

Versatile Synthesis of Polysaccharide Hydrogels Using the Passerini and Ugi Multicomponent Condensations

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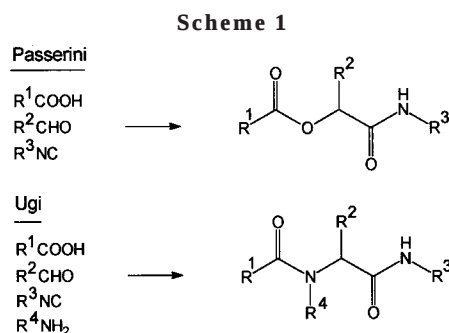
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Introduction. Synthetic hydrogels find widespread use in such diverse areas as prosthetic materials, contact lenses, and controlled drug release.¹ For many applications, it is desirable that the synthetic hydrogels be biodegradable.² From this point of view, polysaccharides are suitable starting substrates, also since they are renewable nontoxic materials, usually of low cost. Thus, over the past years we have been investigating methods for the synthesis of chemical hydrogels based on polysaccharides.³ In this communication we report on the use of the Passerini and Ugi multicomponent condensations⁴ for this purpose (Scheme 1). In the Passerini three-component condensation a carboxylic acid and an aldehyde or ketone are condensed with an isocyanide to yield an α -(acyloxy) amide. In the Ugi four-component condensation an amine is added to this reaction mixture to yield an imine, which, together with the carboxylic acid, condenses with the isocyanide to yield an α -(acylamino) amide. Since carboxylic, amine, or carbonyl groups are already present in many polysaccharides or can easily be introduced, it occurred to us that these reactions may be used as a general method for the synthesis of polysaccharide hydrogels when one of the components is a bifunctional linker. The Ugi reaction has been used previously in a similar manner for the immobilization of an enzyme in an alginate network.⁵

Experimental Section. Materials. (Carboxymethyl)cellulose (CMC) with a degree of substitution of ~ 0.75 and a viscosity of ~ 1500 cP (1% solution, 20 °C) was obtained from BDH Chemicals, Poole, England. Sodium hyaluronate was from Fidia SpA, Abano Terme, Italy; scleroglucan (Actigum CS 11) was from CECA, Carentan, France; pullulan was from Hayashibara, Okayama, Japan; and chitosan ($M_r \sim 70\,000$) and cyclohexyl isocyanide were from Fluka, Buchs, Switzerland. All other chemicals were commercially available products and were used without prior purification.

General Procedure for the Formation of Networks. To a clear viscous solution of the polysaccharide in water was added diluted HCl to obtain a pH of about 3.5–4. The other components were added at ambient temperature, with the bifunctional linker determining the theoretical cross-linking density and the other components in moderate excess. The solution was well stirred until it became too viscous to stir. In most cases this happened within 15–30 min after the last component, generally the isocyanide, was added. The gel was



left overnight and was subsequently dialyzed against doubly distilled water.

Network from Partially Oxidized Polysaccharides. The polysaccharide was partially oxidized by a selective TEMPO-mediated oxidation for primary alcohols according to a previously described procedure.⁶ The proportion of carboxylates of the products was 20% and the proportion of aldehydes was about 10%.⁷ After the oxidation, which was performed at pH 9.2 at a concentration that allows formation of a gel, the pH was brought to 3.5–4 by adding dilute HCl and isocyanide was added, after which a gel formed.

Chitosan-Containing Network. 1-(Deoxylactit-1-yl)chitosan⁸ was prepared by a reductive amination of chitosan in dilute HCl. To a solution of chitosan (1.0%, 100 mL) at pH 5 were added 3.5 g of lactose and 0.1 g of NaBH_3CN . After 4 days, when all NaBH_3CN had reacted, part of the reaction mixture was brought to pH 4 and the other components were added as above.

Results and Discussion. In Table 1 a summary of the results is given. Polysaccharides bearing carboxylic (entries 1–15), aldehyde (entries 14 and 15), and amine (entry 16) functions have been used. (Carboxymethyl)-cellulose (CMC) was chosen as a model polymer. Throughout this work we used cyclohexyl isocyanide as the isocyanide component for it is the most widely available one. This gives for the Ugi reaction amide linkages between the polysaccharide chains, whereas the Passerini reaction yields ester linkages. Since esters are much more easily hydrolyzed under alkaline conditions than amides, this is a useful distinction. For the Passerini reaction the dialdehyde glutaraldehyde was used (entries 1–6) and for the Ugi reaction 1,5-diaminopentane was used as the bifunctional agent (entries 10–12). In Chart 1 examples of possible cross-links are shown where, for the sake of clarity, only one function of the bifunctional reagent has been engaged.

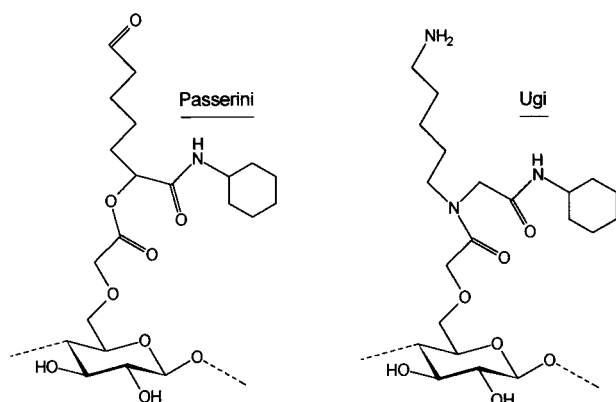
Both in the case of the Passerini reaction and in the case of the Ugi reaction, completely transparent hydrogels were obtained. It is noteworthy that when, instead of dilute HCl (see Experimental Section), phosphate buffers of varying pH were used, the gels were white instead of transparent. The reasons for this will be further investigated. To study the behavior of the different gels at alkaline pH, we immersed some swollen gels in a bicarbonate buffer at pH 9.5. The results are shown in Figure 1. Due to the fact that the gels are brought from distilled water in a salt solution, a rapid shrinking occurs since the charges are shielded by the salt. After this initial shrinking, the Passerini gel (entry 6) is quickly degraded by the alkaline solution. As

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Table 1. Synthesis and Partial Characterization of Various Polysaccharide Hydrogels

entry	carboxylic acid	aldehyde	amine	polysaccharide ^a (w/v)	cross-link ^b (%)	swelling ^c	Youngs modulus ^d (10 ⁻³ Pa)
1	CMC	glutaraldehyde		2	6	13.3	
2	CMC	glutaraldehyde		2	8	11.2	
3	CMC	glutaraldehyde		2	10	9.5	
4	CMC	glutaraldehyde		2	15	4.1	30
5	CMC	glutaraldehyde		3	5	10.3	21
6	CMC	glutaraldehyde		3	8	4.3	30
7	CMC	glutaraldehyde	NH ₄ Cl	3	8	4.9	28
8	CMC	glutaraldehyde	NH ₄ Cl, excess	3	8	4.4	32
9	CMC	glutaraldehyde		3	10	3.4	41
10	CMC	formaldehyde	1,5-diaminopentane	3	5	4.7	
11	CMC	formaldehyde	1,5-diaminopentane	3	7.5	3.4	
12	CMC	formaldehyde	1,5-diaminopentane	3	10	2.4	
13	hyaluronic acid	glutaraldehyde		6	10	3.0	73
14	pos ^e	pos		0.8	~10	1.2	94
15	pop ^f	pop		3.5	~10	4.6	
16	L-tartaric acid	formaldehyde	1-(deoxylactit-1-yl)chitosan	~3	10	5.5	

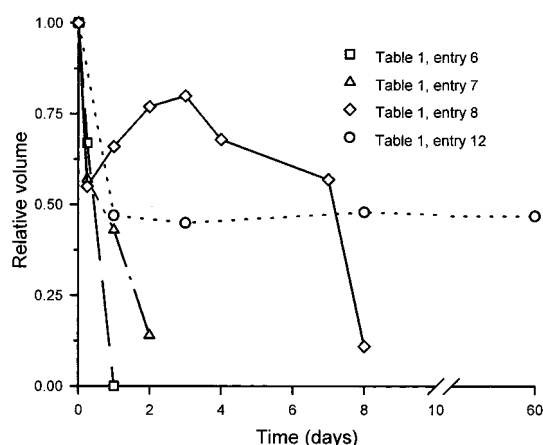
^a Initial concentration of polysaccharide. ^b Theoretical proportion of cross-links. ^c Swelling of the network as given by the weight after 10 days in water divided by the initial weight of the network at 25 °C. ^d Youngs modulus at 25 °C determined according to ref 9. ^e Pos, partially oxidized scleroglucan. ^f Pop, partially oxidized pullulan.

Chart 1

expected, the Ugi gel (entry 12) is completely stable under those conditions. It was tried to shift from a Passerini reaction to a Ugi reaction by simply adding NH₄Cl to the reaction mixture. The gel (entry 7) synthesized with a moderate excess of amine (50 mol % amine with respect to the monomeric units of the polymer) degrades rapidly. Only when a large excess of amine is used (entry 8, 500 mol % amine) is a more resistant gel obtained. After initial shrinkage, the latter gel swells due to slow hydrolysis until this gel also completely dissolves, indicating that it consists partly of amide and partly of ester linkages. We did not succeed in synthesizing hydrogels that were completely resistant to alkali this way by further changing the reaction conditions.

In Table 1 also the influence of different initial concentrations of CMC and bifunctional linker is shown. The measured swelling behavior and the compression modulus are in qualitative agreement with the expected behavior; that is, gels formed at higher polymer concentration with an equal degree of cross-linking yield more rigid gels that take up less water (compare entries 2 and 3 with entries 6 and 9, respectively). The same is true for gels formed at equal polymer concentration but with a higher degree of cross-linking, as is seen from entries 1–4, 5, 6, and 9 for Passerini gels and entries 10–12 for Ugi gels.

Polysaccharides containing aldehyde groups were obtained after partial oxidation of the primary alcohol groups of pullulan and scleroglucan with hypohalite

**Figure 1.** Behavior of several water-swollen hydrogels in 0.1 M bicarbonate buffer (pH 9.5) at ambient temperature.

mediated by TEMPO.^{6,7} Since after this partial oxidation two of the components are present in the polysaccharide chain, a bifunctional linker is not necessary and a gel was obtained by adding only the isocyanide. The scleroglucan gel (entry 14) was much more rigid than the pullulan gel (entry 15) despite its lower concentration of polysaccharide. This is probably due to the fact that scleroglucan is an exceptionally stiff polymer in the triple helix form,¹⁰ whereas pullulan is a very flexible one.¹¹ One may note that the oxidation of scleroglucan up to a low degree of oxidation probably does not rupture the triple helix structure since it forms a rigid gel at an initial concentration of less than 1%.

Chitosan is an amine-containing polysaccharide that is generally dissolved in dilute acetic acid. Thus we expected that two of the four components would already be present in such a solution. However, upon addition of glutaraldehyde and cyclohexyl isocyanide, an intensely yellow gel was formed. Upon addition of only glutaraldehyde, a less rigid but also yellow gel was formed. It was thought that this color was due to imine formation before the Ugi reaction could take place. Therefore we changed the cross-linking agent to a dicarboxylic acid, tartaric acid or adipic acid, with formaldehyde as the aldehyde component. Indeed, color formation was not observed in this case, but the gel was only scarcely swellable and precipitated during dialysis. Therefore we used a more soluble derivative of chitosan,

partially substituted 1-(lactit-1-yl)lactose. In this case we got transparent stable hydrogels with tartaric (entry 16) as well as with adipic acid.

In conclusion, a mild method has been presented that is very simple to perform and allows the rapid formation of chemical hydrogels from a variety of polysaccharides. The reactions described should, in principle, also be applicable to functionalized synthetic polymers. Furthermore, the reactions are regioselective and properties of the hydrogels may be tuned by changing the components and their relative proportions.

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