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Studies on the Chemical Stability of Propylene Glycol Alginate Esters

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ABSTRACT

The chemical stability of propylene glycol alginates (PGAs) has been examined. Under acidic conditions the ester groups in PGA are stable to hydrolysis but hydrolytic degradation of the glycosidic linkages in the polysaccharide backbone occurs. Under alkaline conditions the ester groups are hydrolysed with the primary 2-hydroxyprop-1-yl ester groups being more susceptible than secondary 1-hydroxyprop-2-yl ester groups, with little degradation of the polysaccharide backbone. Sodium carbonate–bicarbonate buffer was a much more effective hydrolysing reagent than sodium hydroxide at the same concentration and pH, and the rate of hydrolysis was greatly accelerated by increasing the hydrolysis temperature. Acetate, citrate and phosphate ions accelerated the rate of hydrolysis of the ester groups in PGA when added to the sodium hydroxide hydrolysing reagent. Hydrolysis of the ester groups in PGA with sodium hydroxide was unaffected by the addition of imidazole. However hydrolysis of the ester groups in PGA with sodium hydroxide in the presence of 1-aminobutane led to the formation of an alginate amide in which only the primary 2-hydroxylprop-1-yl ester groups were present, suggesting that a nucleophilic substitution of primary ester groups by amine groups is involved in the reaction.

1 INTRODUCTION

Propylene glycol alginate (PGA) is a commercially available product prepared by treatment of alginic acid with propylene oxide at elevated temperatures and pressures at acid pH. In a previous communication (Kennedy *et al.*, 1989) we reported the characteristics and distribution

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of the ester groups in PGA based upon NMR spectroscopic data and chemical saponification studies. It was concluded that PGA contains two types of ester groups, namely 2-hydroxyprop-1-yl primary ester groups and 1-hydroxyprop-2-yl secondary ester groups. These esters were found to be in equilibrium, the thermodynamically favoured primary ester groups predominating over the secondary ester groups in a ratio of 4:1. We now report herein the chemical stability of the ester groups in PGA to a number of reagents.

2 MATERIALS AND METHODS

2.1 Materials

All samples of PGA were obtained from Kelco International Ltd, and all chemicals used were of analytical grade.

2.2 Methods

2.2.1 Acid hydrolysis of PGA

A sample of PGA1 (1 g) was dissolved in water (50 ml) and the pH of the solution adjusted with hydrochloric acid to pH 3, and then heated for 30 min at 100°C. The hydrolysate was dialysed against distilled water and freeze dried. A solution (1% w/v) of the freeze dried hydrolysate was prepared by the addition of sodium hydroxide (0·02 M) to effect dissolution and treated at pH 12 for 3 h by the constant addition of sodium hydroxide (0·1 M) from an autotitrator (Radiometer TT2 with ABU12 Autoburette). The alkaline hydrolysate was analysed for the propylene glycol by a specific assay as previously described (Kennedy *et al.*, 1989). As a control, PGA1 without added hydrochloric acid was treated similarly.

In addition the ^{13}C NMR spectrum of a 10% (w/v) solution of the freeze dried acid hydrolysate in water was recorded on a Jeol FX 90 FT NMR spectrometer operating at 90 MHz. The width used was 12 μs corresponding to an angle of 75° and 40 000 scans were recorded at a repetition of 0·2 s. Chemical shifts were recorded relative to the internal standard 2,2-dimethyl-2-silapentane-5-sulphonate (DSS).

2.3 Hydrolysis with sodium hydroxide

A solution of PGA1 (4 ml, 10% w/v) in water was adjusted to pH 6·0 by the addition of sodium hydroxide (0·02 M) and was titrated with sodium

hydroxide (0·1 M) from an autotitrator and the pH maintained at pH 10 over a period of 15 min. Aliquots (0·1 ml) of this reaction solution were withdrawn from time to time and analysed for free propylene glycol by a specific colorimetric method as previously described (Kennedy *et al.*, 1989) and for sodium hydroxide consumed.

2.4 Hydrolysis with sodium carbonate–bicarbonate buffer

Solutions of PGA samples PGA7–11 (10% w/v) were adjusted to pH 6 with sodium hydroxide (0·02 M). Sodium carbonate–bicarbonate buffer (0·1 M, pH 10, 2 ml) was added to aliquots (8 ml) of these PGA solutions such that the overall carbonate–bicarbonate buffer concentration was 0·02 M, and the solutions were maintained at 25°C for 3 h. Aliquots (0·1 ml) were withdrawn over this period, added to sodium acetate buffer (0·05 M, pH 6, 1 ml) and analysed colorimetrically for free propylene glycol as before.

The procedure was repeated for PGA11 at 25°C with the exception that the final concentration of the sodium carbonate–bicarbonate buffer was 0·1 M. This procedure was also repeated for a sample of PGA8 with the exception that the temperature was maintained at 45°C.

2.5 Hydrolysis with sodium hydroxide in the presence of phosphate, citrate and acetate ions and imidazole

The hydrolysis procedure described in Section 2.3 was repeated using PGA11 with the exception that 0·4 mmol of phosphate, citrate, acetate and imidazole were added to PGA11 prior to hydrolysis. The hydrolysis was repeated with sodium hydroxide and sodium hydroxide plus acetate at pH 9.

2.6 Reaction of PGA1 with 1-aminobutane under alkaline conditions with polyhexamethylene biguanidinium chloride (PHMB) monitoring

A solution of 1-aminobutane (74 mg ml^{-1}, 1·0 ml, prepared by neutralising 1 ml of 1-aminobutane with hydrochloric acid, 0·5 M to pH 7 and making to a final volume of 10 ml) was added to a solution of PGA1 (1% w/v, 25 ml). The pH of this solution was adjusted to pH 10 and maintained at this pH over a period of hours by the addition of sodium hydroxide (0·1 M). At regular intervals aliquots (0·25 ml) were withdrawn and slowly mixed with PHMB (0·3% w/v, 1·0 ml) with constant stirring for 5 min. After centrifugation, an aliquot (0·1 ml) of the supernatant was withdrawn and diluted to 10·0 ml with distilled water and the absorbance

recorded at 235 nm. When consecutive readings tended to a constant value, the pH of the solution was adjusted to pH 12 by further addition of sodium hydroxide (0·1 M) and further aliquots (0·25 ml) were removed and analysed. Since PGA partially precipitates PHMB, the procedure was repeated using distilled water as a control. The procedure was also repeated without the addition of 1-aminobutane.

3 RESULTS AND DISCUSSION

Acid hydrolysis of PGA1 at 100°C for 30 min (Section 2.2.1) led to a decrease in the viscosity of PGA1 (determined by a Brookfield viscometer, also visually noticeable) relative to the control (PGA1 without added acid). Analysis after dialysis of acid hydrolysed and unhydrolysed PGA1 for propylene glycol by an alkaline saponification procedure gave identical results thereby demonstrating that the ester groups in PGA1 are resistant to acid hydrolysis under the conditions used. Analysis of PGA1 by ^{13}C NMR spectroscopy gave the ratio of primary to secondary ester groups as 78·1:21·9 by virtue of the intensity of the methyl carbon resonances at C3 on the ester groups. This compares well with the primary to secondary ester ratio of unhydrolysed PGA1 of 79·3:20·7 as previously reported (Kennedy *et al.*, 1989). It can therefore be concluded that under acidic conditions the ester groups in PGA1 are both stable to hydrolysis and that no acid promoted rearrangements take place within the ester groups, but that a considerable amount of hydrolytic degradation of the glycosidic linkages in the polymer takes place leading to a decrease in molecular weight which brings about a corresponding viscosity decrease.

Alkaline hydrolysis of PGA1 with free propylene glycol monitoring and base consumption monitoring (Section 2.3) clearly shows that more base is consumed than propylene glycol released (Fig. 1). However no viscosity change was observed and therefore end group degradation of the polymeric backbone to produce terminal saccharinic acids must have taken place (Fig. 2). A viscosity decrease in PGA1 would have indicated chain cleavage of the polymeric backbone by a β-elimination ElCB mechanism (Fig. 3). Clearly under the conditions used, 0·1 M sodium hydroxide at pH 10, the base strength was not great enough for the initial proton abstraction at C5 (BeMiller & Kumari, 1972) of the carbohydrate residues.

Hydrolysis of PGA7–11 with sodium carbonate–bicarbonate buffer (0·02 M at pH 10) with monitoring for free propylene glycol (Section 2.4) gave reaction profiles (Fig. 4) in which the rate of ester hydrolysis was

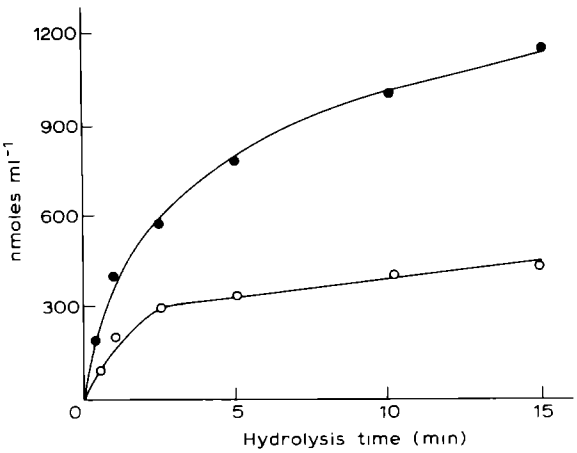


Fig. 1. Alkaline hydrolysis of PGA1 at pH 10. Plot of the amount of propylene glycol released (○) and the amount of sodium hydroxide consumed (●) versus time.

greater than for the hydrolysis of the same samples previously treated with 0·1 M sodium hydroxide alone at pH 10 (cf. Fig. 9, Kennedy *et al.*, 1989). Hydrolysis of PGA11 with 0·02 M, and 0·1 M sodium carbonate–bicarbonate buffer (pH 10) and with 0·1 M sodium hydroxide (pH 10) gives a reaction profile (Fig. 5) which clearly illustrates the effectiveness of the sodium carbonate–bicarbonate system in hydrolysing ester functions. Since we have previously demonstrated that PGA exists as an equilibrium system in which primary ester predominates over secondary ester in the ratio of 4:1 (Kennedy *et al.*, 1989) it follows that the extremely rapid hydrolysis of propylene glycol from PGA11 with 0·1 M sodium carbonate–bicarbonate buffer at pH 10, with 70% saponification within 1 h, illustrates that primary ester groups are particularly unstable to this reagent. However, at the same pH 95% of the total ester group are hydrolysed after 3·5 h illustrating that the conversion of secondary ester groups to primary ester groups was much slower than the consequent hydrolysis of primary ester groups to propylene glycol and alginic acid. Hydrolysis of PGA8 with sodium carbonate–bicarbonate buffer (0·02 M) at the elevated temperature of 45°C (Fig. 6) effected total saponification in 1·5 h, with 80% saponification in 15 min. At the lower temperature of 25°C (Fig. 6) only 80% saponification took place after 3 h. Since the primary ester is more thermodynamically stable, the activation energy for the intramolecular rearrangement of secondary to primary ester in PGA may well be low.

The hydrolysis of PGA11 with catalytic amounts of phosphate, citrate, acetate and imidazole, at pH 10 together with 0·1 M sodium

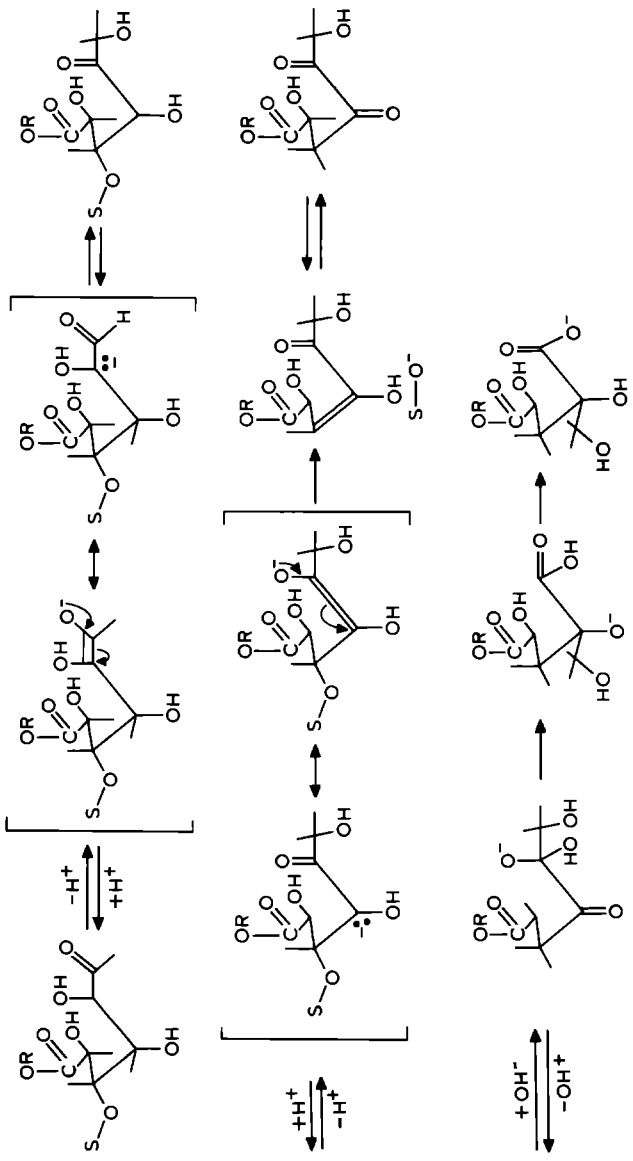


Fig. 2. End group alkaline degradation of polyuronic acid derivatives (S denotes continuation of the polyuronic acid chain).

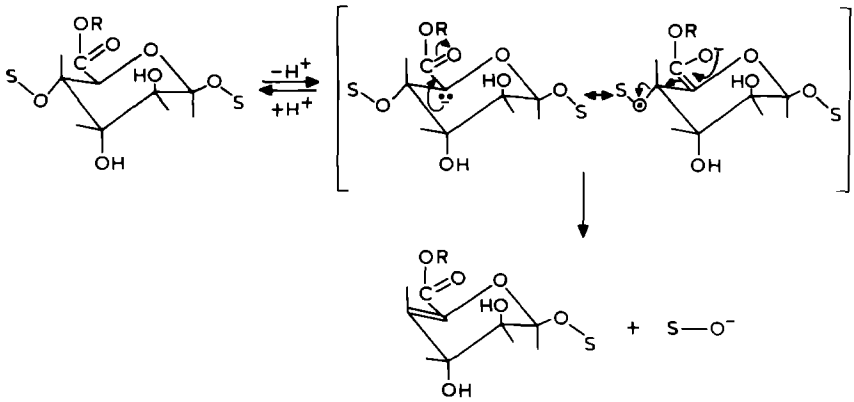


Fig. 3. Chain cleavage of alginate esters by alkali (S denotes continuation of alginate ester chain).

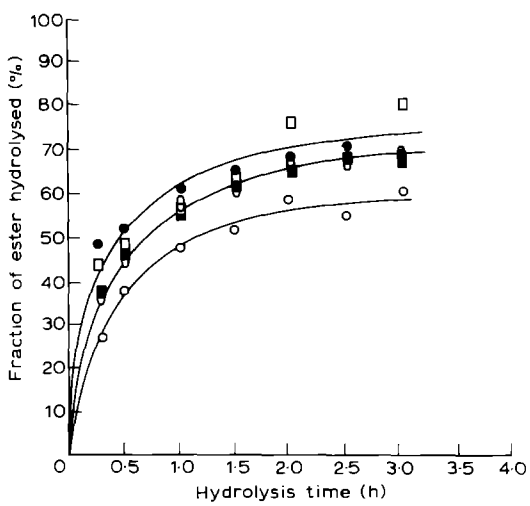


Fig. 4. Reaction profile of the alkaline hydrolysis of PGA samples with sodium carbonate–bicarbonate buffer (0·02 M) at pH 10 and 25°C (■, PGA7; □, PGA8; ○, PGA9; ●, PGA10; ○, PGA11).

hydroxide (Section 2·5) is shown in Fig. 7. It is clear that phosphate, citrate and acetate ions have a pronounced catalytic effect on the hydrolysis whereas imidazole, which is known to catalyse some ester hydrolysis reactions, has no effect whatsoever. The rate of hydrolysis with sodium hydroxide at pH 9 is low but is accelerated in the presence of acetate ions, although not as greatly as sodium hydroxide alone at pH 10 (Fig. 7). This result is as expected since the hydroxide ion is the effective hydrolysing agent.

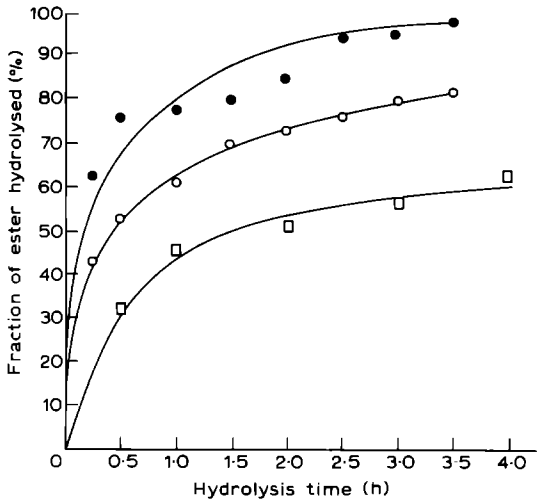


Fig. 5. Reaction profile of the alkaline hydrolysis of PGA11 with sodium hydroxide (0·1 M) (□), sodium carbonate–bicarbonate buffer (0·02 M) (○) and sodium carbonate–bicarbonate (0·1 M) (●) at pH 10 and 25°C.

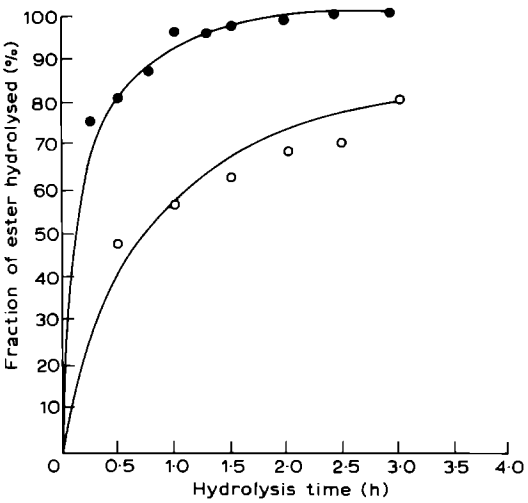


Fig. 6. Alkaline hydrolysis of PGA8 with sodium carbonate–bicarbonate (0·02 M) at pH 10 and at 25°C (○) and 45°C (●).

The hydrolysis of PGA1 in the presence and absence of 1-aminobutane with PHMB assay (Kennedy & Bradshaw, 1986, 1987) monitoring (Section 2.6) is shown graphically in Fig. 8. This assay relies on the precipitation of PHMB by any anionic polymeric species and

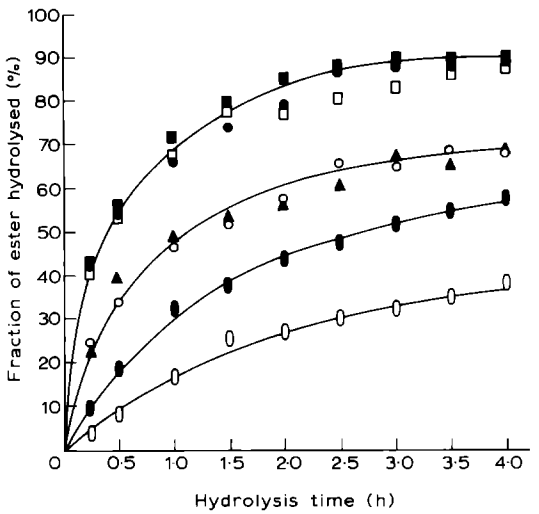


Fig. 7. Alkaline hydrolysis of PGA11 at 30°C in the presence and absence of a number of compounds. ○, sodium hydroxide (0·1 M) at pH 9; ●, sodium hydroxide (0·1 M) plus acetate ions (0·4 mmol) at pH 9; ○, sodium hydroxide (0·1 M) at pH 10; ▲, sodium hydroxide (0·1 M) plus imidazole (0·4 mmol) at pH 10; ●, sodium hydroxide (0·1 M) plus phosphate (0·4 mmol) at pH 10; □, sodium hydroxide (0·1 M) plus citrate (0·4 mmol) at pH 10; ■, sodium hydroxide (0·1 M) plus acetate (0·4 mmol) at pH 10.

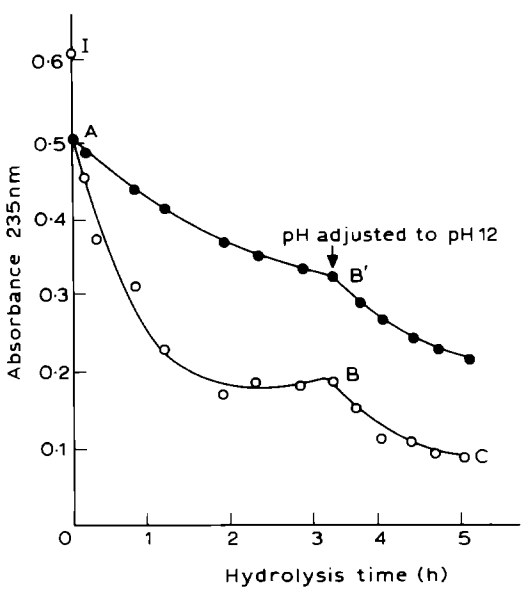


Fig. 8. Alkaline hydrolysis of PGA1 with sodium hydroxide (0·1 M) (○) and sodium hydroxide (0·1 M) plus butylamine (●) with PHMB assay monitoring.

measurement of residual PHMB by ultraviolet spectrophotometry. Hydrolysis of the ester groups in PGA1 will generate free carboxyl groups on the carbohydrate backbone of the polymer thereby precipitating PHMB from solution. It is immediately clear from Fig. 8 that a rapid decrease in the absorbance of residual PHMB is observed in the hydrolysis of PGA1 in the absence of 1-aminobutane indicating rapid hydrolysis of the primary ester groups (Kennedy *et al.*, 1989). On adjusting the pH to 12 a further rapid decrease in the absorbance of PHMB is observed indicating the rapid hydrolysis of the secondary ester groups in PGA1. However, hydrolysis of PGA1 in the presence of 1-aminobutane at pH 10 gives a much lower decrease in the absorbance of residual PHMB, whereas the reduction in absorbance at pH 12 was similar in magnitude to the reduction in absorbance at pH 12 for PGA1 in the absence of 1-aminobutane. Clearly hydrolysis of PGA1 at pH 10 does not generate free carboxyl groups in the presence of 1-aminobutane.

Since the magnitude in the reduction of residual PHMB absorbances at pH 12 were equal both in the presence and absence of 1-aminobutane it would appear that only primary 2-hydroxyprop-1-yl ester groups are involved in the reaction with PGA1. The most likely explanation for this observation is nucleophilic displacement of primary ester groups in PGA1 by the amino group in 1-aminobutane leading to the formation of neutral amide, which was unable to precipitate PHMB from solution. From Fig. 8 it is possible to calculate the following parameters using the expressions

$$\text{Primary ester} = \frac{A - B}{A - C} \times 100$$

$$\text{Secondary ester} = \frac{B - C}{A - C} \times 100$$

$$\text{Esterification (\%)} = \frac{A - C}{I - C} \times 100$$

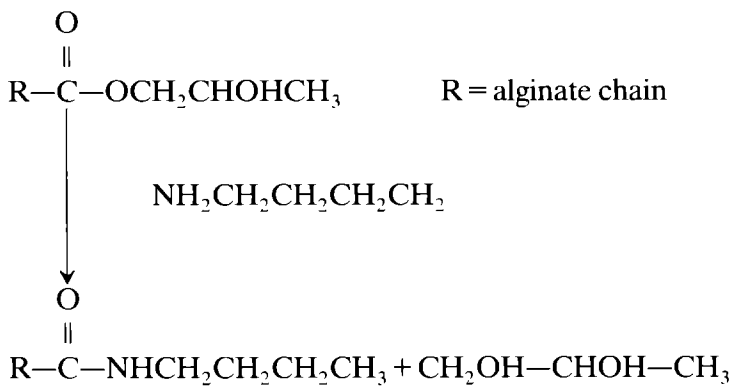
$$\text{Amide formation (\%)} = \frac{B' - B}{A - C} \times 100$$

(by nucleophilic displacement of primary ester groups by 1-aminobutane)

where

A = OD^{235} of PGA1 before hydrolysis
B = OD^{235} of PGA1 after hydrolysis at pH 10
C = OD^{235} of PGA1 after hydrolysis at pH 12
I = OD^{235} of control (i.e. no PGA1 added to PHMB)
B' = OD^{235} of PGA1 in presence of 1-aminobutane after hydrolysis at pH 10.

Using these expressions the degree of esterification in PGA1 was 82% corresponding to 18% free carboxyl groups in the polymer. The primary ester content was 76% of the total ester component whereas the secondary ester content was 24%. Hydrolysis of PGA1 by sodium hydroxide in the presence of 1-aminobutane gave a neutral alginate amide in which 33% of the primary ester groups were catalytically displaced by a nucleophilic substitution reaction, i.e. derivatised with 1-aminobutane.



4 CONCLUSIONS

It has been shown that the ester groups in PGA are stable to extreme conditions of acidity but that hydrolytic degradation of the polysaccharide backbone occurs. Under alkaline conditions the ester groups in PGA are hydrolysed with the primary 2-hydroxy-prop-1-yl ester groups being more susceptible than the secondary 1-hydroxyprop-2-yl ester groups, and that end group degradation of the polysaccharide backbone to saccharinic acids is likely to occur. The addition of catalytic amounts of acetate, phosphate and citrate ions during alkaline hydrolysis of the ester groups in PGA accelerate the hydrolysis considerably,

whereas imidazole did not have any effect on the hydrolysis. Sodium carbonate–bicarbonate buffer was found to be a much more effective reagent than sodium hydroxide at the same concentration and pH for hydrolysing the ester groups in PGA. The hydrolysis of the ester groups in PGA with sodium carbonate–bicarbonate buffer was greatly accelerated by increasing the hydrolysis temperature.

Alkaline hydrolysis of PGA in the presence of the primary amine 1-aminobutane appears to lead to the formation of amides in which, initially at least, the primary ester groups in PGA alone are involved.

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