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SOLUTIONS OF POLYSACCHARIDES IN N-METHYL MORPHOLINE N-OXIDE (MMNO)

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ABSTRACT

A mixture of N-methyl morpholine N-oxide and a small amount of water was found to be a general solvent system for underivatised polysaccharides with the exception of chitin. With the stiffer polysaccharide molecules, the most concentrated solutions were anisotropic, indicating a liquid crystalline order. On the other hand, more flexible molecules gave only isotropic solutions. In several instances, the anisotropic solutions could be spun or extruded easily and upon regeneration gave access to well oriented polysaccharide films or fibres.

INTRODUCTION

Over the past years, several patents have described the dissolution of cellulose in a series of aliphatic or cyclic tertiary amine oxides (Graenacher & Sallman, 1939; Johnson, 1969; Franks & Varga, 1979; Franks & Varga, 1980). These compounds form strong hydrogen bonds between their N–O appendages and primary or secondary hydroxyl groups. This behaviour, which was characterised on model compounds (Chanzy *et al.*, 1982; Maia & Perez, 1982; Maia *et al.*, 1981), is believed to be the key to the dissolving power of amine oxides. Remarkably, natural or synthetic substances, rich in internal hydrogen bonding, become soluble in amine oxide systems whereas they are totally insoluble in most organic solvents. Amine oxides and specifically N-methyl morpholine N-oxide (MMNO) are able to dissolve several plant and animal polysaccharide materials; solutions of starch, amylose and glycogen in MMNO have been patented (Johnson, 1969) while it was reported that this amine oxide could also give solutions of plant hemicelluloses, bacterial polysaccharides (Joseleau *et al.*, 1981) (1 → 3)-β-D-xylan (Utille, 1979) and (1 → 3)-α-D-glucan (Ogawa *et al.*, 1981).

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Our work with amine oxides has so far dealt with the dissolution of cellulose in MMNO (Chanzy *et al.*, 1979; Chanzy *et al.*, 1980). It was shown that highly viscous, concentrated solutions, containing up to 55% of cellulose could be obtained in optimal circumstances when the water content of the solutions was properly monitored. The concentrated solutions were anisotropic and upon simple spinning or casting followed by regeneration gave well-oriented fibres and films.

The present work was devised to see whether similar properties could be transposed to other polysaccharides made of either flexible or rigid chains. The goal was thus to prepare concentrated solutions of a variety of polysaccharides and to detect the occurrence, if any, of a liquid crystalline order within the solutions. All experiments were performed using MMNO in order to establish a good correlation between this work and the related studies already in the literature.

EXPERIMENTAL

Materials

MMNO, H_2O was prepared by recrystallising in dry acetone MMNO obtained from Eastman Kodak Company. It had a melting point of 74°C and contained 13·3% water as determined by the Karl Fisher method.

Anhydrous MMNO was prepared by vacuum sublimation of MMNO, H_2O over a temperature range which was slowly increased from 65 to 90°C.

Locust bean gum (galactomannan), guar gum (galactomannan), xanthan gum (grade II), amylose (type I and III), potato starch and κ-carrageenan were purchased from Sigma and were dissolved as such without purification.

Nigeran from *Penicillus crustosum* (Bobbit & Nordin, 1978) was a gift from Dr R. H. Marchessault. It had a reported molecular weight of 10^6.

Pullulan was purchased from BDH Biochemicals.

Chitin from Sigma was dissolved in NN-dimethyl acetamide saturated with LiCl (Austin, 1977). It was then filtered, reprecipitated by dialysis against water and freeze dried before testing for dissolution in MMNO.

Glucomannan from *Amorphophallus konjac* was purchased from Senn Chemicals. It was purified according to the method of Sugiyama *et al.* (1972) and deacetylated with aqueous ammonia.

Curdlan (1 → 3)-β-D-glucan with a degree of polymerisation ($\overline{DP}$) of 460 was a gift from Dr K. Ogawa. It was used without further purification.

Pustulan from Calbiochem was bleached according to the method of Wise *et al.* (1946) and deacetylated for 7 days in water saturated with ammonia. It was then recovered and dried.

Dextran B 512 and T 2000 were purchased from Pharmacia.

Lichenin was a gift from Dr M. Vincendon.

Scleroglucan (trade name Actigum CS 11) was kindly provided by the Ceca Company.

(1→3)-β-D-Xylan was a gift from Dr J. P. Utille (1979) and was extracted from *Penicillus dumestosus* (Taylor, 1960).

(1→4)-β-Mannan A was prepared from ivory nut meal following the method of Meier (1958).

(1→4)-β-D-Mannan B, prepared from *Codium fragile*, was a gift from Mr A. Grosrenaud.

Amylose, of high molecular weight (origin AVEBE), was a gift from Mr A. Buléon. It had a reported DP of 2000.

Solution Preparation

All mixing and handling were performed in confined dry environments. The first step towards dissolution consisted of homogenising at room temperature amounts of dry disintegrated polysaccharides together with MMNO monohydrate and anhydrous MMNO. The mixtures were then positioned inside capped vessels equipped with a stirrer. The vessels were heated in an oil bath at temperatures ranging from 90 to 140°C while stirring. Total dissolution normally resulted within minutes. The solutions were then solidified by cooling and stored in a dry atmosphere until further use.

Physical Characterisation

Optical microscopy: Fragments of the solidified solutions were placed on glass slides which were inserted inside a Mettler hot stage maintained at a temperature of 80°C. The fragment softened and a coverslip was pressed on top of the liquid. The hot stage was then fitted on the rotary stage of a Zeiss universal polarising microscope which was used to examine optical anisotropy as a function of temperature.

X-Rays on regenerated fibres: Hot solutions of polysaccharides were spun with a small laboratory extruder working at temperatures ranging from 90 to 150°C. The fibres were then cooled and regenerated by immersion in water or ethanol. The regenerated fibres were studied by wide angle X-rays using a flat film vacuum Wahrus X-ray camera.

RESULTS AND DISCUSSION

With the exception of chitin, which did not dissolve to a significant extent, all the polysaccharides dissolved readily in MMNO. As already found with cellulose (Chanzy *et al.*, 1979), good dissolution was obtained when the water content was kept as low as possible by a proper mixing of MMNO monohydrate and anhydrous MMNO. The mixtures had melting temperatures which increased with a decrease in water content and ranged from 74°C (m.p. of MMNO monohydrate) to 172°C (m.p. of anhydrous MMNO). It was found that beyond 150°C, the solutions were no longer stable and presented indications of decomposition and blackening.* For this reason 150°C was

* Higher dissolution temperatures, especially in the presence of metallic ions, can lead to fast exothermic decomposition of MMNO, and consequent fire hazard.

set as an upper temperature limit. This necessitated a water content of at least 4% within the solution in order to have the solvent in the liquid state at this temperature.

Table 1 gives a list of the maximum concentration which can be reached with the various polysaccharide samples. As with cellulose, solutions of high concentration (30% and above) could be obtained readily with all samples tested, except chitin. In general, the solubility of a given polysaccharide material increased when its molecular weight was decreased. This was the case for cellulose and was confirmed here with materials such as mannan, amylose or dextran for which a variety of molecular weight samples were tested for dissolution. Another factor leading to a high solubility is the flexibility of the polysaccharide chain. This is well illustrated with a specimen such as dextran which is well known for its chain flexibility and multiple possibilities of conformation (Tvaroska *et al.*, 1978; Brant & Burton, 1981) as opposed to stiffer molecules such as cellulose. Typically T 2000 dextran with a $\overline{DP}$ of 12 500 is about twice as soluble as cellulose of a corresponding $\overline{DP}$.

Some of the most concentrated solutions of polysaccharide have the interesting property of being anisotropic when observed with a polarising microscope operating with crossed nicols. The anisotropy, which denotes a liquid crystalline order, is best illustrated with scleroglucan and curdlan which qualitatively yield solutions having the

TABLE 1
Dissolution parameters of polysaccharides in N-methyl morpholine N-oxide, H_2O[a]

Polysaccharide material	*Maximum concentration*[b] *(weight polysaccharide per weight of solvent, water included) (%)*	*Order of the solution*
Chitin	Insoluble	–
Agarose	30	Anisotropic
Galactomannan: locust bean gum	35	Anisotropic
Galactomannan: guar gum	35	Anisotropic
Konjac mannan	35	Anisotropic
Xanthan gum	35	Anisotropic
Mannan B	35	Isotropic
Lichenin	35	Isotropic
(1→ 3)-β-D-Xylan	35	Isotropic
Pustulan	35	Isotropic
Nigeran	40	Anisotropic
High molecular weight amylose	40	Anisotropic
κ-Carrageenan	40	Isotropic
Mannan A	45	Anisotropic
Scleroglucan	50	Anisotropic
Potato starch	50	Anisotropic
Pullulan	50	Isotropic
Curdlan	55	Anisotropic
Dextran T 2000	50	Isotropic
Dextran B 512	60	Isotropic

[a] For optimum dissolution, the solvent composition was 4–8% (w/w) water.
[b] Maximum concentration at which there are no insoluble particles detectable by optical microscopy.

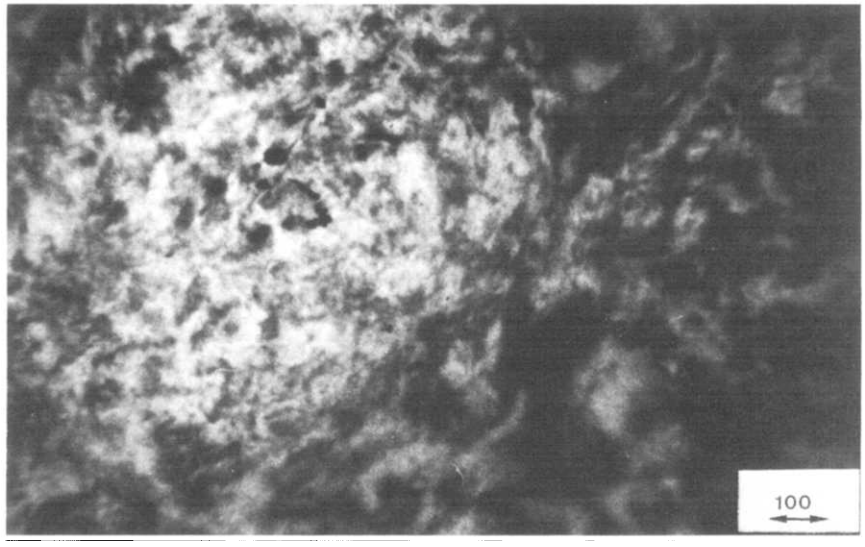

Fig 1. Photomicrograph of an anisotropic solution containing 50% scleroglucan in MMNO-water mixture.

highest birefringence when dissolved at high concentration in MMNO. Figure 1 is an illustration of such a solution of scleroglucan. The solution consists of a large number of small birefringent and highly coloured domains intertwined with dark areas of equally small sizes. The illuminated domains are shapeless and have dimensions in the order of one to several microns. There is no clear, evident pattern in the organisation of the bright and dark domains and for this reason, it is not possible to decide whether the solutions are nematic or cholesteric solutions. A preparation such as that presented in Fig. 1 loses its birefringence upon heating above 130°C. When lost, this birefringence is, however, slowly restored by cooling the solution down to 70°C or below. Besides the temperature, other parameters which influence the order of the solution are the concentrations of the polysaccharide solutions, the water content in the solution and the $\overline{DP}$ of the dissolved material. A high concentration of the polysaccharide, a low water content and a high $\overline{DP}$ favour the formation of birefringent solutions while the reverse, for one or all of these parameters, tends to give isotropic solutions. None of these parameters uniquely determine whether liquid crystalline order forms but rather a combination of all parameters is involved.

For instance, the anisotropy of the scleroglucan preparation shown in Fig. 1 disappears at a much higher temperature if the water content of the solution is lowered or if the concentration of the solution is increased. On the other hand, the anisotropy disappears at a lower temperature, with a lower $\overline{DP}$ or with a high water content. Conditions can even be found where no anisotropy at all is obtained (e.g. by addition of too much water).

On surveying in Table 1 the list of the polysaccharides which are amenable to the formation of a liquid crystalline order when dissolved in MMNO, it is evident that the flexibility and the type of the chain linkages in the various materials are important. In general, the stiffest chains have the highest mesogenic character. This is seen for instance with the (1 → 4)-β-linked polysaccharide molecules which easily yield anisotropic solutions when the proper conditions of temperature, water content and concentration are met. Such behaviour contrasts with that of a material like pullulan or dextran which did not yield birefringent solutions under any conditions. Both dextran and pullulan chains contain (1 → 6)-α-glucan linkages which are renowned for their flexibility and multiple low energy conformations (Tvaroska *et al.*, 1979). Other cases, where polysaccharide chains possessed different types of linkages, were less predictable. A survey of a number of solutions with different molecular weights and various solution parameters is necessary before giving firm conclusions on the relationship between mesophase formation and rigidity of the various polysaccharide chains under investigation. The case of curdlan and scleroglucan, the (1 → 3)-β-linked glucans is interesting since they are the polysaccharides most prone to give a mesophase in MMNO. Intrinsically, the isolated chain of (1 → 3)-β-linked glucan is rather flexible and less extended than a corresponding chain linked (1 → 4)-β. It forms helices which are expected to occur in various states of extension (Rees, 1977). However, (1 → 3)-β-D-glucan, as in curdlan, is believed to be associated in triple helices, both in solution and in the solid state (Fulton & Atkins, 1980). This molecular association greatly enhances the molecular rigidity of curdlan and may explain the propensity of this polysaccharide to yield mesophases when dissolved in MMNO. It can be assumed that similar reasoning also applies to scleroglucan.

One interesting aspect of polymer mesophases is their tendency to become oriented under moderate shearing. When regenerating the polymer from the oriented solution, one may obtain films and fibres of superior mechanical properties. In particular, this procedure gives access to high strength fibres of great commercial importance (Kwolek, 1972; Morgan, 1976). With MMNO as a solvent, it was shown that the birefringent solution of cellulose could be spun readily, and after solvent removal highly oriented cellulose films and fibres were obtained (Chanzy *et al.*, 1980). In the present study, similar behaviour was observed with mesophase solutions of (1 → 4)-β-linked polysaccharides. This is illustrated, for instance, with konjac mannan which gave a mesophase when a 35% solution was made in MMNO. This solution, when spun without post-stretching followed by regeneration in water, gave konjac mannan fibres of good orientation. A typical bundle of such fibres, when tested in an X-ray fibre diagram experiment denoted a fairly good orientation or crystallinity as shown in Fig. 2. The diagram corresponds to an oriented fibre X-ray diagram of mannan II as described by Frei & Preston (1968) with its fibre axis parallel to the length of the fibres. As normal with mannan II the crystallinity of the sample in Fig. 2 is dramatically increased when the X-ray experiment is performed under hydrated conditions. Other polysaccharide material having the same type of linkage (mannan, galactomannan etc....) behaved

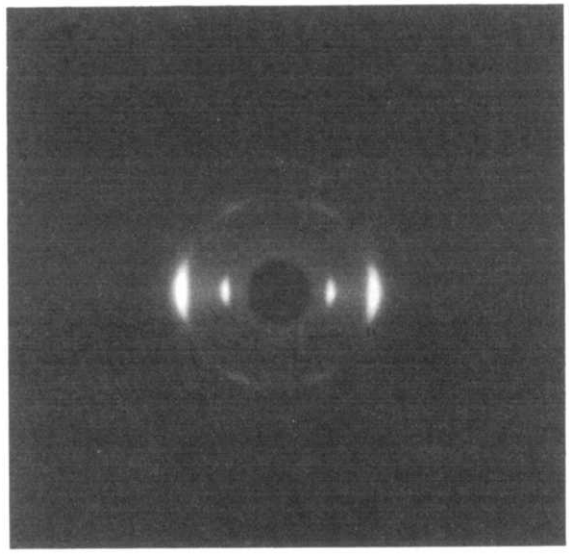

Fig. 2. X-Ray fibre diagram, performed under hydrated conditions from a bundle of fibres spun from a 20% anisotropic solution of konjac mannan in MMNO. The fibres have a vertical axis and were regenerated in methanol prior to the X-ray experiment.

identically and yielded bundles of well oriented crystalline fibres of the corresponding material.

With anisotropic solutions of polysaccharide having linkages other than the $(1 \rightarrow 4)$-β, the behaviour was different. The spinning of the MMNO solutions itself created no specific problem; it was achieved normally and gave highly birefringent, solvent-polysaccharide composite fibres. However, this birefringence was lost upon regeneration in water or methanol and the fibres became brittle. These fibres were crystalline but un-oriented when examined by X-ray diffraction. This behaviour which occurred with amylose, nigeran and others, is particularly significant with curdlan and scleroglucan which are the best cases of polysaccharide liquid crystals in MMNO. With these two $(1 \rightarrow 3)$-β-linked samples, it is most likely that the conformation in the crystalline solid state is different from that achieved in the mesophase. This conformation in solution could be due to either a specific solvation of MMNO on the polysaccharide or to a specific conformation related to the liquid crystalline order. Upon regeneration, the dissolved molecules revert to their solid state minimum energy conformation which is different from the one occurring in the mesophase system. Thus, the regeneration involves a conformational change in the polysaccharide chain which is accompanied by a loss of the initial orientation. This contrasts with the case of $(1 \rightarrow 4)$-β-linked polysaccharides where the chain conformation in the oriented mesophase and the corresponding solid state are either identical or closely related. All

these observations are being further evaluated at present and will be reported in the near future.

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