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Oxidation *vs.* degradation in polysaccharides: Pullulan – A case study

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ABSTRACT

Several oxidation protocols using TEMPO applied on various polysaccharides to convert the primary OH groups into carboxylic ones are reported, without taking into account the potential side reactions (mainly depolymerization) likely to occur when longer reaction times are employed. In this paper, water-soluble pullulan has been used as a model polysaccharide to study the influence of the reaction time on the macromolecular chain degradation during the TEMPO-oxidation. The oxidation reaction was performed at room temperature, pH = 10.5, using water as solvent, in the presence of sodium hypochlorite, sodium bromide and TEMPO. Ten samples of oxidized pullulan were collected, at different reaction times. The high amount of the sodium hypochlorite, ensures a fast conversion of all the accessible C6-OH to carboxylate groups, even at very first collected sample, according with ^{13}C NMR spectra. All the isolated samples were purified by diafiltration through a Millipore ultrafiltration membrane, recovered by freeze-drying and analyzed by FTIR, ^{13}C NMR, gel permeation chromatography, and zeta potential measurements. The intrinsic viscosity of the native and oxidized pullulan samples was determined in aqueous solution by using a new model proposed by Wolf. The Mark-Houwink relationship was established for fully oxidized pullulan samples: $[\eta] = 1.472 \times 10^{-5} M_w^{0.8475}$ (dL/g), the exponent showing very good polymer-solvent interactions. The stability of the polysaccharide backbone was discussed by means of viscosity and molecular weight evolution during oxidation reaction. The present data show a strong effect of the reaction time on the molecular weight and viscosity of the oxidized products.

1. Introduction

The oxidation reaction applied to polysaccharides has become one the most important routes both in lab and industrial scale, applied to convert neutral macromolecules into polyelectrolytes, which represent valuable products for a wide range of applications. This reaction has been studied for more than seventy years, since the pioneering work of Yackel and Kenyon [1]. A revolutionary step in this field was done by de Nooy et al. [2–4], by introducing the stable 2,2,6,6-tetramethylpiperidine-1-oxyl-radical (TEMPO), as mediator for the selective oxidation of the primary hydroxyl groups in natural polysaccharides in the presence of sodium hypochlorite and sodium bromide. The advantages of this method over those using nitrite/

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nitrate in concentrated phosphoric acid [1,5], rely on the higher selectivity, shorter reaction time, better-controlled reaction, and milder reaction conditions. This method was initially applied to water-soluble polysaccharides [2–4,6,7], such as starch, pullulan, inulin, alternan, chitosan, amylodextrin, amylopectin and galactomannan. The success of using TEMPO-NaCl-NaBr oxidation to water-soluble polysaccharides was further extended to water-insoluble ones, such as cellulose, amylose and chitin [8–11]. Fig. 1 shows the general representation of the TEMPO mediated oxidation in polysaccharides.

It is generally accepted that the conversion of the primary OH groups of polysaccharides during TEMPO mediated oxidation proceeds via aldehyde groups, the final products being represented in the large majority only by the carboxylic ones. Despite of the many advantages conferred by the TEMPO-mediated protocol for polysaccharides oxidation, there are serious issues linked with the severe degradation of the oxidized products. To have a better insight of this sensitive subject, we gathered some examples from literature presenting the degree of polymerization of the starting and oxidized polysaccharides (Table 1).

As it can be observed from Table 1, severe degradation phenomena are accompanying TEMPO-oxidation. For example, cellulose, as the most studied polysaccharide, shows a wide range of data, depending on its source, type, origin, and crystallinity. In relation with these factors, the degree of polymerization of the oxidized celluloses drop sharply for the native or regenerate cellulose, and less pronounced for the nanocellulose. The same behavior is observed also for other polysaccharides: curdlan and paramylon. It can be pointed out that the DP of the oxidized curdlan is less than 2% as regard with the starting material! For other two polysaccharides included in Table 1, the only two water-soluble, xanthan and gellan, the drop of the DP after TEMPO-oxidation seems to be as pronounced as in the case of those water-insoluble, since for example, in the case of gellan, the oxidized sample has a DP which is only 4% from the unoxidized sample.

Several factors influencing the macromolecular chain degradation during oxidation are known, such as: the nature and the source of the polysaccharide, reaction time, degree of oxidation (amount of the aldehyde/carboxylic groups introduced), reaction temperature, the crystallinity of the starting material, and pH-value of the reaction media. In principal the chain breaking of the polysaccharides during TEMPO-mediated oxidation can be attributed to β-elimination caused by the alkaline pH (10) of the reaction medium and/or the presence of the aldehyde groups formed at early stages. It has also been reported that carboxylic groups on the cellulose chains serve as catalytically active sites for the scission of the neighboring β-1,4-glycosidic bonds [14,21]. In a recent paper, Carlsson et al. [15], performed a control experiment to ensure that the degradation of cellulose using TEMPO-mediated protocol is mainly caused by the oxidation reaction and not by the β-elimination. In this manner, the possibility of the β-scissions of the D-glucose units was ruled out. After this experiment the above mentioned authors, stated that: "*the depolymerization indeed took place during the TEMPO-mediated oxidation*" [15]. Other very recent works showed that carboxyl groups formed during oxidation have activation and inductive effects on the cleavage of the 1,4-glycosidic bonds inside the cellulose chains [22]. Confused by the abundance of the controversy data regarding the degradation of the polysaccharide chain during TEMPO-mediated oxidation, we performed a careful investigation of the oxidation kinetics by characterizing the sample intrinsic viscosity at different reaction times. Due to the continuous

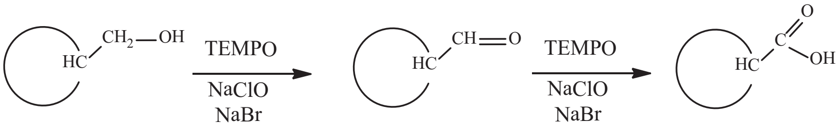

Fig. 1. Schematic presentation of the TEMPO mediated oxidation of polysaccharides.

Table 1
Degree of polymerization (DP) of the original (unoxidized) and TEMPO-mediated oxidized polysaccharides samples.

Polysaccharide	DP of the original (unoxidized) sample	DP of the oxidized sample	DP percent of the oxidized product as compared with the starting sample (%)	Refs.
Cellulose Avicel	170	50–85	29–50	[12]
Cellulose cotton linters	850	80–120	9–14	[12]
Cellulose bacterial	1450	55–130	4–9	[12]
Cellulose primary wall	980	110–130	11–13	[12]
Cellulose Borregaard	450	80–105	18–23	[12]
Cellulose rayon	360	65–100	18–28	[12]
Cellulose regenerated	680	39	6	[13]
Cellulose native	1270	38	3	[13]
α-Cellulose	1177	117	10	[14]
Cladophora nanocellulose	740	570	77	[15]
Curdlan	6790	86	1.3	[16,17]
Paramylon	1670	68	4	[17]
Chitosan oligomer	22	17	77	[18]
Xanthan	2136	665	31	[19]
Gellan	3084	112	4	[20]