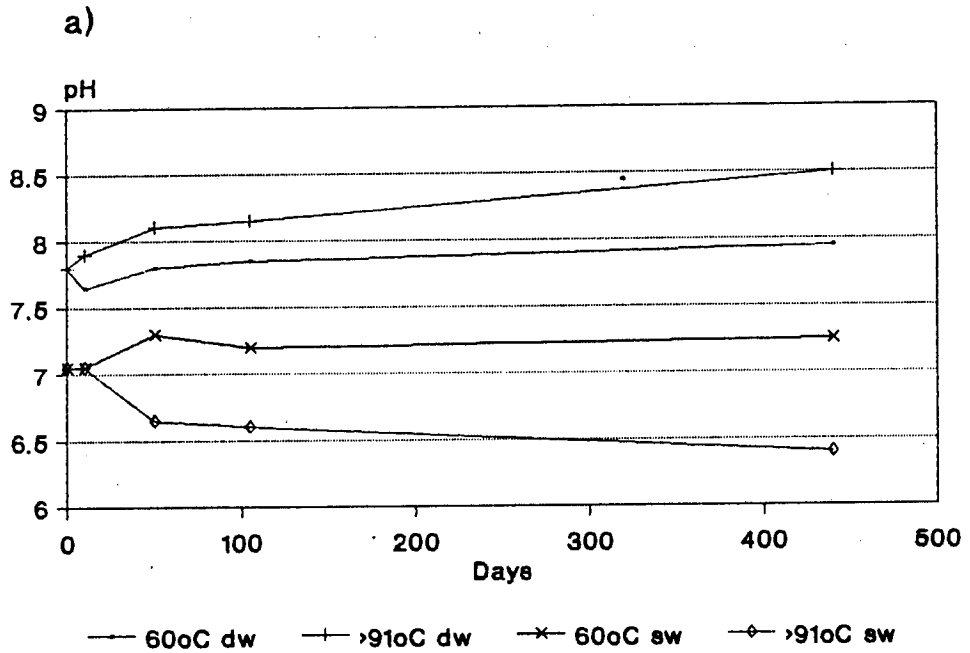
method of determining the uronic acid residues is limited in its accuracy, but has certain advantages over other uronic acid assay procedures. It seems however that the substitutions of the uronic acids remain unchanged during the storage at higher temperatures (not shown). It was intended to check the glucuronic acid contents quantitatively by HPLC, but technical problems were encountered.

F. THE pH OF SOLUTIONS

1. Storage in seawater

With the salts present, the solutions were thought to be fairly well buffered. They maintained their original pH with small changes throughout the experimental period. Flocon in seawater showed an initial pH of 7.0 and the pH increased slightly up to 440 days at 60°C when it was measured to 7.2. (fig. 3.11). In the samples in which more severe degradation occurred, at 91°C and above, a slight fall in pH was noted. The final measurements showed the 91°C sample to have a pH of 6.4 and the samples at the two higher temperatures a pH of 6.5. The pH is thus not thought to affect the polysaccharide degradation rate in any negative way when in seawater.

Fig. 3.11. The pH of the Flocon solution (a) and the xanthan 1128 (b) upon storage. (sw=seawater/dw=distilled water)



The fairly sharp fall in pH during the first 50 days at 91°C corresponds fairly well with the loss of acyl groups during the same period and may reflect release of acetic acid into the solution.

For xanthan 1128, the pH remains more or less unchanged in seawater during the whole storage period for all temperatures concerned. The pH is found to be 6.6 when preparing the ampoules, and this remains at 6.5 during the subsequent measurements.

2. Storage in distilled water

More variations were found when storing the polymers in distilled water. The Flocon had a pH of 7.8 to start with. For the 60°C sample, this changed only slightly to 8.0 over 440 days. A larger change was noted for the higher temperature samples. The pH of the 91 and 96°C samples were 8.5 and 8.2 respectively when the last measurements were done. Why the initial pH is so high and why there is an increase rather than a fall in pH is not understood.

In the solutions of xanthan 1128 in distilled water, the pH starts very much lower than for the Flocon sample. The 60°C samples decrease in pH from 5.4 to 5.0 during the 540 day period. For the solutions stored at above 90°C, pH values of 5.0-5.2 were reached after only 10

days. Why there is such a discrepancy between the two preparations in distilled water is not understood. The pH of the distilled water was not checked before dissolving the polymers in them. The difference still remains an enigma.

G. ELECTRON MICROGRAPHS

Holzwarth and Prestridge (1977), produced electron micrographs depicting xanthan. Members of the Rinaudo group (Callet *et al.*, 1987b), claim that EM is not a valid method for looking at conformational properties of biopolymers in solution (see p. 27). Any results must therefore be regarded with caution.

The rigidity of different polymers can be distinguished by their persistence lengths (q). Stiffness increases with increasing q values. Stokke *et al.* (1987), prepared electron micrographs of xanthan and other polysaccharides which showed a great variation in stiffness. Values for some of them are given in table 3.1 on the following page.

Table 3.1

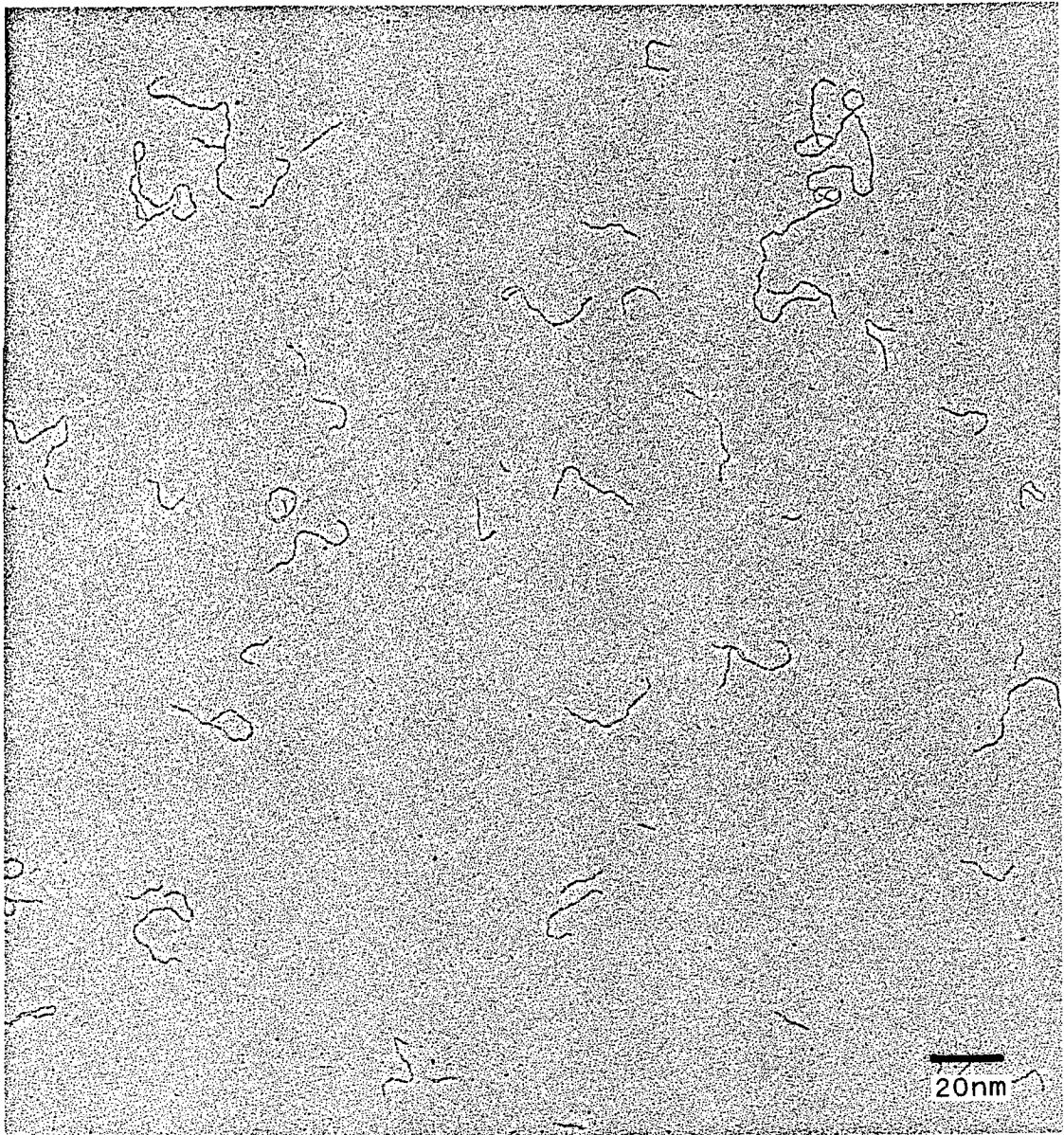
The persistence length as calculated from EM micrographs by Stokke *et al.*, 1987.

	q
human bronchial mucin	15 nm
alginate	16 nm
xylinan	45 nm
single-stranded xanthan	60 nm
double-stranded xanthan	150 nm

These values for the persistence lengths are given for the polymers when they are in their monovalent ion form. The differences between the various molecules are interesting and correspond to the values found by physical methods (Stokke *et al.*, 1987). Also of interest is of course the difference noticed between the single-stranded and double-stranded form of xanthan. The former was a xanthan produced by a strain of *X. campestris* which was known to be in the single-stranded form under the ionic conditions used (ie. 2 mM w.r.t. salts).

The values given above thus give the EM micrographs some authenticity in determining the physical state of the polymer in solution. Stokke has however also taken some pictures of xanthans that have been heat treated. One of

Fig. 3.12. A Flocon sample which has been at 100°C for two months in seawater. Compare this with its original structure in fig. 1.11.



these is given in figure 3.12 and should be compared with its original state as given in figure 1.11 (p. 26). From being very long strands in the native form, the heat treatment shows the strands to be very much shortened. This indicates that there has been a marked amount of

chain scission during this period. The viscosity had fallen to 15% of its original value.

H. AUTOHYDROLYSIS AND PAPER CHROMATOGRAPHY

Autohydrolysis occurs due to the action of the acidic groups of the polysaccharides when in the solution in the proton form. At this low pH (about 3), degradation of the polysaccharides occurs rapidly and there are no "foreign" acid residues to remove after hydrolysis. Thus, for pure analytical purposes, autohydrolysis is a useful tool.

A sample of Flocon was autohydrolysed according to the procedure given in the materials and methods (43 hours at 100°C). The resultant solution was 0.45 μm filtered before filtering through the MW cutoff filters. The total amount of carbohydrate found in the filtrates was about 20% lower than for the total in the original solution. The molecular weight distribution upon filtering it through various MW cutoff filters is given below in table 3.2. Only very low MW size products were left after this time of hydrolysis. It was not confirmed if any larger MW material was left on the 0.45 μm filter. After 0.45 μm filtration, the solution passed eventhrough the 30,000 MW cutoff filter without any problems. Of the lower MW fractions denoted a, b and c above in

Table 3.2

The amount of carbohydrate that are retained by various MW cutoff filters. Flocon autohydrolysate, heated for 43 hours.

MW size (filter)	% carbohydrate
x > 100'000 daltons	0.1%
100'000 > x > 30'000 ----"----	0.1%
30'000 > x > 5'000 ----"----	27% (a)
5'000 > x > 500 ----"----	36% (b)
500 > x ----"----	37% (c)

table 3.2, samples were prepared for the analysis of neutral sugars by HPLC. The results are given in table 3.3.

TABLE 3.3

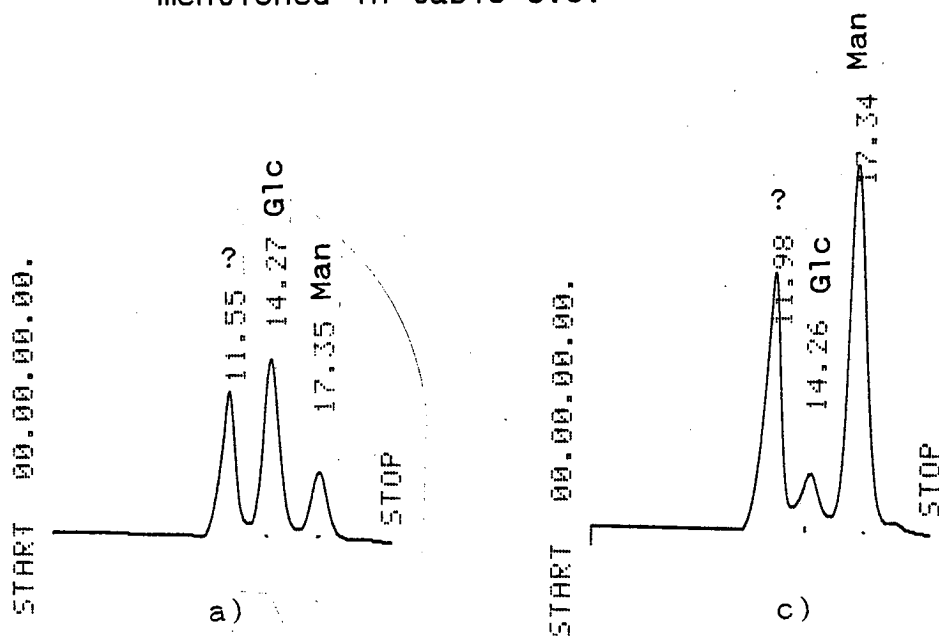
The glucose (Glc) to mannose (Man) ratios of the various MW fractions separated by cutoff filters as in table 3.2. determined by HPLC.

Sample	Glc/Man ratio
a)	1.00/0.39
b)	1.00/0.46
c)	1.00/7.66

From the values in table 3.3, it can be seen that there is a distinct difference in the glucose/mannose (glc/man) ratio when comparing the different samples (for an HPLC trace of sample a and c see fig. 3.13).

These results confirm that the mannose is much more liable to be cleaved off on hydrolysis than the degradation of the "cellulosic" backbone to glucose monomers. The larger molecular weight fractions (a) and (b) thus contain mannose-poor material and (c) contains much free mannose.

Fig.3.13. HPLC traces of the autohydrolysates which had been put through molecular weight cutoff filters. The samples correspond to those mentioned in table 3.3.



Solutions that were autohydrolysed were also subjected to paper chromatography. These could be run in solvent B (see materials and methods) for the separation of oligo-

saccharides (120 hours running time). The fractions produced are given in table 3.4.

TABLE 3.4

Oligosaccharide separation by paper chromatography run in 18:3:1:4 (ethylacetate-acetic acid-formic acid-water) for 120 hours. (R_{CB} = relative position to cellobiose)

R_{CB}	0.09	0.33	0.93	1.13
Size weighting	1	4	4	1
Man ratio (Glc=1)		1.04	0.32	1.13

The glc/man ratio was obtained by running a preparative paper chromatogram, using cellobiose (CB) as a standard, and eluting and hydrolysing the fractions rather than developing them by the Trevelyan silver-staining method. The fractions were then hydrolysed for HPLC analysis (see table 3.4) and analytical paper chromatography. From viewing this developed chromatogram, the fractions (glc/man) were estimated to have a ratio of 7/10, 10/1 and 10/8 for the fractions 0.33, 0.93 and 1.13 respectively. Analytically, HPLC is quantitatively superior, but due to small peak sizes obtained for the 1.13 fraction, this HPLC ratio might be too high. The fractions seem however to confirm the fact that both fractions 0.33 and 1.13 contain both glucose and mannose, whereas the 0.93 fraction is almost certainly cellobiose.

It was hoped to use a similar procedure for the elucidation of small breakdown products (ie dialysable material) when storing the polymer at high temperatures with a protection package present. This would probably have shown up some of the weaknesses of the polymer under high temperature stress, and would have produced some smaller fractions that could be analysed analytically. As salts were present in all the preparations used, these would have to be removed before proceeding any further. The salts would ruin HPLC columns, and paper chromatograms develop white spots in the presence of salts when staining it. It was attempted to remove these salts by running samples on desalting gels (Sephadex G-10, for MW fractions up to 700 daltons). This method did not remove sufficient salts to allow analysis by HPLC. As salts are not very soluble in alcohol, whereas carbohydrates are, methanol and ethanol extractions at 60°C were also attempted. This removed a lot of salts, but some was still left as was shown by the silver-staining technique (Trevelyan, 1950). Removal of the remaining salts by mixed-bed resin removed carbohydrate material as well.

I. ENZYMIC METHODS

Enzymes have the ability to hydrolyse xanthan into smaller fractions when it is in the unordered confor-

mation (Rinaudo and Milas, 1980) and Sutherland (1984). β -glucosidase (EC 3.2.1.21) is one such enzyme. The products produced by this hydrolysing enzyme could then be determined by the glucose oxidase (EC 1.1.3.4) method, which is a very sensitive method to measure the amount of glucose in solution (0-4 μ g). The colourimetric development was through the reduction of ABTS (2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid). Upon addition of this colourimetric substance to the reaction mixture, the colour, instead of being enhanced in the presence of glucose, faded immediately. Thus, some byproducts produced in the ampoules at high temperatures obviously produce some very strongly oxidising agents that react with the ABTS. This was also so in trials with auto-hydrolysis, where no salts were present. So an elaboration of the smaller MW material by using these enzymes was not successful.

J. MOLECULAR WEIGHT DETERMINATION

The TSK G4000 PW column is especially well adapted to the size exclusion chromatography of water soluble polymers. The pullulan standards proved to be very successfully separated on the TSK G4000 PW column. These pullulan standards had a very low polydispersity index, all lying between 1.06 and 1.14. Pullulan is however a polymer

with great conformational freedom due to the maltotriose (and some maltotetraose) units being α -(1 \rightarrow 6) linked. Therefore the elution pattern of these MWs might not mirror the elution pattern for the xanthan which is a more ordered and stiffer polymer. At present, MW standards prepared from xanthan are not available. The calibration curve for pullulan with some samples analysed are given in fig. 3. 14.

Fig. 3.14. The pullulan standards run on the TSK G4000PW column. Their elution pattern is shown above the calibration curve. There were four standards in each solution analysed, the peak at 20.7 minutes in the second run probably being oligo-saccharides or salts.

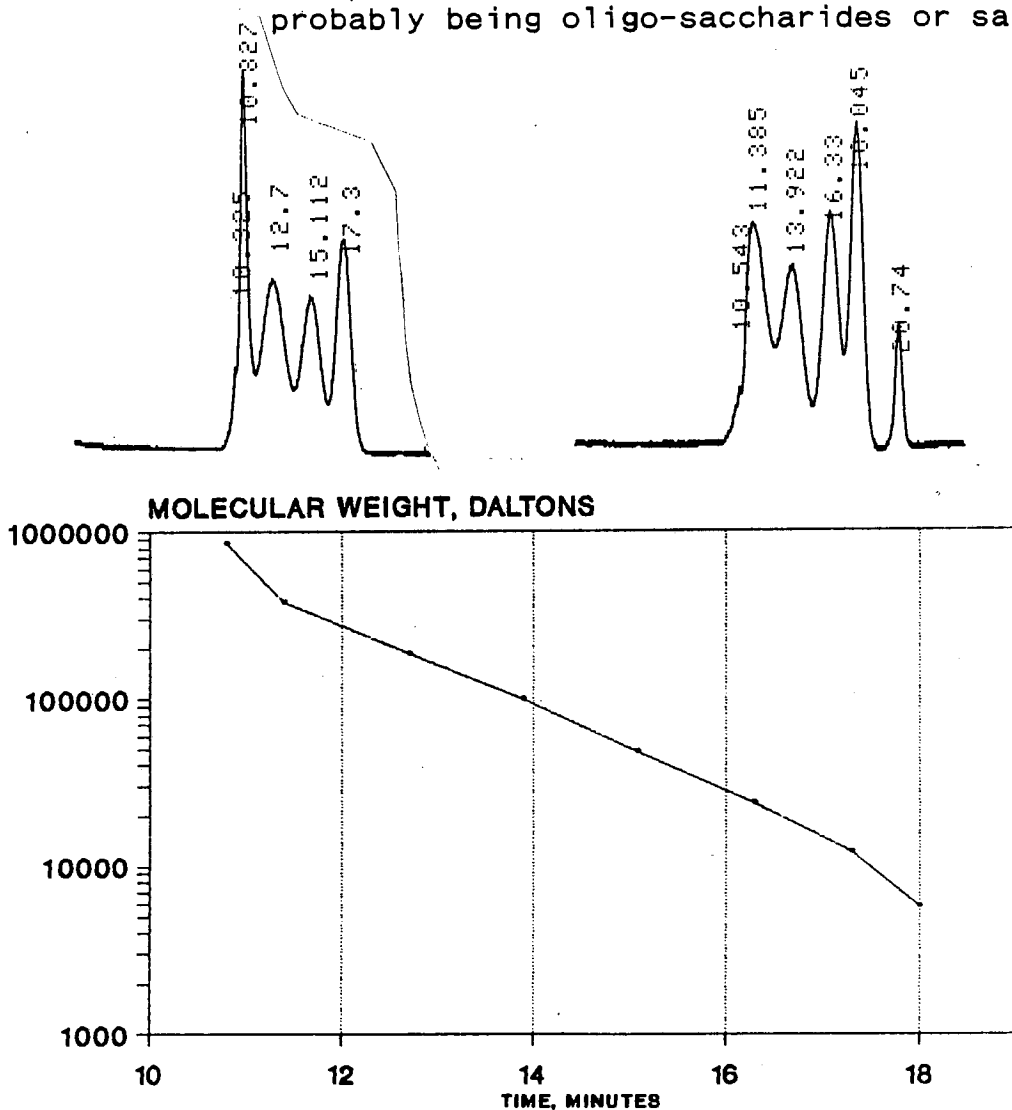
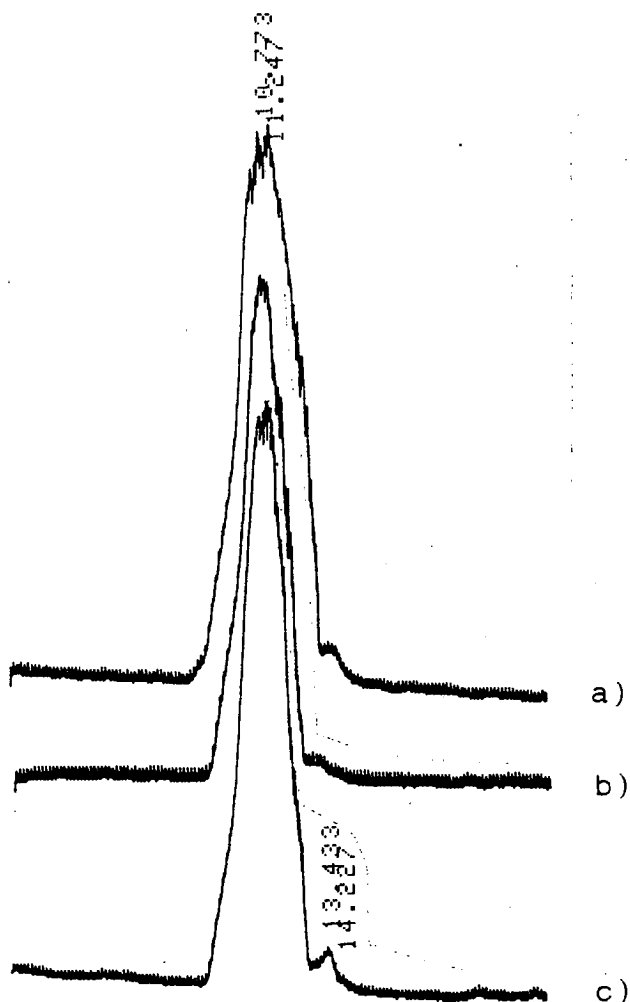


Fig. 3.15. Flocon samples stored for 10 (a) and 440 (b) days at 60°C, and 94 days at 100°C (c), run on a TSK G4000PW column.

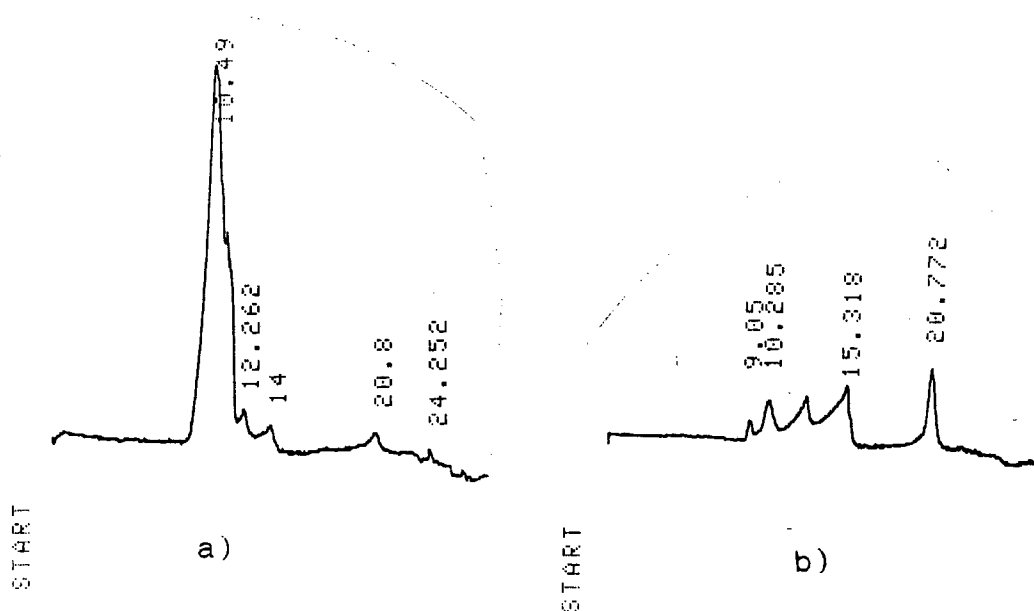


To avoid putting the column under too much stress with respect to salts, only dialysed samples were analysed. Thus only a single peak was observed at the start of an analysis. Originally it was thought that some smaller MW material would be produced and that these would show up in the chromatograms running at greater retention times than the original preparation. In fig. 3.15 are shown the chromatograms of Flocon stored for 10 and 440 days at 60°C, and 94 days at 100°C. A peak narrowing and increa-

sing height is observed, but otherwise no intermediate fractions of xanthan are revealed in the elution pattern. This is similar to results obtained when running similar heat treated fractions on the LKB AcA34 gel by straightforward gel permeation chromatography (Kierulf and Sutherland, 1988).

From the differences of the polysaccharide conformation in the electron micrographs made by Stokke, as shown in figs. 1.11 (p.26) and 3.12 (p.89), heat treated material seems to have gone through quite a substantial lowering in MW. This would be expected to show up in the elution profile in the size exclusion chromatography.

Fig. 3.16. Flocon samples from an earlier study run on the TSK G4000PW. Sample a), 100°C for 5 months, b), 100°C for 12 months.



In figure 3.16, there are two chromatograms of samples run for size exclusion chromatography on a TSK G4000PW gel column. It is from the same Flocon preparation as shown in figures 1.11 and 3.12. These two chromatograms in figure 3.16 show that the Flocon solution after 5 months at 100°C still has a large, high MW peak (at 10.49 min.). Compare this with the chromatogram for the 12 month sample at 100°C. This shows no large peaks for any MW size, but rather smaller peaks between the 9 and 15 minute period. This corresponds to a MW of 40'000 and above. The 20.7 min. fraction is a small amount of salts (and low molecular weight oligosaccharides) left after dialysis.

Thus the intermediate MW material, ie residual polymer, but smaller than ~800,000 daltons, does not really seem to show up before there has been total degradation of the polymer. The viscosity of the solutions given in figure 3.16, were measured to be 3 and 1 (shear rate $1s^{-1}$) in the 5 and 12 month samples respectively. As there is nearly no viscosity left in any of the samples, it might indicate that there could be some association between the large and small molecules of xanthan upon gel permeation chromatography. Ie. the smaller MW material would run with the high MW material. It seems that there has to be very extensive degradation of the polymer before the peak at the exclusion volume is broken down sufficiently to show a wider MW spectrum of the breakdown products.

K. CONCLUSIONS

At temperatures of 60°C and above in distilled water, neither of the xanthans tested shows any rheological stability. The Flocon was less stable than expected when stored in seawater. A large viscosity loss is even observed at 60°C. This probably has to do with the ageing of the polymer concentrate before preparing the solutions for long term high temperature storage. For xanthan 1128, rheological stability of the polymer was relatively good up to 90°C. The large initial increase in viscosity is most probably due to the microgels becoming completely dissolved at higher temperatures. This rate of dissolution increases with increasing temperature. At 96°C and above, after the initial increase in viscosity, there is a slight fall in viscosity. Thus a further drop in viscosity is expected at these temperatures. Xanthan 1128 is thus probably stable for EOR use up to 91°C (to 100 days evaluated here) under closely controlled conditions. If it is stable for any longer at this temperature needs further evaluation.

Upon storage at high temperatures, the amount of residual carbohydrate remains more or less constant. This is rather surprising when considering the corresponding high loss in viscosity. Thus the backbone scission is the most likely reason for loss in viscosity. It might again

indicate that there is some association between the large and small MW material, and that thus little dialysable material shows up at any one time. Loss in carbohydrate is probably due to the loss of the side-chain terminal mannose residues.

A change in reducing sugar concentration is not found in the 60°C samples. At 91°C and above, Flocon experiences an immediate loss in reducing sugar material. The rate of loss in reducing sugar material seems to be diminishing as less material becomes available for degradation. A difference is observed with the xanthan 1128 polymer, where the formation rate of the reducing sugar is higher than their initial degradation rate. This is probably due to the quality of the polymer used as the pH of both the Flocon and xanthan 1128 solutions are similar. With the microgels being present, it is quite possible that a large part of the xanthan 1128 molecule is available for degradation from the outset. Thus this xanthan has a high rate of reducing sugar formation to start with whereas Flocon has not. Again, this is thought to be due to using two different polymer preparations, one a concentrate and the other a freeze-dried preparation.

Pyruvate groups are not lost at 60°C, but at 91°C and above, these acyl groups are lost fairly rapidly in both polymers. Acetate is however lost even at 60°C, and this is again the same for both polymers. This means that

although pyruvate, has a destabilising effect on the polymer, it does ^{not} affect the stability of polymer for very long at the higher temperatures. Acetate on the other hand is supposed to stabilise the polysaccharide in its ordered conformation. Its loss means that it may rather lower the pH of the solution due to the formation of acetic acid and thus aggravate the stability of the polymer at higher temperatures.

A loss in MW size can be assumed from the lowering in viscosity of the solution. This MW decrease does however not show up in the elution profiles of samples put through the TSK G4000PW gel. There seems to be either some aggregation of the polysaccharide in running through the column, or the amount of intermediate MW material is so low that the refractive index monitor is not able to detect it. Only dialysed samples were used for size exclusion chromatography, so the production of small oligosaccharides would not be revealed in the elution profile. From previous runs on LKB AcA34 gels and subsequent analysis of carbohydrate by the phenol sulphuric method, no large production of small MW material was observed. Thus it seems certain that the small MW material produced is degraded to products that do not react with this assay method.

The overall conclusion for the use of xanthan in EOR is that at temperatures over 90°C, the xanthan is definitely

too unreliable. A maximum temperature of 80°C in the presence of salts is probably more recommendable, but even then the omission of oxygen is crucial to the stability of the polymer. Various xanthans show differences in stability at higher temperatures and salt concentrations, and if new strains are used, they will again have to be evaluated for their thermal stability. This is shown by the surprisingly high loss in viscosity of Flocon at 60°C, where previously it had shown good stability at 80°C for 800 days.

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