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Synthesis and characterisation of polyglucuronan

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Several samples of polyglucuronan have been prepared by oxidation of cellulose with nitrogen oxides. Optimisation of the reaction conditions in terms of degree of oxidation and molecular weight of the products was carefully performed.

INTRODUCTION

The reaction of nitrogen oxides with cellulose to yield polyglucuronic acid is well known (Unruh *et al.*, 1942; Painter, 1977). The oxidised derivative has found application in medicine as a wound healing aid (Linsky *et al.*, 1987; Diamond *et al.*, 1987). Recently new interest in this kind of derivative was aroused by the report of immunostimulant properties of polyglucuronic acid (US Patent, 1992). The biological activity seems to be related to the degree of oxidation and to the molecular weight of the products.

MATERIALS AND METHODS

The oxidised samples were obtained from different starting materials including cellulose powder (OC1, OC2), cotton linters (OC3, OC4) and bacterial cellulose (OC5).

^{13}C-NMR spectra were recorded with inverse-gated decoupling using a Brucker AM 300 WB spectrometer equipped with a 10 mm broad band probe. ^{13}C-NMR chemical shifts were referenced to internal acetone (31.07 ppm). The degree of oxidation of the samples was determined by comparing the integral values of the anomeric signals at 104–101 ppm with those of the C-6 signal at 62.2 ppm.

The molecular weight and molecular weight distribution were determined for each sample by means of HP-SEC using a Jasco HPLC Model 990 PU pump with a Reodyne 9125 injector and with a serial set of TSK PWXL G6000, G5000 and G3000 columns (Toso Haas) thermostated at 40°C. A refractive index detector (Waters 410) was used. Column calibration was performed via broad standard calibration procedure using an oxidised cellulose sample previously characterised by means of LALLS (LCD Chromatix CMX 100) (Gamini *et al.*, 1992). The viscosity was measured at 25°C using automatic AVS 440 Schott-Geraete equipment with an Ubbelohde capillary viscometer.

RESULTS AND DISCUSSION

The reaction of nitrogen oxides with cellulose suspended in phosphoric acid results in oxidation to the carboxylic group of the primary alcoholic function on C-6 (Fig. 1). To a very minor extent, the oxidation of secondary alcoholic groups also occurs. The keto-groups produced by the oxidation of secondary alcoholic functions are selectively reduced with sodium borohydride after extraction of the oxidised polysaccharide from the reaction mixture. In our laboratories, several experiments have been carried out and reaction parameters were controlled and modified. The aim of these sets of experiments was to identify the reaction conditions in terms of preparation of oxidised cellulose samples with high degrees of oxidation and high molecular weights. In fact, it was observed that the degree of oxidation at the C-6 position and the extent of depolymerisation of the starting polymer are related to the reaction conditions. Table 1 reports the reaction parameters applied for the preparation of some oxidised samples that have been characterised.

Cellulose was swelled in H_3PO_4 for a period of time between 0 and 5 h at low temperature (0°C) before starting the oxidation reaction. This step appeared to be important because better results were obtained, also in terms of yield, when the reaction mixture was homogeneous. Conversely, swelling time longer than 5 h has a negative impact on the degree of polymerisation of the derivatives. A period of time between 1 and 5 h was finally chosen.

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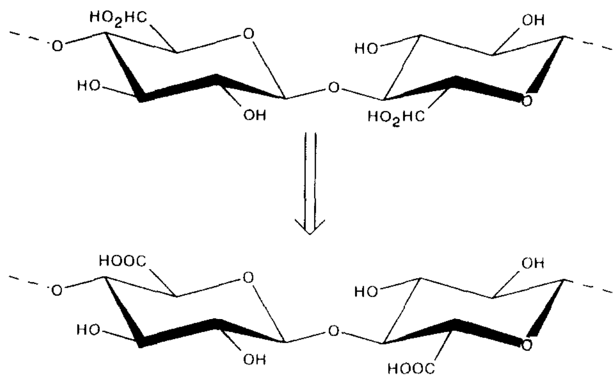

Fig. 1. Structure of cellulose and C-6 oxidised cellulose.

Nitrogen oxides were produced outside the reaction vessel and added to the mixture, which was kept at low temperature to assure an efficient distribution of the oxides in the reaction mixture (Italian Patent Application, 1992). It was found that the optimum temperature during the addition of the oxidising agent ranges between −8 and 4°C. In particular, in most of the reactions that provided the most interesting derivatives in terms of molecular weight and oxidation degree, this temperature was set at 0°C. After the addition of the oxides, the optimised temperature was between 4 and 10°C. Higher temperature should be avoided in order to reduce cellulose depolymerisation. Stirring of the reaction mixture was also an important parameter for the degree of substitution and had to be maintained during the whole reaction period to ensure a high degree of oxidation. A reaction time longer than 16 h did not lead to further increases in the degree of substitution.

Table 1. Reaction parameters applied for the preparation of polyglucuronan samples

Sample	Time 1 (h)	Temp. 1 (°C)	Temp. 2 (°C)	Time 2 (h)
OC1	5	−10	4	16
OC2	5	0	4	16
OC3	1	4	4	16
OC4	5	0	4	16
OC5	5	−8	4	16

Time 1 is the period of swelling in H_3PO_4 85% at 0–4°C; Time 2 is the reaction time after addition of nitrogen oxides; Temp. 1 is the operating temperature applied during addition of nitrogen oxides; Temp. 2 is the temperature during the reaction after addition of nitrogen oxides.

Several sources of cellulose have been used for the

Table 2. Oxidation degree and molecular weight (M_W) of polyglucuronan samples

Sample	Oxidation degree	M_W
OC1	84	26 700
OC2	87	26 300
OC3	93	51 000
OC4	93	60 500
OC5	78	44 700

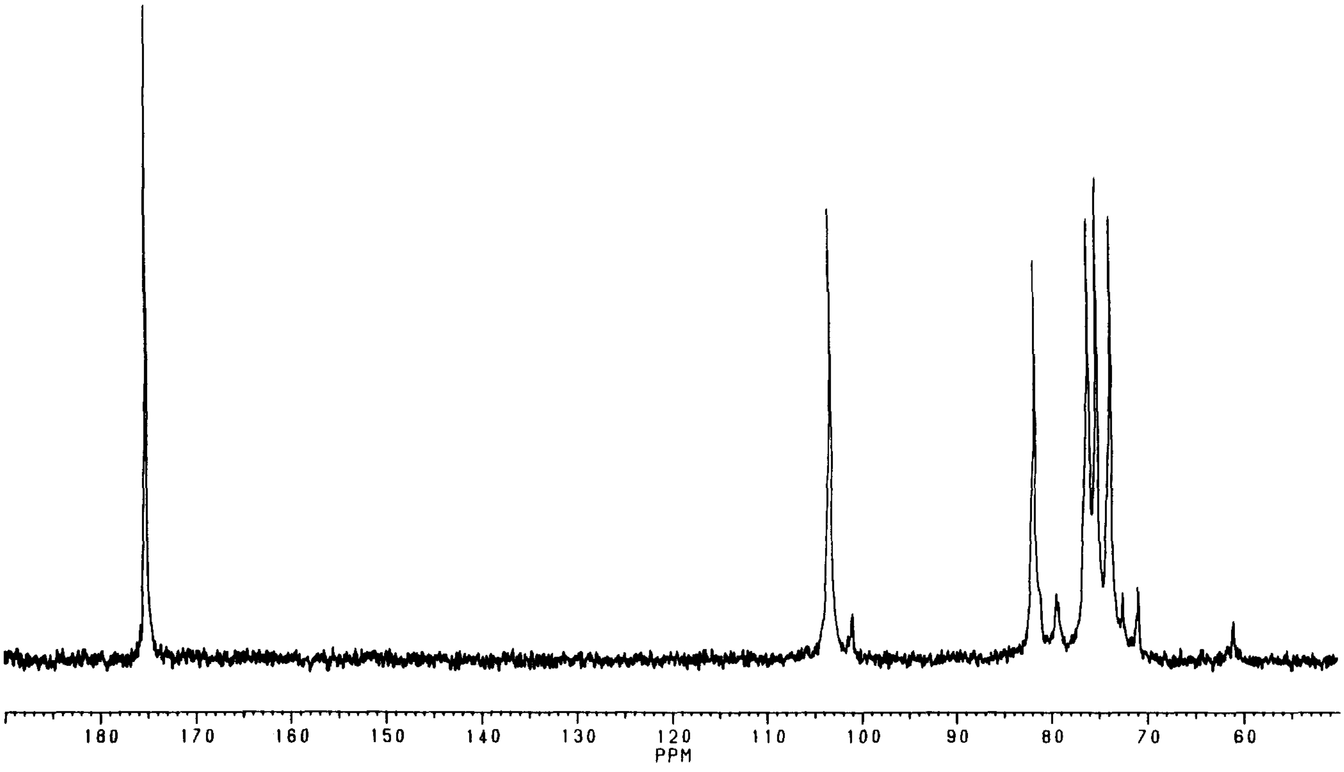

Fig. 2. ^{13}C-NMR spectrum of OC1.

production of different oxidised cellulose. The samples obtained as previously described are listed in Table 2.

The ^{13}C-NMR spectra of OC1 is shown in Fig. 2. Assignment (C-6 ox: 175.5 ppm; C-1: 104–101 ppm; C-4: 82.1 ppm; C-5: 76.5 ppm; C-3: 75.4 ppm; C-2: 73.9 ppm; C-6: 62.2 ppm) was obtained by D2-NMR techniques and the values for the degrees of oxidation calculated by NMR are shown in Table 2.

Molecular weight (M_W) values have been determined for all samples (Table 2). It is obvious that these values are not only dependent on the reaction conditions but also on the degree of polymerisation of the starting material. Therefore, the origin of cellulose is also an important aspect that has to be considered for the production of high M_W of oxidised samples of cellulose. In fact, cellulose with a high degree of polymerisation (cotton cellulose) yielded the oxidised sample characterised by the highest M_W (OC3, OC4).

The intrinsic viscosity data as a function of the inverse of square root of ionic strength for oxidised cellulose samples exhibit a linear dependence. This is typical of polyelectrolytes not undergoing a salt-induced co-operative conformational transition. Moreover, the intrinsic viscosity data were used to estimate the chain stiffness according to Smidsrød and Haug (1971). The values of empirical stiffness parameter *B* (ranging from 0.05 to 0.065) were found to be similar to those reported in the literature for carboxymethylcellulose (Smidsrød & Haug, 1971) indicating that the stiffness exhibited by the two polyelectrolytes is mainly due to conformational constraints and is not related to the type of ionisable groups.

CONCLUSIONS

Polyglucuronans were prepared from different samples of cellulose including cellulose powder, cotton linters and bacterial cellulose. Characterisation, with respect to their M_W, degree of oxidation and viscosity of the samples, was used for the optimisation of reaction conditions. It was found that M_W was both dependent on the polymerisation of the starting material and on reaction conditions. Furthermore, it was observed that a careful control of the reaction parameters has to be applied in order to obtain derivatives with high degrees of oxidation (up to 93%). Two crucial parameters were identified: temperature and time. Low temperature during swelling and during reaction is essential for minimising the degradation of the polymer. The optimal period of time during swelling and reaction was also determined in order to achieve a high oxidation and low degradation of the starting cellulose.

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