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Preparation, characterization and biodegradation studies on cellulose acetates with varying degrees of substitution

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Several samples of cellulose acetate polymers with varying degrees of substitution (*DS*) between 0.7 and 1.7 have been prepared and tested for their biodegradation potential. The original and biodegraded polymers were characterized using chemical techniques as well as nuclear magnetic resonance, Fourier transform infra-red spectroscopy, gel permeation chromatography, solution viscosity and X-ray diffraction. *DS*, solubility and crystallinity have been investigated as possible factors affecting biodegradability. © 1997 Elsevier Science Ltd.

(Keywords: cellulose acetate; degree of substitution; biodegradation; crystallinity)

INTRODUCTION

Cellulose acetate (CA) is a polymer of great importance whose industrial uses include yarn for the textile industry, filters, photographic films, transparent and pigmented sheeting and plastic compositions such as those used for compression, extrusion, injection moulding and, to a lesser extent, surface coatings[1].

The degree of substitution (*DS*) of CA, i.e. the average number of acetyl groups per anhydroglucose unit, can range from 0 in the case of cellulose, to 3 for the triacetate. CA polymers with high *DS* values (>1.7) have been synthesized and studied extensively using n.m.r. and solution viscosity[2–14]. The reason for this is that higher-*DS* polymers are acetone-soluble and can be spun easily. The CA polymers with a *DS* below 1.7, on the other hand, have not been examined in as great a depth, and there is still some controversy about their physical properties[2,15]. This is partly because they do not dissolve in acetone, and, hence, cannot be spun under existing conditions.

Environmental factors, and in particular biodegradability, now are important considerations in determining applications of polymers. The biodegradation potential of CA with a high *DS* has been investigated by several authors[16–20]. However, there is conflicting evidence about its biodegradability. Some authors claim that CA with a high *DS* is biodegradable, while others dispute this. Some claim that chemical modification is needed before biodegradation becomes viable[21–23]. Our work proves that there is a strong link between *DS* and biodegradability; the lower the *DS* the more biodegradable CA becomes. This fact, therefore, could make the lower-*DS* polymers very important in the following years in CA copolymers and/or blends.

CA polymers with low *DS* values have been synthesized by various workers, but they have not followed conventional industrial methods[24–27]. The aim of this paper is to prepare CA polymers with varying values of *DS* and to characterize them using chemical, spectroscopic and chromatographic techniques.

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EXPERIMENTAL

Materials

The starting material was cellulose diacetate flake (Courtaulds, Spondon, UK) with a *DS* value of 2.5. Deionized water was used throughout the experiments. All other chemicals were of reagent grade and used without further purification. All bacteriological media were supplied by Oxoid (UK) and prepared as per the manufacturer's instructions.

Preparation of CA samples with varying DS

CA (60 g) was dissolved in glacial acetic acid (1200 ml) in a 2 l B34 'Quickfit' conical flask fitted with a magnetic stirrer and a condenser. Concentrated sulfuric acid (24 g) was added to the mixture followed by water (132 ml). The water was added slowly so as to avoid precipitation of the CA. The temperature was then raised to 80°C for the required amount of time depending on the desired value of *DS*.

Finally, a 21% aqueous solution of magnesium acetate (650.4 g) was added to the reaction to neutralize the sulfuric acid. The solution was then filtered so as to remove the precipitate of magnesium sulfate, and the product was precipitated either from water or from isopropanol depending on the final *DS* (see *Table 1*). The product was then washed free from glacial acetic acid using an appropriate solvent and dried in a vacuum oven at 55°C overnight. The product was finally ground to a fine powder and then characterized.

The biodegradation of CA of given DS *using the fungus* Aspergillus fumigatus

Nutrient agar plates containing 1% of CA with a *DS*

Table 1 Hydrolysis times and precipitation media used to isolate the various chemically synthesized CA samples

	*DS*2.0	*DS*1.7	*DS*1.5	*DS*1.0	*DS*0.7
Hydrolysis time (min)	20	25	30	90	120
Precipitation medium	Water	Water	Water	Isopropanol	Isopropanol

Table 2 Table of the ratio of OH (peak height) over C=O (peak area) for CA with given *DS* values as calculated from the *FT*i.r. spectra

DS	C=O	OH	OH/C=O ($\times 10^3$)
2.5	42.375	0.099	2.34
1.7	18.471	0.106	5.75
1.5	20.893	0.129	6.18
1.0	13.491	0.108	8.00
10*DS*1.5	7.126	0.0607	8.518

10*DS*, *DS* after 10 days of biodegradation

of 1.7 were inoculated with garden soil and allowed to grow for 48 h. Colonies were subcultured on plates containing Czapek Dox medium and allowed to grow for 3 days at 37°C. The process of subculturing and purifying the fungus colonies was repeated three times. A strain of the purified fungus was then sent for identification to the International Mycological Institute at Kew, where it was identified as *Aspergillus fumigatus*, a common soil species.

The inoculation of the CA-containing medium with A. fumigatus

A basal medium made up as follows was prepared:

KH_2PO_4	0.15 g
$MgSO_4$	0.20 g
$CaCO_3$	0.025 g
Yeast extract	0.025 g
$(NH_4)_2SO_4$	1.00 g
Tap water	1 l

The pH of the medium was adjusted to pH 7.0. The medium was sterilized by autoclaving at 121°C for 15 min, and sterile magnesium sulfate solution was added when cool to give the above concentration.

CA (1%) was added to the medium as the sole carbon source. The CA was added to the medium asceptically after autoclaving in order to avoid any thermal degradation of the CA. The CA polymers used in this study were of the following *DS* values: 2.5, 1.7, 1.5, 1.0 and 0.7.

The medium was then inoculated with *A. fumigatus* and was incubated at 30°C in a rotary incubator for up to 10 days. At the end of every incubation period, the CA was isolated from the medium and was characterized using *FT*i.r., n.m.r. and gel permeation chromatography (g.p.c.).

*Chemical characterization (*DS *determination)*

Approximately 1 g of the dry CA sample was weighed accurately in a weighing bottle. The sample was then transferred to a 250 ml Erlenmeyer flask, and the bottle was reweighed to determine the exact sample weight. Ethanol (40 ml of 75% (v/v)) was then added to each sample, and reagent blanks were set up and carried through the rest of the procedure.

The flasks, loosely stoppered, were heated for 30 min at 60°C. Then, a sodium hydroxide solution (40 ml, 0.5 N) was accurately measured with a Jencons 'Digitrate' digital dispenser and was added to each of the flasks, which were then heated again at 60°C for 15 min. The flasks were then stoppered tightly and allowed to stand at room temperature (below 30°C) for 72 h.

The excess alkali was then titrated with hydrochloric acid (0.5 N) using phenolphthalein as indicator. An excess of acid was added (1 ml), and the alkali was allowed to diffuse from the regenerated cellulose overnight. The disappearance of the pink colour indicated the complete neutralization of the alkali. The small excess of acid was then back titrated with sodium hydroxide (0.5 N) to the phenolphthalein end-point. After the solution had acquired a faint pink colour, the flask was stoppered and shaken vigorously. Because the colour might fade because of acid diffusing from the cellulose, the addition of alkali and the shaking were continued until the faint pink end-point persisted.

The DS was then calculated as follows:

$$\% \text{ acetyl} = [(A - B)N_b - (C - D)N_a] \times 4.3/W$$

and

$$n = (3.86 \times \% \text{ acetyl})/(102.4 - \% \text{ acetyl})$$

where A is the amount in millilitres of sodium hydroxide added to the sample, B is the amount in millilitres of sodium hydroxide added to the blank, N_b is the normality of the sodium hydroxide solution, C is the amount in millilitres of hydrochloric acid added to the sample, D is the amount in millilitres of hydrochloric acid added to the blank, N_a is the normality of the hydrochloric acid solution, W is the weight of the sample in grams, 4.3 is a factor to calculate the % acetyl, and $n = DS$ = average number of acetyl groups per anhydro-D-glucose unit of cellulose.

Spectroscopic characterization

FT*i.r. spectroscopy*. The instrument used was a Nicolet 20DXC *FT*i.r. spectrometer with Omnic software. Samples were run as thin films on NaCl discs. The films were prepared as follows. Dilute solutions of the CA samples in *N,N*-dimethylacetamide (DMA) were prepared (2%). A few drops of the solution were placed on a clean, polished NaCl disc, and the disc was placed in a vacuum desiccator. The disc remained under vacuum overnight. The disc was then removed from the desiccator and placed in an electric oven (65°C) for a further 24 h to dispel any remaining atmospheric moisture from the film. A transmission spectrum of the sample was then obtained. Two peaks were of interest: the carbonyl peak at 1750 cm^{-1} and the OH peak at 3460 cm^{-1}. The peak area of the carbonyl peak was then recorded as well as the peak height for the OH peak in absorbance units (see *Table 2*). For the CA samples whose *DS* was already determined by the chemical method, a graph of the ratio of the height of the OH peak for the carbonyl peak *versus DS* could be plotted. A calibration curve was thus obtained, from which the *DS*

Table 3a The *o*-acetyl peaks of the starting polymers as calculated from the ^{1}H n.m.r. spectra

DS	Position	δ (ppm)	Intensity	Intensity ratio
2.5	6	2.07	16.8	1.8
	2	1.99	9.2	1
	3	1.94	23.6	2.6
1.7	6	2.07	16.8	1.8
	2	1.99	9.2	1
	3	1.94	30.4	3.3
1.5	6	2.07	9.3	1
	2	1.99	13.3	1.4
	3	1.94	18.1	1.9
1.0	6	2.07	5.1	1
	2	2.01	12.0	2.4
	3	1.94	10.9	2.1
0.7	6	ND	ND	0
	2	2.01	10.0	2.3
	3	1.95	4.4	1

Table 3b The *o*-acetyl peaks of the biodegraded polymers (10 days) as determined by ^{1}H n.m.r.

10 *DS*	Position	δ (ppm)	Intensity	Intensity ratio
2.5	6	2.07	21.0	1.8
	2	1.99	11.6	1
	3	1.94, 1.96	30.1	2.6
1.7	6	ND	ND	0
	2	1.99	5.9	1
	3	1.94, 1.95	10.1	1.7
1.5	6	ND	ND	0
	2	2.01	5.4	1
	3	1.94, 1.95	6.8	1.3
1.0	6	ND	ND	
	2	ND	ND	
	3	ND	ND	
0.7	6	ND	ND	
	2	ND	ND	
	3	ND	ND	

ND, not detectable

of any CA sample could be calculated once its spectrum was available (see Graph 1).

^{1}H n.m.r. spectroscopy. The samples (20 mg) were dissolved in 0.8 ml deuterated dimethyl sulfoxide (DMSO) in a 5 mm diameter n.m.r. tube. After dissolution, they were run at 25°C in an SRC WH-400 instrument. The results are summarized in *Tables 3a* and *3b*.

Table 4 The ^{13}C chemical shifts for the starting CA samples (0 days) and the biodegraded samples (10 days) (given in parts per million using tetramethylsilane as the shift reference compound)

DS	C=O	C-1	C-4	C-2, C-3, C-5	C-6	CH_3
DS 2.5	170.6	101.6	ND	73.6	63.4	20.8
	(15)	(62)	(62)	(62)	(62)	(25)
10 *DS* 2.5	171.0	101.3	ND	73.5	62.6	20.9
	(15)	(61)	(61)	(61)	(61)	(24)
DS 1.7	171.3	102.0	ND	73.5	63.2	21.0
	(14)	(64)	(64)	(64)	(64)	(22)
10 *DS* 1.7	171.3	102.0	82.0	73.3	63.0	20.9
	(12)	(62)	(62)	(62)	(62)	(26)
DS 1.5	171.3	102.0	ND	73.5	63.3	21.0
	(13)	(66)	(66)	(66)	(66)	(21)
10 *DS* 1.5	171.5	102.1	82.0	73.3	64.2	20.9
	(12)	(66)	(66)	(66)	(66)	(23)
DS 0.7	171.7	104.5	82.9, 81.0	73.3	64.6	21.0
	(12)	(68)	(68)	(68)	(68)	(20)
10 *DS* 0.7	171.2	ND	ND	72.9	ND	20.8

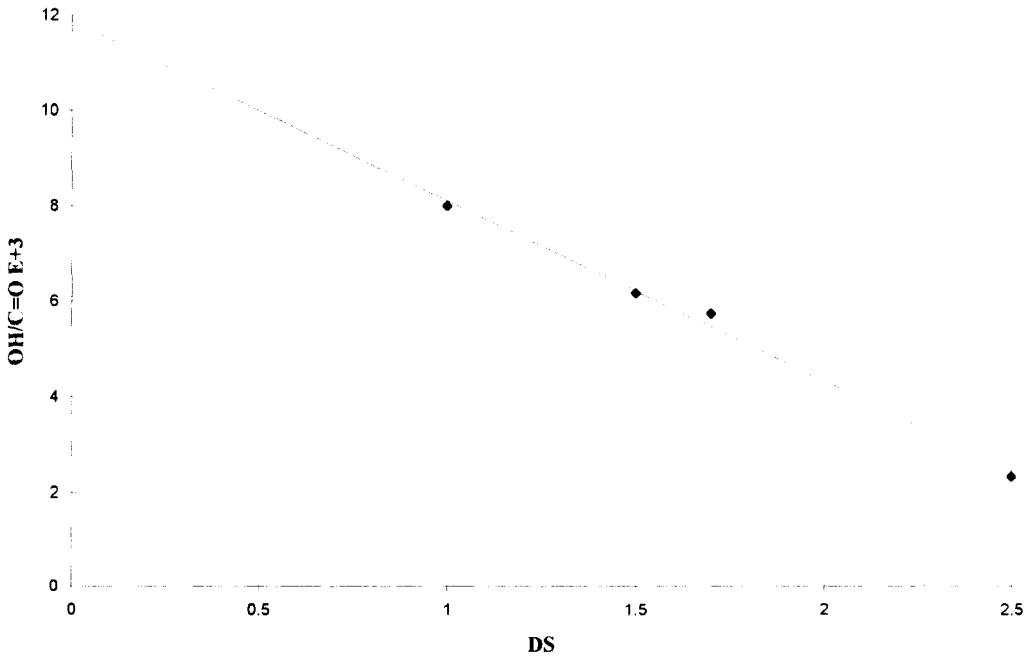
The numbers in parentheses represent the relative intensities of the peaks

Solid state ^{13}C n.m.r. spectroscopy. The samples were ground to a fine powder and were placed on a 7 mm diameter rotor and then spun at the 'magic angle' of 54.7°. The instrument used was a Varian Unity Plus, 300 MHz, with a Doty Scientific probe, at the University of Durham (see *Table 4*).

Chromatographic characterization (g.p.c.)

The g.p.c. system used was as follows:

Columns:	3 × PLgel 10 μm MIXED-B 300 × 7.5 mm
Eluent:	dimethylacetamide with 0.5% (w/v) LiCl
Flow rate:	1 ml min^{-1}
Temperature:	60°C
Detector:	differential refractometer
Data handling:	PL Caliber GPC software



Graph 1 Graph of OH/C=O vs *DS*

Table 5a The molecular weights (M_n) of the starting CA with varying *DS* and their corresponding *DP* using PEO/PEG calibration standards

Standard	*DS*2.5	*DS*1.7	*DS*1.5	*DS*1.0	*DS*0.7
PEO/PEG	42000	39200	31500	9300	6600
DP	167	137	130	42	32

DP, degree of polymerization

Table 5b The molecular weights (M_n) of the CA with varying *DS* after 10 days of biodegradation using PEO/PEG calibration standards

Standard	10 *DS*2.5	10 *DS*1.7	10 *DS*1.5	10 *DS*1.0
PEO/PEG	35500	34600	27900	11500

The samples were prepared as 1–2 mg ml^{-1} solutions in an aliquot of the eluent. Gentle heat and stirring were used to aid dissolution. All solutions were filtered over a 0.45 μm membrane prior to injection. An injection volume of 100 μl was used, and samples were analysed in duplicate.

The column set was calibrated using narrow polydispersity poly(ethylene oxide)/glycol (PEO/PEG) standards (Polymer Laboratories Ltd, UK) (see *Tables 5a* and *5b*).

Solution viscosity characterization

The viscometer used was a Schott-Geräte AVS 310 model (Camlab UK) with a Schott-Geräte Type 531 01 (0.53 mm capillary) capillary viscometer.

In viscometry, the following equations are used:

$$\eta_r = t/t_0 \tag{1}$$

where t is the run time of the solution, and t_0 is the run time of the solvent, and

$$\eta_{sp} = \eta_r - 1 = (t - t_0)/t_0 \tag{2}$$

$$\eta_{sp}/c = [\eta] + k_1[\eta]^2 c \qquad \text{(Huggin's equation)} \tag{3}$$

where $[\eta]$ is the limiting viscosity.

By plotting η_{sp}/c against c, $[\eta]$ becomes the intercept and $k_1[\eta]^2$ the slope. The following should also hold:

$$1.2 \leq \eta_r \leq 2, \text{ or } 0.2 \leq \eta_{sp} \leq 1. \tag{4}$$

Finally, the Mark–Houwink–Sakurada (MHS) equation gives a molecular weight expression:

$$[\eta] = KM^a \tag{5}$$

where K and a are constants specific to every solvent at a given temperature.

The polymer solutions were made up in a DMA/LiCl solution (0.05%, w/v), and their viscosity recorded at 25°C. In all cases appropriate polymer concentrations were used so as to satisfy equation (4).

X-ray diffraction analysis on the starting CA polymers

The X-ray diffraction spectra were obtained from a Philips PW 1130 generator, using Cu_α radiation (λ = 1.5418 Å), coupled with a Hiltonbrooks motor drive.

RESULTS AND DISCUSSION

CA preparation and chemical DS *determination*

Table 1 shows the relationship between hydrolysis time, *DS* and the precipitation medium. Using water as the precipitation medium is a great advantage, as it is a convenient method of precipitating CA from solution. However, as the *DS* value drops below 1.5, water is no longer a non-solvent for CA, and a replacement must be used. After many unsuccessful attempts, isopropanol was used, which works well. Although the precise reason for its success is not known, it is interesting to note that straight-chain alcohols (propanol and butanol) were not successful.

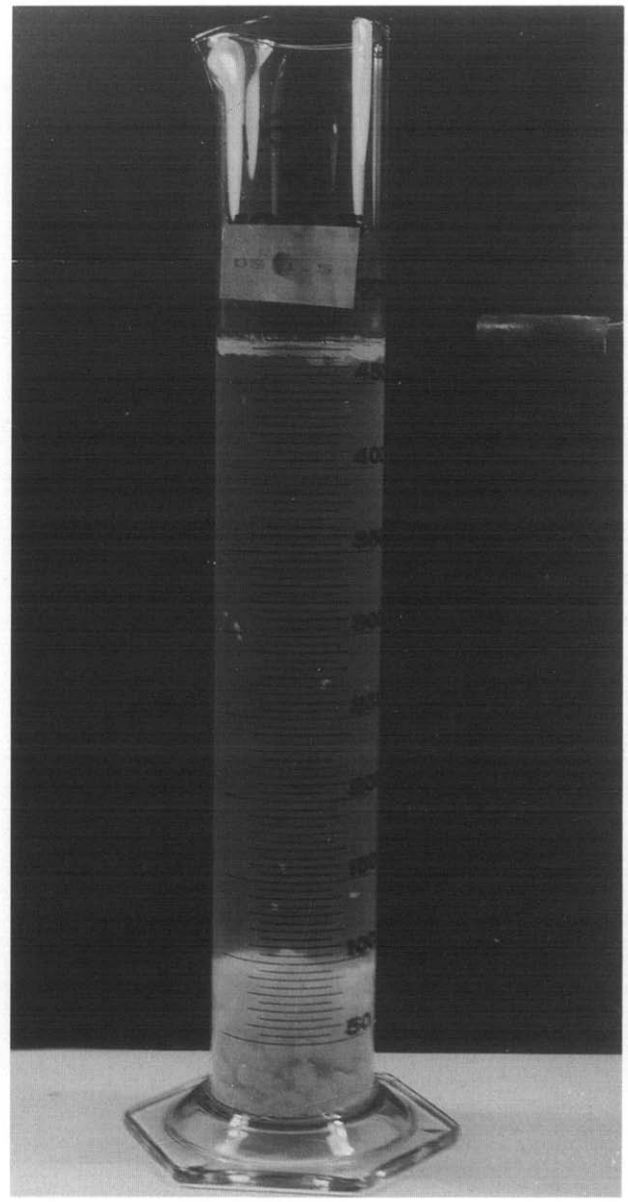


Figure 1 CA with a *DS*1.5 after being in contact with the fungus for 10 days

Another important observation was that none of these polymers were water-soluble. It is generally thought that CA polymers with *DS* values ranging from 0.5 to 1.1 solubilize in water[26]. This, however, is an over-simplification of a more complex state of affairs. Water solubility can only be achieved if the three positions on the anhydroglucose unit are roughly equally substituted as reported by Kamide *et al.*[2]. The preparation method is also of great importance. The general debate currently going on regarding the CA polymers with lower *DS* values simply illustrates the point that there are many aspects of their chemistry that still need very thorough investigation.

The biodegradation of CA of given DS *using* A. fumigatus

The chemically hydrolysed cellulose acetates were subjected to biological hydrolysis in order to establish a possible biodegradation pathway for CA. The literature seems to be unclear as regards the mechanism of the biodegradation of the CA, i.e. whether deacetylation precedes depolymerization, or vice versa, or if both processes are happening simultaneously. By following the biodegradation of the CA with the lower *DS* values over a period of time, it was possible to study the biodegradation mechanism in some detail. The medium used contained CA as the sole carbon source, and it was inoculated with the fungus *A. fumigatus*, as this was the fungus that was isolated and purified from a consortium of micro-organisms that were initially found on discarded cigarette filters. The hydrolysis time was 10 days at 30°C.

The problem that was encountered initially was the isolation of the CA at the end of the biodegradation period. After 10 days, the fungus had grown in such a way that it formed a large number of globules, leaving a very small amount of CA at the bottom of the flask. In addition, the medium had acquired a faint yellow colour. When the CA was filtered and weighed, it represented a very small amount of the initial 5 g that was added to the flask at time zero.

This implied one of two things: the CA had been hydrolysed to soluble derivatives, or the CA was adhering to the fungus. In order to test this, glacial acetic acid was added to the fungus, and the resulting solution was left overnight. If there was any CA adhering to the fungus, it would dissolve in the glacial acetic acid and, after filtering off the fungus, the CA could then be isolated as described in the previous section. Following this method, more CA was isolated, proving the association of the polymer with the fungus.

This method again proved the resistance of CA with a *DS* of 2.5 to microbial attack. After 10 days it was the only CA sample that did not associate with the fungus, the liquid remained colourless, and the whole 5 g was recovered, allowing for a small quantity (0.3 g) that was lost in the recovery of the polymer. The amounts recovered for *DS* 1.7, 1.5, 1.0 and 0.7 samples ranged from 3 g for the *DS* 1.7 sample down to 0.7 g for the *DS* 0.7 sample. This experiment also showed that the lower the *DS* the greater the chance of biodegradation. After the initial experiment, the contents of each flask were transferred to measuring cylinders. It was immediately obvious that the amount of fungus increased with decreasing *DS*. A representative example is the action of the fungus on the polymer with a *DS* of 1.5, and is illustrated in *Figure 1*.

A problem that arose, however, was the determination of the *DS* value for the biodegraded CA samples. The chemical method described above was used, but the results obtained were unreliable. The *DS* values for the degraded products appeared to have significantly larger values than the equivalent starting polymers. A possible explanation for this behaviour was that the chemical method could not be employed successfully if the chain had been cleaved below a certain degree of polymerization (*DP*). This was overcome by using the *FT*i.r. method.

CA that had been in contact with the micro-organisms for 10 days and with a starting *DS* of 1.5 (10*DS* 1.5), was analysed using the above technique, and its final *DS* was deduced to be 0.9.

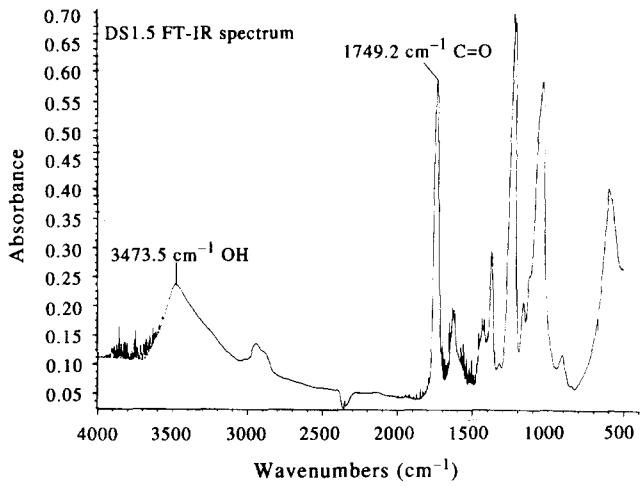


Figure 2 *FT*i.r. spectrum of the *DS* 1.5 sample

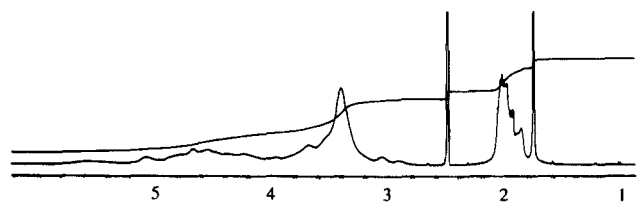


Figure 3 ^{1}H n.m.r. spectrum of the *DS* 1.5 sample

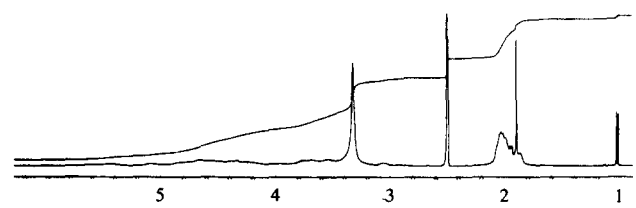


Figure 4 ^{1}H n.m.r. spectrum of the 10 *DS* 1.5 sample

Spectroscopic characterization

FT*i.r. spectroscopy.* *FT*i.r. is a very powerful tool in the characterization of CA as it provides an alternative method for calculating the *DS*. A typical example of the *FT*i.r. of *DS* 1.5 is shown in *Figure 2*. *Table 2* shows the relationship between the *DS* and the ratio of the hydroxyl peak height to the carbonyl peak area. From the data, the graph of the ratio of the peaks versus the *DS* was plotted (*Graph 1*). This serves as a calibration curve from which the *DS* of any CA sample can be calculated once the *FT*i.r. spectrum is available.

^{1}*H n.m.r. spectroscopy.* The assignment of the peaks was first reported by Goodlett *et al.*[11] and has been confirmed by other workers[28,29]. *Tables 3a* and *3b* show the distribution of the *o*-acetyl peaks of the original and biodegraded polymers, respectively, as determined by the ^{1}H n.m.r. spectra. The proton spectrum of the *DS* 1.5 sample is shown in *Figure 3* and that of the biodegraded sample 10*DS* 1.5 is shown in *Figure 4*.

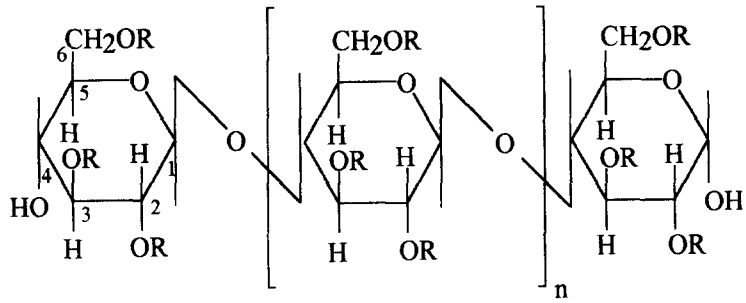


Figure 5 Structure of cellulose and cellulose triacetate (R = $COCH_3$ (cellulose triacetate) or H (cellulose))

The following conclusions can be made for the starting polymers. As far as the conformation of the polymers is concerned, Frommer *et al.*[12] looked at the n.m.r. spectra of cellulose triacetate in deuterated chloroform at various temperatures. At room temperature and below, the three acetyl peaks were clearly separated, and in good agreement with the assignments made by Goodlett *et al.*[11]. On heating, the peaks for the acetyl protons in the 2-position and 3-position (see *Figure 5*) merged to a single peak at a temperature of approximately 95°C. With subsequent lowering of the temperature, separation of the two peaks was again observed. This could be explained by assuming that the acetyl protons in the two positions became equivalent above 95°C. At lower temperatures, each anhydroglucose unit had, therefore, to exist in the chair form with free rotations of the C_2–O and C_3–O linkages being forbidden due to steric hindrance. This would make the acetyl protons of the two positions non-equivalent. In order to allow the free rotations of the two linkages, each anhydroglucose unit had to exist in the boat form at elevated temperatures. It can be assumed, therefore, that the anhydroglucose units of the CA polymers in this study adopted the chair conformation.

Secondly, a statement can be made about the hydrolysis of the three ester groups. In theory, the ester group in the 6-position (the primary site) should be attacked first. The ester group in position 2 should be attacked second and the 3-position should be attacked last, as the position 2 ester would be closer to the (O)-link, and also more exposed than the 3-position (see also CA structure).

However, by looking at the intensity ratios in *Table 3a*, it can be seen that the picture is more complicated and does not always follow the theoretical trend.

Position 6 should have been the most vulnerable position and therefore one would have expected that it would have the lowest intensity ratio in the series. This was only true for the lower-*DS* polymers (1.5, 1.0 and 0.7, where in fact the signal disappears completely). The two highest-*DS* polymers (2.5 and 1.7) favour the 2-position. Similar anomalies are observed for the positions 2 and 3. Very marked is the discrepancy in the *DS*0.7 polymer, where the peak intensity for position 2 is nearly two and a half times greater than that of position 3.

The other peaks on the spectra are due to the solvent (δ 1.87 and 1.89) and the cellulose (δ 2.5–5.1).

Overall, the primary acetate is hydrolysed first, and it is the first signal to disappear. There seems to be some discrepancy with the theory about the other two esters which are not hydrolysed in the order that would be theoretically predicted.

Furthermore, the relationship between uniformity of acetylation and water solubility is illustrated. The three sites in the low-*DS* polymers are far from equally substituted and, therefore, no water solubility is observed.

As far as the biodegraded polymers are concerned, the resistance to biodegradation of the CA with a *DS* of 2.5 is obvious as the spectra at zero time and 10 days are identical (see also the relative peak intensities in *Tables 3a* and *3b*). The polymers of interest are the higher-*DS* ones. As mentioned before, the biodegraded sample with an initial *DS* value of 2.5 did not show any difference from its starting polymer. However, the position 6 peak disappeared from the 10 *DS* 1.7 and 10 *DS* 1.5 polymers. As for the position 2 and 3 signals, the ratio of position 2 to position 3 was as expected (i.e. smaller intensity for position 2 than for position 3); however, as we go down this 10 *DS* series there was a further reduction in the position 3 signal, while the signal due to position 2 remained the same. This again was in contradiction to the theory, as one would have expected that after the disappearance of the position 6 peak, the position 2 peak would have greatly reduced.

The other peaks on the spectra were due to the solvent (δ 1.87 and 1.89) and the cellulose (δ 2.5–5.1).

To conclude, therefore, on both series, overall, the primary acetate was hydrolysed first, and it was the first signal to disappear from the starting series, and also from the 10 *DS* 1.7 and 1.5 polymers. There seemed to be some discrepancy with the theory about the other two esters, and it was believed that they were not hydrolysed in the order that was predicted by theory. The biodegraded polymers with low *DS* (10*DS* 1.0 and 10*DS* 0.7) had biodegraded to such an extent that their acetyl content had been completely removed.

^{13}C n.m.r. spectroscopy. All the samples displayed a very reduced solubility in the common n.m.r. solvents, and it was impossible to obtain any useful carbon spectra in solution. Therefore, solid state ^{13}C n.m.r. was performed. However, many authors have claimed solubility of CA with various *DS* values in n.m.r. solvents[13,24,26,30–32]. The conflicting evidence proves again that the method of synthesis and the relative substitution of specific sites are very important in CA solubility.

Doyle *et al.*[25] studied the ^{13}C n.m.r. spectra of various CAs in solution and in the solid state. The synthesis used involved a heterogeneous acetylation of cotton linters to the appropriate *DS*. For the solution spectra, DMSO was used for the CAs with a *DS* greater than 0.5, whereas a mixture of DMSO and *N*-methylmorpholine-*N*-oxide was needed for the cellulose sample and the CA with a *DS* of 0.5. The authors found that for CA with a *DS* value greater than 0.5, irrespective of the *DS*,

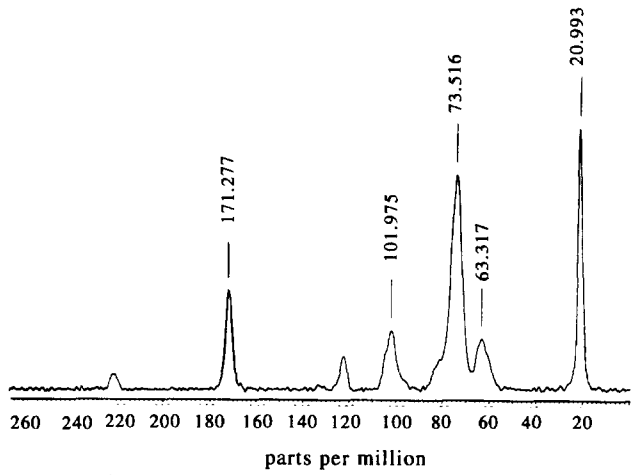


Figure 6 ^{13}C n.m.r. spectrum of the *DS* 1.5 sample

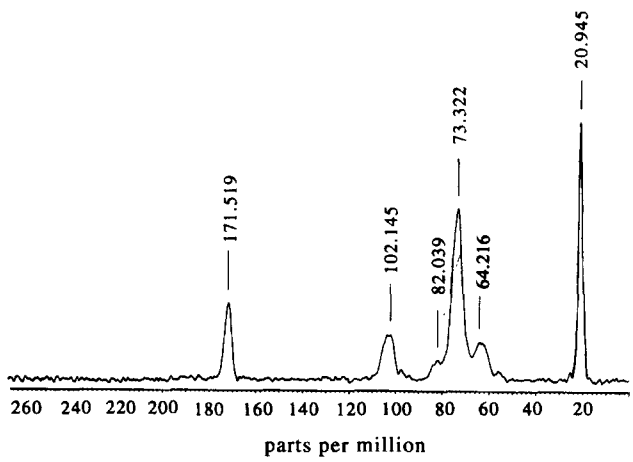


Figure 7 ^{13}C n.m.r. spectrum of the 10 *DS* 1.5 sample

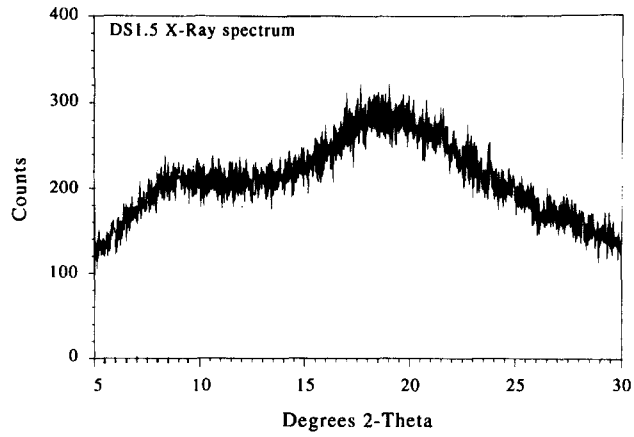


Figure 8 X-ray spectrum of the *DS* 1.5 sample

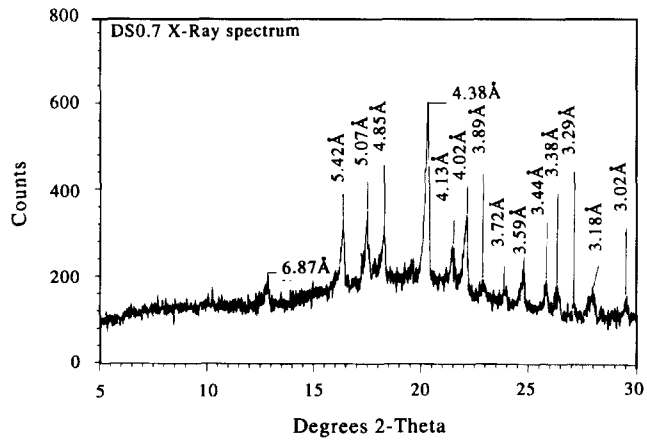


Figure 9 X-ray spectrum of the *DS* 0.7 sample

the spectrum obtained was that of the triacetate, and there was no evidence for partially substituted or unsubstituted cellulose, despite the fact that chemical analysis clearly showed that acetylation was far from complete. By comparing their results with results obtained by other workers on nitration of cellulose, Doyle and co-workers concluded that, using their conditions, the rates of acetylation were not controlled by the reactivity of the particular sites but rather by accessibility.

The authors also compared their *DS* 2.5 sample with a commercially available sample of the same *DS*, and found the two to be different. They further concluded that the commercial sample was generated under such conditions that the basic cellulose structure was destroyed and the observed *DS* of 2.5 was achieved by subsequent hydrolysis of the triacetate, which is indeed the method used commercially. The commercial sample spectrum displayed both substituted and unsubstituted cellulose features.

They finally concluded that the initial acetylation in their samples occurred in the disordered accessible regions of the cellulose. Once this had been completed, further acetylation occurred in the ordered regions without them losing their integrity. This meant, therefore, that the solutions subsequently obtained contained dispersions of these ordered regions and hence the spectra corresponded to the solubilized part of the cellulose structure only. An important implication was, therefore, that DMSO and *N*-methylmorpholine-*N*-oxide might not be true solvents for cellulose, but rather were capable of achieving dispersions of the ordered regions. It was thus possible that such regions survived to a large extent in fibrous CA and that the solution spectra corresponded to solubilized surface groups which occurred in amorphous areas. *Table 4* summarizes the results for the original and biodegraded polymers (see also *Figures 6* and *7* for the carbon spectra of *DS* 1.5 and 10 *DS* 1.5, respectively).

Several points can be made about the results obtained. Firstly, some spectra, especially that of the 10 *DS* 0.7 polymer, were noisy. This was due to the small quantities of sample that were available for analysis. Normally 0.5 g is needed for a good solid state ^{13}C n.m.r. spectrum. However, in some cases, only 0.3 g or less was available.

Some of the samples, despite having been ground were not fine enough for ^{13}C n.m.r. and were too hard to grind further. In order to obtain a spectrum for these polymers, they were packed in talc to help spinning. In these cases the spinning was still relatively slow (2600 Hz compared to 3600 Hz), so a sideband suppression sequence (TOSS) had to be used to improve resolution.

It is also important to note that the relative intensities of the peaks as detailed in *Table 4* can be compared sample to sample, but the intensities within one spectrum do not necessarily have a 1 : 1 correlation with the number of carbons they represent. This is due to the cross-polarization technique used to obtain the spectra.

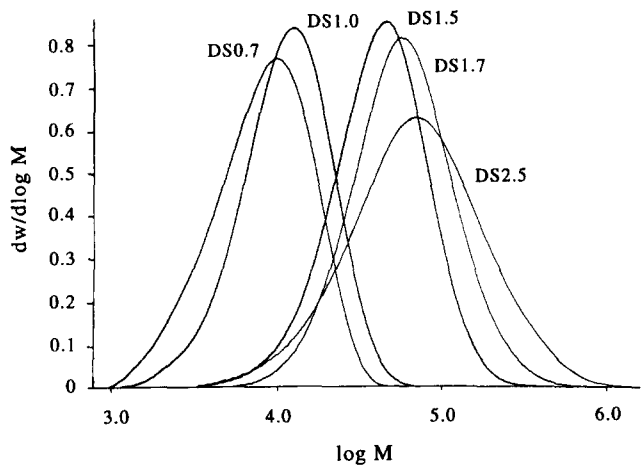


Figure 10 Overlaid g.p.c. chromatograms for the starting polymers

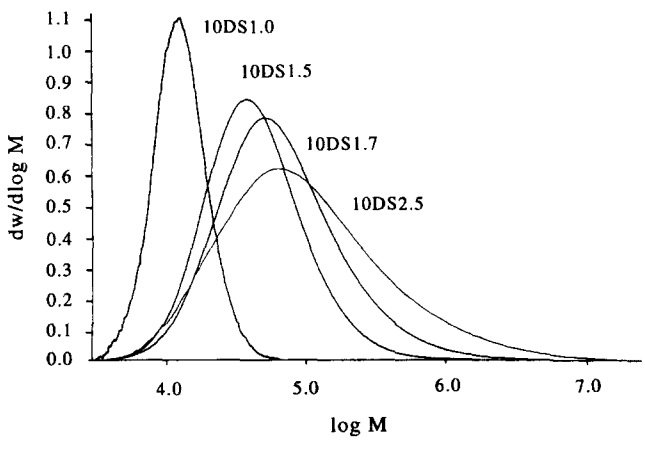


Figure 11 Overlaid g.p.c. chromatograms for the biodegraded polymers

The technique involves the magnetization of the protons adjacent to the carbons being transferred to the appropriate carbons. Therefore, if there are more protons around a particular carbon atom, then the intensity will be higher than if there are fewer or no protons close to the carbon atom.

By taking these factors into consideration, the *DS*2.5 material does not show any marked differences before or after biodegradation. This confirms the previous evidence as to its resistance to biodegradation. The biodegraded *DS*1.7 and 1.5 polymers, however, do show some changes from their starting compounds. In both cases a C-4 peak appears. Taking into account that crystalline cellulose displays a peak at about 88 ppm, this could imply that the biodegraded polymers may have acquired some crystalline content and that some deacetylation had occurred. The fact that the *DS*0.7 polymer features a peak at the same resonance would also emphasize the point. The biodegraded polymer with an initial *DS* of 0.7 did not give any significant results as the background noise was extremely marked due to the lack of material present in the rotor.

Furthermore, the starting polymers show a decreasing trend in the intensities of the carbonyl and methyl peaks. This is the expected trend, as there is a decrease in the acetyl content with decreasing *DS*. Furthermore, there is an increase in the C-1, C-2, C-3, C-5, C-6 and, importantly, C-4 intensities. This again shows that the crystalline content increases with decreasing *DS*.

Table 6 MHS constants for given *DS* values (DMA, 25°C)

DS	$K \times 10^3$ (ml g^{-1})	log *K*	*a*
0.49	191	−0.72	0.60
1.75	95.8	−1.02	0.65
2.50	39.5	−1.40	0.738
3.00	26.4	−1.58	0.750

Table 7a Polymer flow times (in seconds) with varying concentrations (DMA/LiCl, 25°C)

Concentration	*DS*2.5	*DS*1.7	*DS*1.5
Solvent		254.62	
6 mg ml^{-1}	486.24	450.30	429.23
5 mg ml^{-1}	445.50	415.39	398.45
4 mg ml^{-1}	402.67	382.46	367.56
3 mg ml^{-1}	363.38	345.68	338.36

Table 7b Ten day biodegraded polymer flow times (in seconds) with varying concentrations (DMA/LiCl, 25°C)

Concentration	10 *DS*1.7	10 *DS*1.5
Solvent	254.62	
2 mg ml^{-1}	300.17	279.37
1.5 mg ml^{-1}	287.06	272.01
1 mg ml^{-1}	274.57	265.92
0.5 mg ml^{-1}	261.70	258.36

As far as the biodegraded samples are concerned, the decreasing carbonyl trend continues, as does the increasing crystalline content. There seems to be a discrepancy in the methyl intensities, however, as there seems to be a rise for the 10 *DS*1.7 polymer before it decreases again for the 10 *DS*1.5 polymer. This might be due to the reasons noted before, as to the use of talc in order to spin the samples and having to use a slower spin rate and also to the fact that some corrective software had to be used (TOSS). These factors could have made direct comparisons less accurate.

X-ray diffraction analysis of the starting polymers

The X-ray spectrum for the CA with a *DS* of 1.5 (*Figure 8*) was typical for the higher-*DS* polymers (*DS*2.5, 1.7 and 1.5). These polymers were amorphous. However, as the *DS* decreased, an increase in the crystallinity was observed (see also *Figure 9* for the *DS*0.7 polymer). This was due to the fact that as the acetyl content of these low-*DS* polymers decreased, a more 'cellulose-like', semi-crystalline structure was adopted. This behaviour was also indicated by the ^{13}C n.m.r. spectra of the lower-*DS* polymers as well as for the biodegraded ones.

The increased crystallinity would make biodegradation more difficult, i.e. the time needed for these polymers to biodegrade would be longer than if no crystalline content was present.

Chromatographic characterization

Tables 5a and *5b* illustrate the molecular weights obtained for the starting and biodegraded CA polymers respectively, using g.p.c. calibrated with PEO/PEG standards (see *Figures 10* and *11*).

It can be seen that for the higher-*DS* polymers (*DS*1.7 and 1.5), there is some decrease in the *DP* accompanying the decrease in the *DS*.

The drop in molecular weight in the last two polymers

Table 8 Molecular weight values of some starting and degraded polymers

DS	$\overline{M}_w$
2.5	60 000
1.7	53 000
1.5	50 000
10 *DS* 1.5 (final *DS* 0.9)	8500

with *DS* values of 1.0 and 0.7 is much greater, accompanied by a much larger decrease in the *DP*. This trend illustrates a well-known problem, i.e. that chemical hydrolysis under these conditions also promotes some chain scission. However, for the higher-*DS* polymers, the problem is not too marked.

One has to treat these results with caution, however. This is due to a well-known problem with g.p.c., and that is the possible association between the reversed-phase g.p.c. column and the polymer in solution. The very big drop in the low-*DS* polymers highlights the problem. As the *DS* drops, the solubility of the polymer decreases. This in turn increases the adsorption of the polymer to the column packing. This means that the polymer elutes later than expected, giving lower *MW* values than expected[33]. This could, therefore, mean that the low-*DS* polymers may have a higher molecular weight than indicated in *Tables 5a* and *5b*.

The trends in the biodegraded polymers follow the trends for the starting polymers, i.e. there is a drop in the molecular weight, becoming very marked with decreasing *DS*. The molecular weight drop in the 10*DS* 1.7 and 10 *DS* 1.5 polymers is generally small, and can be attributed to the loss of acetate groups. The large subsequent drop in the 10 *DS* 1.0 polymer, however, shows that depolymerization has also taken place.

Solution viscosity characterization

Equation (5) shows the relationship between limiting viscosity and molecular weight. The two constants *K* and *a*, which are specific for every solvent/polymer/temperature system have to be known, however. In the literature, these constants are only available for the DMA solvent in the absence of LiCl at 25°C[34]. Because of this, it is impossible to obtain absolute molecular weight values, but comparisons can be made for the trends in the molecular weights exhibited by the various polymers. The constants are shown in *Table 6*. The flow times for the starting CA polymers are shown in *Table 7a*. Using equations (1) and (2), the flow times can be converted to viscosities, and appropriate graphs can be drawn so as to satisfy equation (3). The solution viscosity measurements for the biodegraded polymers are shown in *Table 7b*.

The 10 *DS* 2.5 polymer gave identical results to the starting material. This was further proof that the CA with a *DS* of 2.5 did not biodegrade.

By observing *Tables 7a* and *7b*, it can be seen that two different sets of concentrations were used. The reason is that the biodegraded 10*DS* 1.7 and 10*DS* 1.5 polymers have a much poorer solubility in DMA than the original polymers. As mentioned previously, there are no MHS constants for the DMA/LiCl system, so absolute molecular weight values cannot be calculated. However, relative comparisons can be made by using the MHS constants for DMA alone at 25°C. *Table 8* summarizes the results.

The trends seen above seem to be in agreement with the results obtained by the g.p.c. DMA/LiCl system in *Tables 5a* and *5b*. There seems to be a relatively small drop in the molecular weight values of the high-*DS* polymers consistent with a loss of acetate groups. However, there is a dramatic fall in the biodegraded sample, which would be consistent with not only a loss of acetate groups (the *DS* drops from 1.5 to 0.9 after 10 days), but also with significant chain scission.

Furthermore, from the data available in the literature[5,7], the radii of gyration were calculated for the *DS* 2.5 and 1.7 polymers using the following equations:

$$\text{For the } DS\,2.5 \text{ polymer}: (s^2)^{1/2} = 0.68 \times 10^{-8} M_w^{0.53} \text{ (cm)} \quad (6)$$

$$\text{For the } DS\,1.7 \text{ polymer}: (s^2)^{1/2} = 0.38 \times 10^{-8} M_w^{0.52} \text{ (cm)} \quad (7)$$

By substituting the molecular weight values from *Table 8*, the radii of gyration were deduced to be 2.32×10^{-6} cm for the *DS* 2.5 polymer and 1.09×10^{-6} cm for the *DS* 1.7 polymer. This shows that DMA becomes progressively a 'poor' solvent for CA. This is in agreement with this work. As mentioned earlier, the *DS* 1.0 and 0.7 polymers were hardly soluble in DMA and no meaningful results were obtained from their viscosity measurements.

CONCLUSIONS ON THE BIODEGRADABILITY OF CA POLYMERS

By considering the biodegradation of chemically synthesized CA polymers with lower *DS* values, an accurate picture of the CA biodegradation was obtained.

The *DS* was a very significant factor in the biodegradability of these polymers.

The lower the *DS* the easier the biodegradation.

The higher-*DS* polymers were amorphous, and the crystallinity increased with decreasing *DS*. This was demonstrated by the X-ray spectra as well as by the ^{13}C n.m.r. spectra with the appearance of the C-4 peak. This meant that the predominant factor that hindered biodegradation in high-*DS* polymers was the *DS* alone. It was originally thought that crystallinity in the high-*DS* polymers played an important role in the reduced biodegradability.

The *FT*i.r. technique made the *DS* determination easier and faster than the chemical titration method. The other problem with the titration method was the fact that below a certain *DP* the method broke down and gave incorrect results. With the *FT*i.r. technique, the *DS* of any *CA* polymer could be determined with the aid of the calibration curve.

The n.m.r. work also gave an insight into the biodegradation mechanism. From the theory, it was expected that the primary 6-position would be more vulnerable to hydrolysis, followed by the position-2 and finally by position-3. The actual removal of acetates proved to be more complicated, but, in general terms, the primary 6-position was more likely to be hydrolysed, and, in fact, it disappeared completely from the polymer with a *DS* of 0.7 as well as from the biodegraded polymers with initial *DS* values of 1.7 and 1.5.

It can be seen by looking at the molecular weights that the biodegraded polymer with an initial *DS* of 1.5 (which

was investigated in greater detail) had not only deacetylated (final *DS* was 0.9 as determined by *FT*i.r.), but it had also significantly depolymerized (initial *MW* was 50 000 compared to 8500 after biodegradation).

After all the evidence was assessed, the following mechanism of biodegradation is proposed. A consortium of micro-organisms is responsible for the biodegradation. An esterase is needed to deacetylate the polymer, and a cellulase is also present to depolymerize it. As the fungus does not biodegrade cellulose acetate, one can assume that the esterase must have acted first. Once the *DS* drops below a certain level, then enough room is created around the chain for the cellulase to attack. The fact that the CA with a *DS* of 2.5 does not biodegrade can be attributed to some steric phenomenon, whereby there is not enough room for the esterase to attack.

This is in agreement with common microbiological knowledge that, in such cases, there should be at least two neighbouring unsubstituted glucose molecules before biodegradation can commence. This also agrees with the findings in this work that biodegradation is more difficult for the high-*DS* polymers.

In order to quantify the probability of having two adjacent unsubstituted glucose molecules in the CA chain, a mathematical model has been devised in association with the Mathematics Department of Loughborough University, and is presented in another paper[35].

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