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Morpholine-Induced Gel Formation with Propylene Glycol Alginate Solutions

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ABSTRACT

Treatment of solutions of propylene glycol alginate (PGA) with morpholine resulted in the formation of stable gels. Gelation occurred with a PGA concentration as low as 0·8%, in contrast to that required (approx. 3%) for gelation by the high pH mechanism. Furthermore, morpholine-induced gelation was achieved over the pH range 8·0–10·5, and the gels produced were more thermally stable than a carbonate-induced gel prepared at pH 9·25.

It is concluded on the basis of elemental analysis and infra-red studies that the gelation is the result of replacement of the propylene glycol groups to give morpholine amide groups on the alginate chains.

Thiamorpholine was the only other secondary amine tested which also resulted in gel formation with PGA.

INTRODUCTION

Alginate, a charged polysaccharide containing β-D-mannuronic acid and α-L-guluronic acid units (Chapman, 1970), is found in all types of *Phaeophyceae* and, in addition to its role as a structural polysaccharide, appears to prevent desiccation of the seaweeds exposed to air at low tides. Due to the high viscosity of their solutions and other properties, alginates and their derivatives are of major commercial importance in the food, beverage, detergent, pharmaceutical and textile printing industries (Chapman, 1970; McDowell, 1977; McNeely & Pettitt, 1973).

One derivative of particular interest to us has been PGA alginate which is valuable in its own right as a food thickener and a stabiliser of

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foams and emulsions (McNeely & Pettitt, 1973). It is also useful in that the ester groups have been reported to be reactive to nucleophilic species (Cottrell & Kovacs, 1980) and, using this reaction, new derivatives of alginate may be prepared.

Among the interesting reactions of PGA are two which give rise to the formation of gels: (i) concentrated solutions of PGA, when raised to above pH 8 by the addition of sodium or potassium carbonate, set to gels, almost certainly as a consequence of cross-linking between the polysaccharide chains (McDowell, 1970). This is brought about by trans-esterification between hydroxyl groups on the polysaccharide units (or on the propylene glycol species) and the propylene glycol ester groups; (ii) gelling due to cross-linking may also be achieved by the reaction of PGA with diamines, such as 1,6-diaminohexane or even proteins (Cottrell & Kovacs, 1980; McDowell, 1986). In these reactions the ester groups are replaced by amides.

Pursuing an interest in novel derivatives of alginates, we have examined the reaction of PGA with morpholine in order to prepare alginyl morpholinamides. We were interested to observe that treatment of PGA with morpholine under a range of conditions resulted in the formation of stable gels. This paper reports these results.

MATERIALS AND METHODS

Propylene glycol alginate (PGA) (preparation E/RE; degree of substitution, 80–85%) and sodium alginate (DMB) were kindly provided by Kelco International Ltd. Morpholine was purified by reduced pressure distillation. Piperidine, diethanolamine, glucosamine and thiamorpholine were laboratory reagents obtained from Sigma Chemical Co. Ltd. 2,2′-Oxybis(ethylamine) hydrochloride was obtained from Aldrich Chemical Co. Ltd.

General methods

pHs were measured on a Radiometer Copenhagen pH Meter 28 calibrated with standard buffer solutions.

Infrared Absorption Spectra were obtained with a Pye Unicam SP3-100 Spectrophotometer.

1. Gelling of PGA in the presence of morpholine

Morpholine (20·0 ml; 20·0 g) was mixed, with cooling, with sufficient concentrated hydrochloric acid (15·0 ml) to give a product which gave a pH of 7·0 if added to water. Solutions were prepared as shown in Table

1, the solutions were left at room temperature for the times shown and the observations indicated were made.

2. Effect of pH on the gelation of PGA

Solutions containing PGA (1·67%) morpholine HCl (prepared from morpholine, 1·0 ml) and water (to 15 ml) were prepared at various pH values by adjustment with hydrochloric acid. The results are shown in Table 2.

TABLE 1
Effect of the Presence of Morpholine on PGA Solutions at Alkaline pH

Solution	*Components*	*Quantities (ml)*	*pH*	*Result*
1.1	5% PGA	10	11·5	No change, even after several days
	Water	10		
	20% K_2CO_3	45		
1.2	5% PGA	10	9·8	Gelled in 2 min
	Morpholine (pH 7·0)[a]	10		
	20% K_2CO_3	45		
1.3	5% PGA	10	9·0	Gelled in 1 min
	Morpholine (pH 7·0)[a]	2		
	Water	18		
	20% K_2CO_3	20		

[a]Volume of morpholine used, before adjustment.

TABLE 2
Effect of pH on the Gelling of Solutions of PGA[a]

Solution	*pH*	*Result*
2.1	7·0	No gel, even after several days
2.2	7·5	No gel, even after several days
2.3	8·0	Gel after 8 h
2.4	8·5	Gel within 1 h
2.5	9·0	Invertable gel within 15 min
2.6	9·9	Solid, stable gel within 10 min
2.7	10·5	Solid, stable gel within 10 min
2.8	10·95	No gel
2.9	11·1	No gel
2.10	11·6	No gel
2.11	12·0	No gel
2.12	12·4	No gel

[a]Solutions of PGA (1·67%) and morpholine (6·7%) were set up at different pHs as described in the Materials and Methods section.

3. Minimum concentration of PGA for gel formation
Solutions at pH 9·0 containing morpholine HCl (prepared from morpholine, 1·0 ml), PGA at various concentrations and water to 15 ml were prepared and left for 15 h. The results are shown in Table 3.

4. Effect of morpholine concentration on the gelling of PGA
Solutions containing 1·67% PGA (final concentration) and varying amounts of morpholine were adjusted to approx. pH 9·0 with hydrochloric acid and made up to 15 ml with water. They were then held at room temperature for up to 18 h. The results are shown in Table 4.

5. Effect of heat on various gels prepared from PGA
Solutions, designed to gel, were prepared from PGA solutions as shown in Table 5. On being left for 2 h, they did gel, as expected. They were then heated, in a thermostatted water bath, over a period of 4 h and their physical properties examined visually. The results are shown in Table 5. On cooling solution 5.6 retained some gel-like properties. None of the other solutions did anything other than remain liquid.

6. Products of reaction of morpholine with PGA
Solutions containing, finally, 1·67% PGA were treated with varying concentrations of morpholine as shown in Table 6 at pH 9·0. After 18 h the gels (except 6.5) were treated with water to give mobile solutions and acidified. Addition of acetone caused precipitation. The precipitates were filtered off and washed thoroughly with acetone. The solids were then dissolved in water and reprecipitated with acetone. This was repeated several times to ensure complete removal of morpholine. The washed solids were then dried *in vacuo* at 60° to constant weight. The results of elemental analyses giving nitrogen content are indicated in Table 6. In the case of the preparation obtained using neat morpholine

TABLE 3
Effect of PGA Concentration on Gel Formation[a]

Solution	*PGA concentration (%)*	*Result*
3.1	1·2	Gel, invertable
3.2	1·0	Gel, invertable
3.3	0·8	Gel, invertable
3.4	0·7	Weak gel, not invertable

[a]Solutions of PGA at the concentrations shown, at pH 9·0 (morpholine concentration 6·7%), were left at room temperature for 15 h.

(6.5) a precipitate was obtained rather than a gel. This also was washed copiously with water and acetone to ensure complete removal of morpholine. The infrared spectrum of the product obtained from 6·7% morpholine was taken.

7. Reaction of amines with PGA

Solutions of PGA under the standard conditions (pH 9·0, 1·67% PGA) were treated with various amines (1·0 g) and the formation, or otherwise,

TABLE 4
Effect of Morpholine Concentration on the Gelling of PGA Solutions[a]

Solution	*Morpholine conc. (%)*	*pH*	*Result*
4.1	6·7	9·05	Stable gel rapidly
4.2	3·3	8·95	Stable gel rapidly
4.3	1·7	9·0	Gelled, then liquified after 18 h
4.4	1·0	9·05	No gel
4.5	0·5	9·05	No gel

[a]Carried out as described in the Materials and Methods section.

TABLE 5
Effect of Heating on Gelled Solutions from PGA[a]

Solution	*Composition*	*Result*
5.1	5% PGA, 20% K_2CO_3, pH 8·3	At 34°, became less solid; at 40° began to liquefy; at 44° completely liquid
5.2	2·8% PGA, 20% K_2CO_3, pH 9·25	At 51° began to liquefy; liquid at 57°
5.3	1·7% PGA, 6·7% morpholine, pH 8·0	Did not gel
5.4	1·7% PGA, 6·7% morpholine, pH 9·0	Stable gel, up to 67°, then began to pull away from the sides. At 70°, softened
5.5	1·7% PGA; 6·7% morpholine, pH 10·0	Stable gel up to 75° then liquefied
5.6	2·8% PGA; 6·7% morpholine, pH 9·85	At 75° still a gel

[a]Solutions were prepared and held at room temperature for 2 h. They were then heated in a thermostatted water bath over a period of 4 h.

of gels observed. The products were isolated as described above and the nitrogen contents determined by elemental analysis. The results are shown in Table 7.

RESULTS AND DISCUSSION

The gelling of a solution of PGA alone (by cross-linking due to trans-esterification) requires a minimum, relatively high concentration of the polysaccharide. This minimum has been reported to be approx. 3% (McDowell, 1970). Therefore, in the initial experiment (Table 1) the lack of gel formation in solution 1.1 is not surprising. In this solution, in which potassium carbonate alone is used, the PGA concentration is less than 1%, far lower than the minimum stated above for this process. However, solution 1.2 containing morpholine gelled within 2 min, although its PGA concentration was the same as solution 1.1.

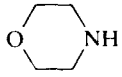
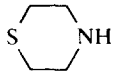
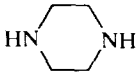
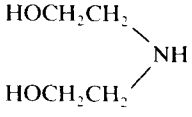
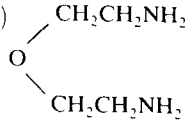
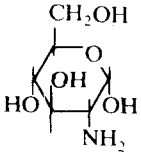
It appears therefore that the gelling in solution 1.2 occurs by a different mechanism. The most likely reaction occurring under these conditions is the attack by the morpholine on the ester groups to yield morpholine amide groups attached to the polysaccharide chains. Other workers (Henkel & Cie, 1957; Cottrell & Kovacs, 1980; McDowell, 1986) have reported the reaction of ammonia and other amines with alginate esters. Henkel and Cie (1957) used conditions involving liquid ammonia in an autoclave. Other reports (Chapman, 1970) have suggested that reaction in aqueous solution of secondary amines with PGA is slower than that of primary amines (Cottrell & Kovacs, 1980; McDowell, 1986) despite the higher basicity of the former. This is probably due to steric restrictions. However, morpholine, in which the amino group is contained within a ring, would be expected to be less

TABLE 6
Nitrogen Content of Products of Reaction of PGA with Morpholine at Various Concentrations

Product No.	*Morpholine in reaction mixture (%)*	*N in product (%)*	*Degree of substitution (%)*
6.1	3·3	1·3	23
6.2	6·67	1·8[a]	34
6.3	13·2	2·2	42
6.4	26·0	2·6	47
6.5	Neat morpholine	3·0	56

[a]Average of five determinations.

TABLE 7
Products Obtained from the Reaction of Amines with PGA

No.	*Amine*	*Physical result*	*N in product (%)*	*Degree of substitution (%)*
7.1	Morpholine 	Gel	2·0	37
7.2	Thiamorpholine 	Gel	1·8	30
7.3	Piperidine 	No gel	n.d.	—
7.4	Diethanolamine 	No gel[a]	2·3	50
7.5	2,2′-Oxybis (ethylamine) 	No gel[a]	5·9	77
7.6	Glucosamine 	No gel[a]	0·9	40

[a]Nor precipitate.
n.d.: none detected.

susceptible to steric hindrance effects and it appears that this reaction occurs under moderate conditions in aqueous solution. Even solution 1.3, at pH 9·0, gelled rapidly in contrast to literature reports which have suggested that a pH as high as 10 is needed for efficient reaction of

primary amines with the propylene glycol ester groups (Cottrell & Kovacs, 1980). Again, the higher reactivity of morpholine is not unexpected.

This preliminary experiment having established that the gelling could occur at a lower PGA concentration than is necessary for the known base-catalysed trans-esterification process, and at a lower pH than was thought necessary for amide formation, further experiments were carried out in order to establish the minimum concentration of PGA needed for gel formation, in addition to the minimum pH and morpholine concentration. Table 2 summarises the results of an experiment to establish the effect of pH on the gelling process.

It is clear from Table 2 that pH 8·0 (solution 2.3) appears to represent the lower limit of pH for gel formation. Furthermore, at pH values above 10·95 gelation did not take place (solutions 2.8–2.12).

At pH values above 10·95 it is likely that ester hydrolysis occurs more rapidly than amide formation. It has been recently reported from this laboratory (Kennedy *et al.*, 1989) that the ester groups in PGA alginate are hydrolysed at a significant rate at pH 10·0 and much more rapidly at pH 12·0.

It is also worth noting that at the high pH values, the probability of chain cleavage due to alkaline degradation is much higher (Niemela & Sjostrom, 1985). This too would prevent highly viscous solutions, and gels, from being produced.

Table 3 shows the effect of changing the concentration of PGA in mixtures at pH 9·0 with a fixed morpholine concentration (of 6·7%).

At PGA concentrations down to 0·8% gelation took place. Even at 0·7% a weak gel was produced (solution 3.4). Over the range 0·8–1·2% PGA visual inspection suggested that the higher polysaccharide concentrations gave stronger gels.

The effect of changes of morpholine concentration was also examined. Table 4 shows that when the morpholine concentration dropped to 1·0% or lower no gelation occurred (solutions 4.4 and 4.5). Solution 4.3 (with 1·7% morpholine) gave a weak gel which liquefied within 18 h and therefore we may define the lower limits for successful gel formation as being 1·7% morpholine, 0·8% PGA and pH 9·0.

In order to obtain stronger, stable gels, higher concentrations of PGA and morpholine at pH 9·0 appear to be needed.

Thermal stability of gels prepared from PGA glycol alginate

In this experiment, gels prepared by carbonate-induced trans-esterification were compared with morpholine-inducd gels produced under

various conditions. The gels or solutions were left at room temperature for several hours, and then all were placed in a water-bath and the temperature raised in steps to 75°. The consequences of these increases in temperature are shown in Table 5. All the mixtures were then cooled to 11° and then left for 62 h. After this time, preparation 5.6, alone, retained its gel-like properties.

Clearly, the morpholine-induced gels are much more thermally stable than those induced by carbonate. The stability of the former is consistent with the presence of amide links which are more resistant to hydrolysis than the ester linkages of the latter. Two other observations are worth noting:

(a) Carbonate treatment of PGA, at a concentration too low to be gelled by the carbonate but high enough for morpholine-induced gelling, followed some hours later by morpholine treatment, gave no gelation. Presumably, the carbonate treatment had hydrolysed the ester groups, thus preventing subsequent formation of amide groups with morpholine.

(b) Sodium alginate did not gel with morpholine under any conditions attempted. This confirms that the gelation by morpholine is not merely the result of the formation of the salt-like morpholinium alginate.

The nature of the reaction product

We have already stated that the most likely consequence of the reaction of morpholine with the ester groups of PGA is the formation of amide links. Elemental analysis of the alginate preparations isolated from morpholine-induced gelation contained nitrogen to varying extents (Table 6). This confirmed the covalent nature of the link between the morpholine and the polysaccharide.

It is possible, by varying the conditions of the reaction, to vary the number of morpholinamide groups present. The results of experiment 6 show (Table 6) that as a result of treatment of PGA with varying amounts of morpholine at pH 9·0, degrees of substitution ranging from 23 to 47% may be obtained. Particularly notable is the result obtained from using a large excess of morpholine (6.5). This gave a product with 56% substitution, and it is interesting that this product did not gel — it was simply insoluble. This result may point to a possible explanation for the gel formation in these reactions.

Infrared absorption spectroscopy afforded further evidence for the presence of amide groups. Films of many alginate derivatives may be

prepared by permitting neutral solutions of the polysaccharides, spread on glass plates, to dry in air. These flexible films gave good sharp infrared absorption bands (other methods of obtaining infrared spectra, e.g. using KBr tablets, gave far less satisfactory spectra). When the film is obtained from neutral solution, the carboxyl groups are fully ionised and absorb typically at approx. 1610–1585 cm^{-1}. Thus sodium alginate and PGA, both of which possess free acid groups, give films cast from neutral solution showing this carboxylate absorption band (Figs 1(a) and 2(a)). The infrared spectrum at neutral pH of the PGA also shows the characteristic ester absorption band at approx. 1715 cm^{-1} (Fig. 2a) as does that of the gel obtained by carbonate-induction which has peaks at approx. 1730 and 1600 cm^{-1} (Fig. 4(a)); this material clearly possesses both ester and carboxylic acid groups.

The infrared spectrum of the morpholine derivative at neutral pH (Fig. 3(a)) shows a small band at 1715 cm^{-1}, indicating a small amount of residual ester, in addition to the substantial carboxylate species (1590 cm^{-1}). This latter absorption band, however, masks the amide carbonyl

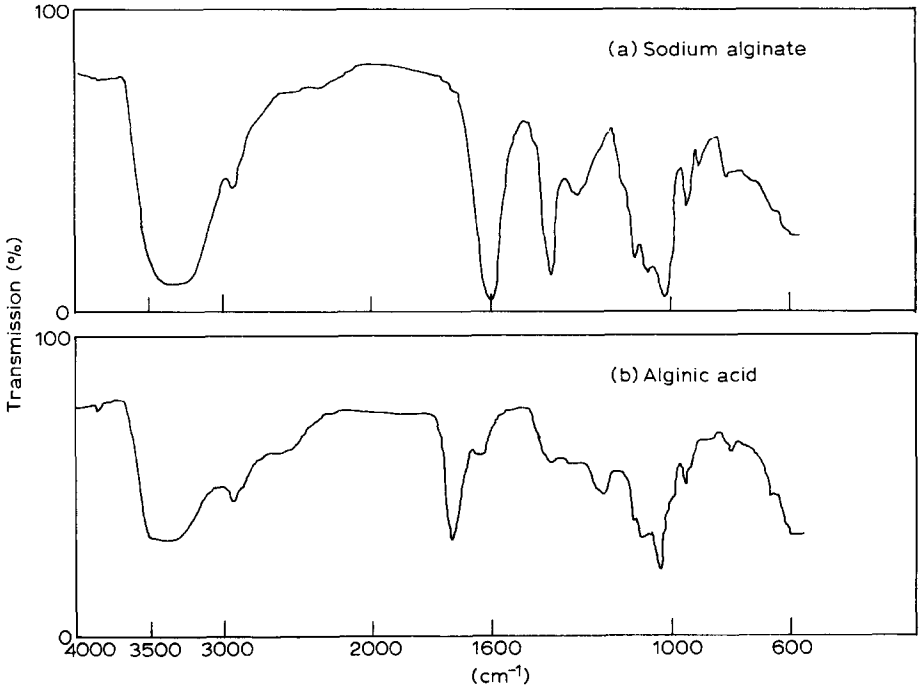


Fig. 1. Infrared absorption spectra of sodium alginate and alginic acid. (a) Sodium alginate (DMB), film cast from neutral solution; (b) alginic acid. DMB was acidified and the alginic acid filtered off, washed and redissolved at neutral pH. The film was then cast and exposed to HCl vapour for 24 h.

absorption band expected at approx. 1635 cm^{-1}. In order to show this, the derivatives were converted into their un-ionised carboxylic acid forms.

Films could not be obtained directly from acid solutions since all the derivatives are insoluble in acid. Suitable films could however be prepared for infrared spectra by exposing films, originally produced from neutral solution, to the acid vapour from concentrated hydrochloric acid. This converted the ionised carboxylate species to un-ionised carboxylic acid groups. The sodium chloride produced as a by-product is of course transparent in the infrared. The result is that the carboxylate absorption band at approx. 1590 cm^{-1} is shifted to approx. 1715 cm^{-1} (see Figs 1(b), 2(b), 3(b) and 4(b)). The amide carbonyl absorption band at 1635 cm^{-1} of the morpholine derivative then becomes visible (Fig. 3(b)). The other materials show only a shoulder or much smaller absorptions in this region (Figs 1(b), 2(b) and 4(b)).

It is clear therefore that the reaction of morpholine with PGA results in a polysaccharide chain carrying three different types of functional

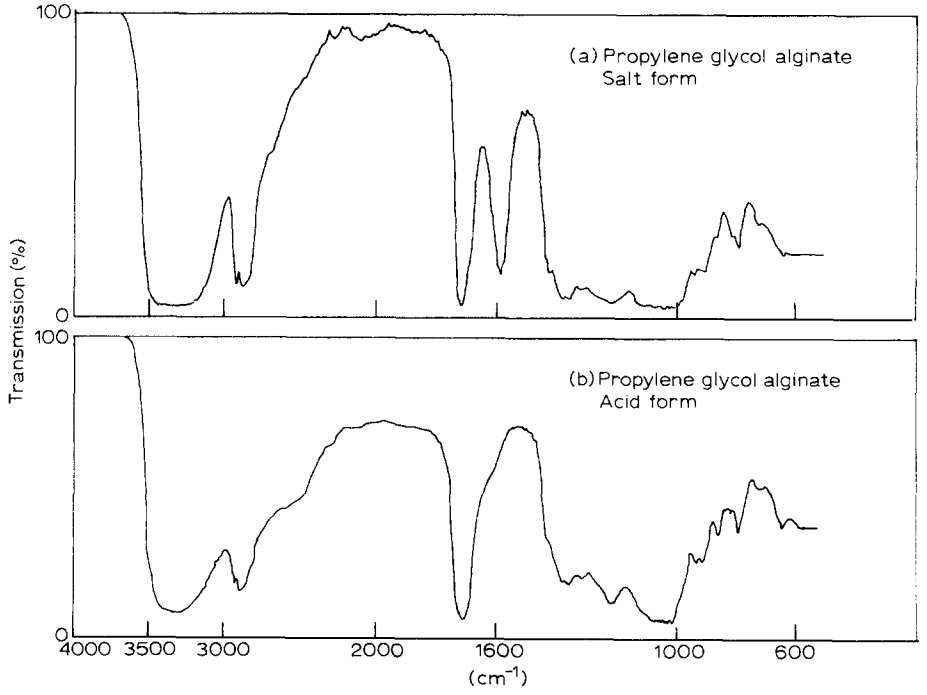


Fig. 2. Infrared absorption spectra of PGA. (a) PGA film cast from neutral solution; (b) film as in (a) above, exposed to HCl vapour for 24 h.

group: (a) a small number of residual ester groups, (b) the morpholinamide groups, and (c) free carboxyl groups.

Long hydrophilic polymers give viscous solutions, the viscosity of which depends on the length and the rigidity of the polymer chains. In the case of the alginates the lengths of the chains and the stiffness of the polysaccharide species result in very high viscosities. (Segments containing guluronic acid appear to be much stiffer than those containing mannuronic acid (Atkins *et al.*, 1970, 1971).) Hermans (1949) stated that gels might be formed from cross-linked networks of polymer chains. The cross-linked alginate derivatives of carbonate- or diamine-induced gelation of PGA referred to above would appear to correspond to this description. However, morpholine cannot bring about covalent cross-linking and another explanation for gel formation is required.

Morris (1986) developed another model for polysaccharide gelation based on the concept that in a polysaccharide gel there are two types of region: conformationally-ordered sections and interconnecting regions

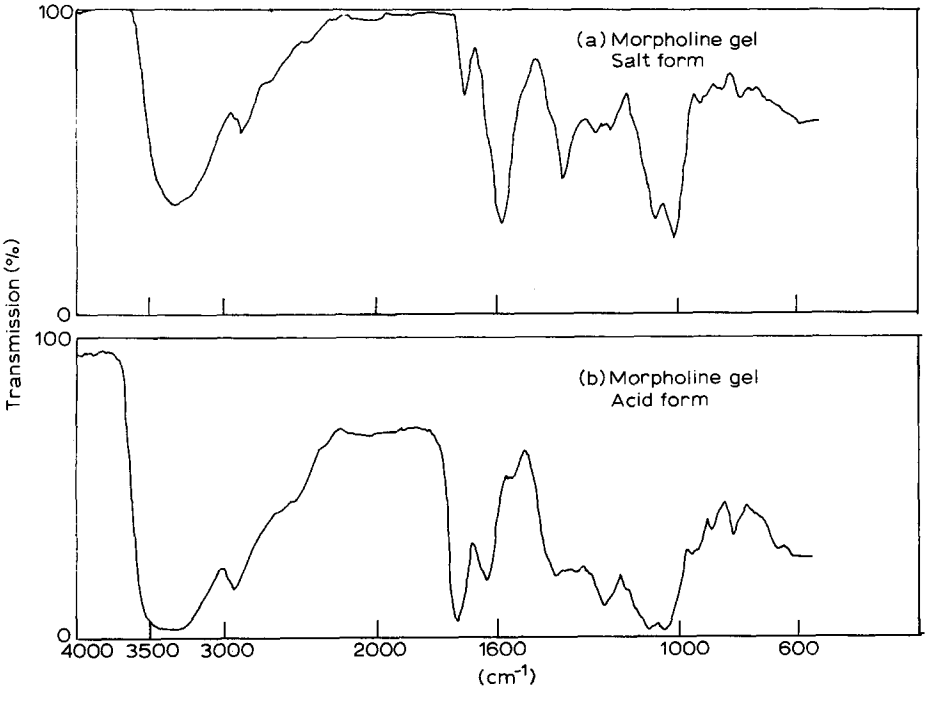


Fig. 3. Infrared absorption spectra of product from morpholine-induced gelation of PGA. (a) Film cast from neutral solution; (b) film as (a) above, exposed to HCl vapour for 24 h.

of essentially random structure. The random arrangements of these interconnecting sections serve to solubilise the whole network. For example, the structure of calcium alginate gel is considered to be built up of ordered dimeric arrangements of oligoguluronic acid segments held together by arrays of bound calcium ions. These segments, which are essentially insoluble (alginate with excess calcium ions actually precipitates out of aqueous solution), are interconnected by sections of polysaccharide chain containing oligomannuronic acid, or mannuronic-guluronic acid, sequences. These latter portions are much more flexible, may take up more nearly random arrangements, and solubilise the network so that a stable, hydrated gel is obtained. However, it is worth noting that calcium alginate prepared from very high levels of calcium (in which a higher proportion of the acid groups are associated with calcium ions) is insoluble, not forming a gel. Similarly, alginate which is highly-substituted by morpholine residues is insoluble, and we may suggest therefore that the gel structure of alginyl morpholinamide arises from

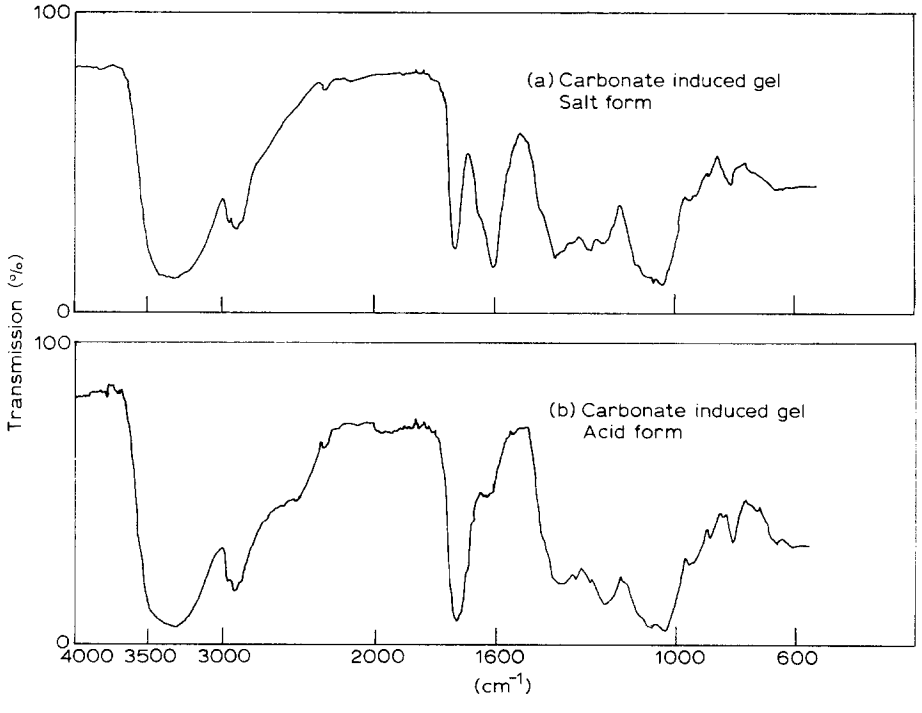


Fig. 4. Infrared absorption spectra of product from carbonate-induced gelation of PGA. (a) Film cast from neutral solution; (b) film as (a) above, exposed to HCl vapour for 24 h.

'insoluble' regions of polysaccharide chain, locally highly-substituted by morpholine, interconnected by soluble chain segments carrying only carboxylic acid or propylene glycol ester groups.

A further experiment was carried out to gain information about which structural features of morpholine were essential for gel formation. PGA under standard conditions was treated with a number of amines to determine whether these would cause gelation. The results are shown in Table 7.

Of the amines tested only thiamorpholine (7.1) brought about gelation. The similarity in structure between thiamorpholine and morpholine is obvious. However, both diethanolamine (7.4) and 2,2′-oxybis(ethylamine) (7.5) have, like morpholine, oxygens three positions away from the reactive amine nitrogens and yet, although they reacted, they did not produce gelation. Presumably in these cases, the amide substituents introduced do not provide sufficient insolubility for the 'insoluble' ordered gel segments to be created. Surprisingly, piperidine appears not to react to give an amide derivative under these conditions. No nitrogen was incorporated and therefore no gel was formed.

Substantial substitution by glucosamine was observed (7.6). In this case also however no gelation took place. Here too we may suggest that the substituents — the pendant sugar groups — are 'too soluble'.

Clearly, further work is needed on the structure of the morpholine-substituted alginate derivative in order to establish more firmly the structure of the gel, its mechanism of formation and the properties and potential applications of the product.

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