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Crosslinking of polysaccharides with activated dimethylsulfoxide

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ABSTRACT

A simple method for the crosslinking of dextran, starch, and several other polysaccharides is described. The crosslinking of polysaccharides is performed with dimethylsulfoxide (DMSO) activated with organic or inorganic acid halogenides or phosphorus pentoxide. The crosslinking level increases with an increase in the acid chloride concentration, the temperature, and the reaction time. A possible crosslinking mechanism is proposed.

The use of natural or synthetic macromolecular matrices for the chromatographic separation of biological compounds in the aqueous phase is a technique developed about 50 years ago. The porosity of these supports, which are insoluble but swell in water, is utilized for the separation of molecules as a function of their molecular weight by simple elution from a column.[1] Porath and Flodin were the first to develop this principle, by using the natural polysaccharide dextran crosslinked by a bifunctional reagent such as epichlorohydrin in a basic medium.[2]

A variety of materials have been used to modulate drug delivery; in this respect polysaccharides and their derivatives represent a group of polymers commonly present in pharmaceutical formulations. Among these, starch and cellulose, with appropriate chemical or physical modifications, are the most frequently employed. Nevertheless, numerous other polysaccharides (alginates, carrageenans, gellan, etc.) have been used for the preparation of controlled release dosage forms.[3] Biocompatible three-dimensional porous scaffolds are also of significant interest for tissue engineering applications[4] as well as for enzymes and cell immobilization.[5,6]

Different chemical and physical methods are available for crosslinking polysaccharides. Crosslinking agents such as epichlorohydrin,[7] alkane dihalides,[3] poly(ethyleneglycol)-diamines,[8] dialdehydes, dihydrazides,[9,10] etc., lead to covalent chain crosslinking. Covalent crosslinking of polysaccharides can also be accomplished by radical polymerization with acrylate monomers in the presence of a crosslinking agent.[11,12] From the reactions of DMSO with polysaccharides, oxidation has been described,[13,14] where the activation of DMSO was carried out with electrophiles such as dicyclohexylcarbodiimide, acetic anhydride, phosphorus pentoxide,[15] or sulfur trioxide-pyridine.[13] Hirano et al.[16] have described the polysaccharide synthesis from mono- and oligosaccharides by the action of phosphorus pentoxide in DMSO. Herein, we report the crosslinking of dextran, starch, and several other polysaccharides with activated DMSO. A possible crosslinking mechanism is proposed.

Crosslinking of polysaccharides

The crosslinking of dextran, starch, or other polysaccharides is performed by the addition of an acid chloride or phosphorus pentoxide as a solution with the polysaccharide in DMSO, or by the addition of activated DMSO (obtained by the reaction of acid chlorides and DMSO) in a solution of the polysaccharide in DMSO (see Supplementary data and Tables 1 and 2). The gelation kinetics depended on the ratio of the reactants. The gelation time was between 2 and 45 min. A very vigorous crosslinking reaction (with a short gelation time) occurred using the acid chlorides: benzoyl chloride, acetyl chloride, thionyl chloride, phosphorus oxychloride, as well as phosphorus pentoxide. Moreover, the crosslinking of polysaccharides could be accomplished with the chlorides of aromatic acids containing electron-withdrawing groups (e.g., with 4-nitrobenzoyl chloride), but the vigor of the reaction was reduced

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Table 1
Relevant data on dextran crosslinking with DMSO activated with an acid chloride or acetic anhydride

Product code[a]	Dextran (g)	DMSO (ml)	Electrophile	$CaCO_3$ (g)	Method of synthesis	Heating		Standing at room temperature (h)	Appearance of the purified product	Yield [g (%)]
						(°C)	Time (h)			
NBC-2.20	5	70	7.9 ml of B[a]	9.6	**1a**, **6a**	—	—	20	Off-white particles	4.35 (87)
NSC-2.76	3	70	3.72 ml of S[a]	10.77	**1a**, **6a**	—	—	20	Off-white particles	2.49 (83)
NAcC-2.78	3	70	3.66 ml of Ac[a]	5.38	**1a**, **6a**	—	—	20	Off-white particles	2.52 (84)
NS-2.**7**8	7.5	100 + 40[b]	9.4 ml of S[a]	0	**1b**, **6b**	50–65	1.5	20	Snow-white particles	5.47 (73)
KS-2.40	5.0	40 + 20[b]	5.4 ml of S[a]	0	**1b**, **6b**	40	1	120	Off-white particles	3.90 (78)
KS-6.00	5.0	40 + 60[b]	13.5 ml of S[a]	0	**1b**, **6b**	50–65	1.5	20	Liquid white amorphous	2.70 (54)
KA-3.41	5.0	40	21.55 ml of A[a]	0	**2**, **7**	40	3	50	Water-soluble particles	3.95 (79)
N_C-0.0	3	70	None	2.0	**3**	—	—	20	No products	—
NChC-0.97	3	70	2.0 g of Ch[a]	2.0	**3**	—	—	20	No products	—

All experiments were performed in triplicate and averaged.
[a] The first letter in the product code indicates the type of dextran: N—native, K—clinical (Mr = 40000). The second letter(s) indicate the electrophile: A—acetic anhydride, Ac—acetyl chloride, B—benzoyl chloride, Ch—calcium chloride, and S—thionyl chloride. A dash indicates that no electrophile was used. The third letter indicates whether calcium carbonate (letter C) was used in the synthesis. The number in the product code indicates the ratio of moles of chloride (or anhydride) and glucosyl residues.
[b] The first and second numbers represent the volume of DMSO in which the dextran and the acid chloride were dissolved, respectively.

Table 2
Relevant data on starch crosslinking with DMSO activated with an acid chloride, phosphorus pentoxide, or acetic anhydride

Product code[a]	Starch (g)	DMSO (ml)	Electrophile	Method of synthesis	Heating		Standing at room temperature (h)	Appearance of the purified product	Yield [g (%)]
					(°C)	Time (h)			
StS-1.85	6	40 + 20	5 ml of S[a]	**1b**, **6b**	50	2	20	White particles	4.0 (67)
StS-1.48	6[b]	40 + 20	4 ml of S[a]	**1b**, **6b**	50	1	20	White particles	5.2 (87)
StS-4.00	5	50 + 20	9 ml of S[a]	**1b**, **6b**	45	1	40	White particles + orange liquid	3.72 (75)
StPc-1.74	6	40 + 20	6 ml of Pc[a]	**1b**, **6b**	40	2	20	White particles	3.02 (50)
StN-1.50	7	40 + 20	12 g of N[a]	**1b**, **8**	40	1	20	Yellowish particles	4.55 (65)
StA-3.43	5	40	10 ml of A[a]	**2**, **7**	40	2.5	20	White amorphous water-soluble particles	4.05 (81)
StA-6.43	4	30	15 ml of A[a]	**2**, **7**	40	2	40	Yellow water-soluble paste	14.9 (75)
StP-1.63	7	40 + 20	10 g of P[a]	**4a**, **6b**	50	3	20	Yellowish particles	3.2 (46)
StP-2.93	7	40	18 g of P[a]	**4b**, **6b**	70	3	20	White particles	5.2 (74)
StB-3.35	5	25[c]	12 ml of B[a]	**5**, **6c**	55	1.5	20	Off-white beads	4.2 (84)

All experiments were performed in triplicate and averaged.
[a] The first letter(s) in the product code indicates the polysaccharide used (St—starch). The second letter(s) indicate the electrophile: A—acetic anhydride, Ac—acetyl chloride, B—benzoyl chloride, N—4-nitrobenzoyl chloride, P—phosphorus pentoxide, Pc—phosphoryl chloride, and S—thionyl chloride. The number in the product code indicates the ratio of moles of chloride (or anhydride) and glucosyl residues.
[b] The reaction was performed with starch, which was dissolved in DMSO with 2 g of tris(hydroxymethyl)aminomethane.
[c] After dissolution of starch, 20 ml of *n*-hexane was added. With benzoyl chloride as the electrophile 15 ml of *n*-hexane was added.

(longer gelation time). After gel formation the mixing was stopped and the reaction mixture was allowed to stand for 1–3 h at a slightly increased temperature (up to a maximum of 70 °C), and for 20–120 h at room temperature. The acid formed during the crosslinking-reaction can be bound by the addition of pulverized calcium carbonate (e.g., a 5% molar excess with respect to the amount of acid chloride), but calcium carbonate is not necessary for the crosslinking process. After completion of the reaction, washing with ether, ethanol, and/or hot water was performed in order to remove the acid halide, DMSO, and other soluble components.

Besides the examples described in Tables 1 and 2, the crosslinking was also successfully performed with other polysaccharides: clinical dextranes T70 and T100, hydroxyethyl-cellulose, and polyvinyl alcohol. On the other hand, the crosslinking of the acidic polysaccharides, carboxymethyl-cellulose, and pectin was not successful under these conditions.

There was no reaction in the control synthesis (N_C-0.0 and NChC-0.97, see Table 1) with native dextran, DMSO, and calcium carbonate (with and without calcium chloride), but without addition of an electrophilic agent which can activate DMSO. This confirmed that activated DMSO was the active agent in the crosslinking reactions.

We found that the initial concentration of the polysaccharide was of significant importance for the crosslinking process and the properties of the final product. We used polysaccharide concentrations in the range of 5–70% of the reaction mixture and obtained successful crosslinking. Lower concentrations resulted in products with a lower degree of crosslinking (i.e., with higher swellability). An increase of swellability can be achieved by reduction of: (i) the molecular mass of the polysaccharide, (ii) the amount of acid halide in relation to the polysaccharide, and (iii) the temperature and/or duration of the reaction.

The crosslinking reaction can also be accomplished in a two-phase system (sample StB-3.35, Table 2). The size of the granules produced is mostly dependent on the stirring speed and less on the ratio of the reactants and solvents used (data not shown). The extent of crosslinking increases with an increase in the acid chloride concentration, the reaction temperature, the reaction time, and the stirring rate.

Properties of crosslinked products

All the crosslinked products were insoluble in water, DMSO, and other solvents (ethanol, acetone, diethyl ether, benzene, petroleum ether, *n*-hexane, etc.). The swellability in water was inversely proportional to the number of reacted hydroxyl groups of the polysaccharide (i.e., the crosslinking-level), while the level of resistance to the action of hydrolytic enzymes was directly proportional to the crosslinking-level (Tables 3 and 4).

Crosslinking of native dextran gave a product (sample NBC-2.20) with a swelling degree of 18 ml/g, which was slowly but incompletely hydrolyzed with dextranase (in contrast with soluble dextran which hydrolyzed quickly and completely). Based on the