Sulfation of cross-linked polysaccharides by the N,N-dimethylformamide—SO_3 complex

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The reactivity of cross-linked starch and microcrystalline cellulose at sulfation is reduced by their preliminary drying which is accompanied by formation of secondary intermolecular bonds. Due to its preswelling effect, dry N,N-dimethylformamide destroys partially the intermolecular bonds thus facilitating the preparation of sulfated derivatives.

Sodium salts of sulfated cross-linked polysaccharides are stable in the air as well as in water. However, in an H form they submit to autohydrolysis with a subsequent loss of weight and deterioration of their colloid properties. Regarding the above-mentioned fact, the possibility of an application of sulfated cross-linked polysaccharides as ion exchangers seems to be limited.

Реактивность сшитого крахмала и микрокристаллической целлюлозы при сульфатировании понижается их предварительной сушкой, при которой возникают новые вторичные связы между макромолекулами. Сухой N,N-диметилформамид своим набухающим действием частично разрушает междумолекулярные связы, в результате чего улучшаются условия приготовления сульфатированных производных.

Натриевые соли сульфатированных сшитых полисахаридов постоянные на воздухе а также в воде. В Н-форме подвергаются в воде автогидролизу, происходит понижение их веса а также ухудшение их коллоидальных свойств. Поэтому применение сульфатированных сшитых полисахаридов в качестве ионообменников ограничено.

A number of methods have been developed for the sulfation of polysaccharides: sulfation by means of sulfuric acid in presence of aliphatic alcohols [1], chlorosulfonic acid in pyridine or tertiary amines [2], complex of dimethylsulfoxide—SO_3 [3]. The complex of N,N-dimethylformamide—SO_3 used by *Schweiger* [4] seems to be the most suitable sulfation agent since it effects only a slight degradation of polysaccharide macromolecules during the reaction. Moreover, the N,N-dimethylformamide—SO_3 complex is stable and N,N-dimethylformamide itself is an excellent solvating agent for many polymers, polysaccharides and their derivatives.

An advantage of the sulfation process involving N,N-dimethylformamide is that the polysaccharide sulfate formation is not accompanied by the liberation of water molecules so that no free sulfuric acid can be formed which makes it possible to

prevent the hydrolytic degradation of polysaccharides during the sulfation. Using the Schweiger's method it is possible to prepare a product with a degree of esterification of polysaccharide greater than 2 without a remarkable degradation of the degree of polymerization by a repeated sulfation.

From a practical standpoint the problem of stability of the sulfated macromolecules with respect to autohydrolysis as well as the stability of bonds between the polysaccharide and sulfate groups appeared to be very interesting since the mixtures of esters of the primary or secondary alcohol groups with relatively bulky substituents of the strong acid. In agreement with the *Schweiger*'s consideration [4] that cellulose as well as starch sulfates are resistant to alkali, *Petropavlovskii* found [1] that the sulfate groups of cellulose ester with the degree of substitution about 0.1 do not splitt off in 6% aqueous solution of sodium hydroxide even if heated at 50°C for 20 hrs. Products with a degree of sulfation about 2.5 do not suffer the desulfation even if heated in 2 M aqueous solution of sodium hydroxide. A prolonged treatment with the solution of sodium hydroxide effected a splitting of about 20% of sulfate groups. The stability of the bonds between polysaccharide and sulfate group is assumed to be a consequence of the steric hindrance of the bulky sulfate substituents as well as of a negative charge of the sulfate groups which thus repulse the OH^- ions. The consideration of *Percival* [5] that a sulfate group is easily removed only in the presence of an adjacent *trans*-hydroxyl group or in the case of location of the sulfate group at the C-6 and free hydroxyl group at the C-3 seems to be incorrect. Accordingly, the mono-substituted glucose units would be alkali labile regardless of the substituent location whereas the tri-substituted and di-substituted glucose units with the substituents at the C-2 and C-3 would be resistant and the further two di-substituted isomers having one of the sulfate groups at the C-6 would be alkali labile, too. These phenomena, however, were not confirmed experimentally.

After a preliminary detailed investigation of the sulfation conditions of uncross--linked polysaccharides (molar ratio cellulose : SO_3, concentration of polysaccharide in reaction mixture, concentration of SO_3 in *N,N*-dimethylformamide, temperature and reaction time) we made some efforts to apply the optimal conditions of sulfation we found both for the cross-linked microcrystalline cellulose and starch [8], with the aim to prepare cation exchangers with sulfate groups under the conditions of a minimum degradation of polysaccharides.

Experimental

Starting substances

Potato starch as well as microcrystalline cellulose (MCC) both cross-linked by epichlorohydrin according to *Kuniak* and *Alince* [6] were used. In average these samples contained one cross-link per six glucose units of polysaccharide. The air-dry sample (humidity 6—8%), sample dried over P_2O_5 under reduced pressure (humidity 0.3—0.5%) and absolute dry one obtained by drying for 4 hrs at 105°C were tested.

N,N-dimethylformamide (DMF) dried over the molecular sieve was used.

Preparation of the DMF—SO₃ complex

Sulfation was carried out by means of 13—15% solution of sulfur trioxide in DMF, prepared by introducing gaseous SO_3 (liberated from oleum by a moderate heating) into

cooled DMF. The reaction is exothermic, therefore the solution was cooled in order to maintain the temperature below 40°C. The content of sulfur trioxide in solution was determined titrimetrically with 0.1 N solution of sodium hydroxide.

Sulfation with DMF—SO₃ complex

Sample of cross-linked polysaccharide (2 g) was mixed with 2—5 ml of dried DMF and allowed to stand in a closed vessel for 90 min at room temperature in order to increase the accessibility. The amount of DMF-SO₃ complex corresponding to the required molar ratio SO₃ polysaccharide (15—30 ml) was added followed by an addition of pure DMF to give a polysaccharide : DMF ratio 1 : 16 (w/v). Reactions were performed at room temperature during 1 to 4 hrs. After the adequate reaction time the reaction mixture was filtered through a fritted glass filter followed by a subsequent washing with DMF (100 ml), methanol (100 ml), and water (200 ml). By means of 0.5 N aqueous solution of sodium hydroxide (100 ml) the sulfated products were converted into sodium salts, filtered, washed with distilled water to neutral reaction, and subsequently dehydrated with acetone. The sodium sulfate salts were air-dried for 24 hrs followed by drying at 60°C.

Determination of the capacity of ion exchanger

The catex in H^+ form was obtained by treating the sodium salt of sulfated cross-linked polysaccharide (1 g) with 0.5 N solution of HCl (100 ml) for 30 min followed by a careful washing with distilled water and subsequently dehydrated with acetone. Resulting substance was then treated with an accurate volume of 0.1 N aqueous solution of sodium hydroxide under stirring for 15 min. The excess of NaOH in solution was determined by titration with 0.1 N-HCl. On the basis of the difference between this titration and the blank the ion-exchanger capacity was calculated.

Autohydrolysis

The samples of cross-linked sulfated polysaccharides were allowed to stand in distilled water for 24 hrs at room temperature, followed by filtration through fritted glass filter and dehydration with acetone. Then the loss of the weight and change in the capacity were determined.

Results and discussion

It is well known that air-dry samples of both starch and MCC contain about 6% humidity. Removal of this humidity either over P_2O_5 under reduced pressure or by drying at 105°C lead to the formation of new intermolecular H bonds and simultaneously to a reduction of the reactivity of polysaccharides. Therefore the influence of drying of polysaccharides upon their sulfation was investigated. It was found out that the sulfation was remarkably reduced when cross-linked polysaccharide, even if partly preswollen in DMF, was dried at 105°C or over P_2O_5 under reduced pressure for 72 hrs (Fig. 1, curve *1* and *2*). Cross-linked starch desiccated over P_2O_5 for 16 hrs (curve *3*) and an air-dry one (curve *4*) sulfated for one hour reached the capacity

values of about 3 mequiv./1 g and increased only to 4 mequiv./1 g when sulfated for next three hours.

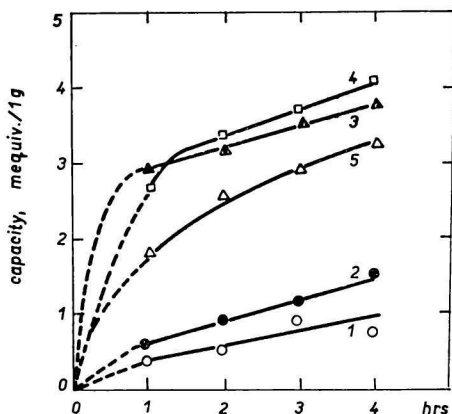Somewhat smaller values of capacity were reached at cross-linked MCC (Fig. 1, curve 5).

Fig. 1. Course of sulfation of cross-linked polysaccharides preswollen in DMF for 1 1/2 hr at molar ratio SO_3 : polysaccharide 5 : 1 at 20°C expressed as ion-exchanger capacity.

1. Starch dried at 105°C; *2.* starch desiccated over P_2O_5 for 72 hrs; *3.* starch desiccated over P_2O_5 for 16 hrs; *4.* air-dry starch; *5.* air-dry MCC.
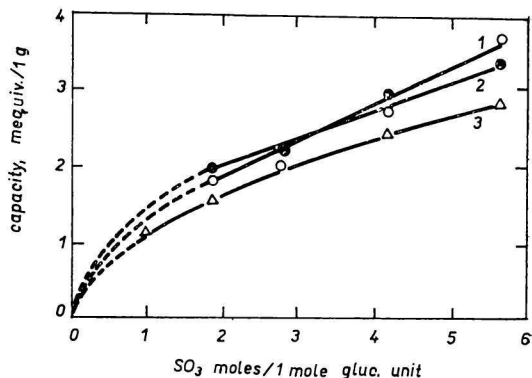
Fig. 2. Sulfation of cross-linked polysaccharides, expressed as ion-exchanger capacity, in dependence on molar ratio SO_3 : polysaccharide at 20°C.

1. Air-dry starch preswollen in DMF for 16 hrs; *2.* air-dry starch preswollen in DMF for 1 1/2 hr; *3.* air-dry MCC preswollen in DMF for 1 1/2 hr.

The presence of water in air-dry polysaccharide (as humidity) seems to hinder the formation of intermolecular bonds thus increasing the reactivity of polysaccharide. In this case the activation effect of DMF seems to be negligible. From Fig. 2 it is obvious that the effect of preswelling of air-dry starch as well as MCC in DMF for 1 1/2 and 16 hrs is negligible provided the samples contain a minor amount of water. However in the case of cross-linked polysaccharide desiccated over P_2O_5 under reduced pressure for 72 hrs the preswelling time (1 1/2 or 16 hrs) affected the sulfation remarkably (Fig. 3). At low SO_3 : polysaccharide molar ratios the sulfation rate of the 16 hrs preswollen cross-linked polysaccharide (Fig. 3, curve *1*) is markedly higher than that of polysaccharide preswollen for only 1 1/2 hr (Fig. 3, curve *2*) and of an air-dry sample (Fig. 2, curve *1* and *2*). It is also obvious that cross-linked polysaccharide desiccated over P_2O_5 and subsequently activated by preswelling in dry DMF attained nearly the same degree of sulfation even if the SO_3 : polysaccharide molar ratio was rised from 3 1 to 5 1. This indicates that by drying the range of the reactivity of polysaccharide has been reduced and DMF was not able to destroy the intermolecular H bonds formed during the drying.

Figs. 2 and 3 also manifest that an increase of molar ratio SO₃ polysaccharide bring about an increase of degree of sulfation which, however, never reaches the conformable value. Analogous conclusions were drawn at the sulfation of cross-linked starch at molar ratios 2.5 1 and 5 1 (Fig. 4). From Fig. 4 it is obvious that at molar ratio 5 1 the exchange capacity of 2.7 mequiv./1 g, corresponding to the degree of substitution 0.44, was attained after 1 hr reaction time, whereas at molar ratio 2.5 1 it was reached only after 3 hrs.
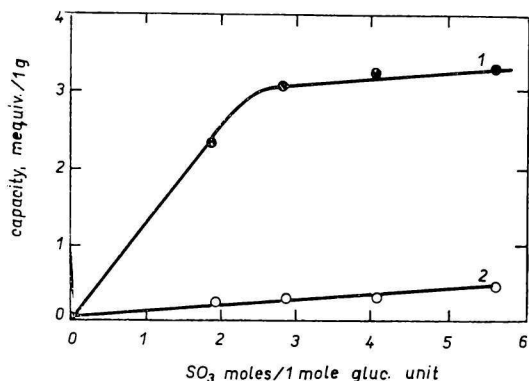

Fig. 3. Sulfation of cross-linked starch desiccated over P_2O_5 for 72 hrs, expressed as ion-exchanger capacity, in dependence on molar ratio SO_3 : polysaccharide at 20°C for 1 hr.
1. Starch preswollen in DMF for 16 hrs;
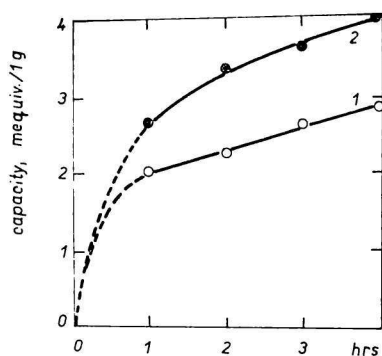*2.* starch preswollen in DMF for 1 1/2 hr.

Fig. 4. Course of sulfation of air-dry starch, preswollen in DMF for 1 1/2 hr at molar ratio SO_3 : polysaccharide at 20°C.
1. 2.5 1; *2.* 5.0 : 1.

Sulfated cross-linked starch is white and sulfated cross-linked MCC slightly yellowish. Samples of cross-linked polysaccharides with the degree of sulfation higher than 3 mequiv./1 g exhibited a beige colour. Flow rates of liquids through both the original cross-linked and sulfated polysaccharide columns were nearly the same.

Sodium salts of sulfated cross-linked polysaccharides are stable in the air atmosphere as well as in water. The changes may occure after a conversion into a H⁺ form. An autohydrolysis in distilled water at room temperature lasting for 24 hrs resulted in splitting of 1,4-glycosidic bonds of polysaccharides and consequently in a loss of weight amounting to about 15%. At the same time the colloid properties of exchangers changed considerably. The original granulated material changed into an amorphous messy substance which was difficult to filter. Ion exchanger on the basis of cross-linked starch changed already when exposed to air for 4—5 days. The weight losses are accompanied by a decrease of the degree of sulfation or of the ion--exchanger capacity. Sulfated cross-linked starch with a capacity of 3.1 mequiv./1 g, corresponding to the degree of sulfation 0.5 exhibited after autohydrolysis followed by centrifugation (it could not be filtered) and dehydration with acetone the exchange capacity of only 1.4 mequiv./1 g which corresponds to the degree of sulfation 0.22.

The presence of SO_4^{2-} ions and saccharide residues was proved in supernatant by means of barium chloride solution and phenol proof, respectively. *Juračka* [7] observed similar autohydrolysis phenomena with a cation exchanger on the basis of styrene containing sulfate groups after washing with hot distilled water.

Regarding the above-mentioned properties of sulfated ion exchangers on the basis of polysaccharides their application is rather limited. Considering the strong reactivity of sulfate group with proteins, the sulfated cross-linked polysaccharides may be used for a separation of proteins from solutions (*i.e.* milk) provided a conversion into a H form was performed immediately before being used. Furthermore, the sulfated cross-linked polysaccharides gelatinized when potassium, rubidium, or cesium ions were bound to their sulfate groups [4]. It may be also assumed that there are still possibilities of application of these sulfated polysaccharides.

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