Iranian Polymer Journal
13 (3), 2004, 235-240

On Enzymatic Degradation of Cellulose Acetate

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Received 2 August 2003; accepted 24 December 2003

ABSTRACT

Cellulose acetates (CA) with different degrees of substitution (DS) and different distributions of substituents within the anhydroglucose unit (AGU) have been synthesized in conventional (acetic acid/water) and unconventional (toluene or benzene/acetic acid/water) media and their susceptibility to enzymatic attack has been investigated using a purified cellulase from Trichoderma reesei. The degradation experiments have been carried out in heterogeneous (water suspension) or homogeneous (water solution) conditions, dependening on the DS of the derivatives. The behaviour of CAs to enzymatic attack has been correlated with their structural characteristics which are dependent on the procedure used for their synthesis. CA Samples with DS value above 1.3 have been treated with enzyme in water suspension for 14 days. It has been observed that the decrease of the reduced viscosities of these acetates solutions in adequate solvents after the enzymatic treatment depends on the procedure and conditions employed for their synthesis. Gel permeation chromatograms show some associations of macromolecules for polymers with DS higher than 1.3. These fractions of apparent high degree of polymerization seem to be preferentially degraded by the enzyme. Water soluble cellulose acetates undergo similar degradation, irrespective of the reaction medium used for their synthesis.

Key Words:
cellulose acetate;
enzymatic degradation; viscosity;
degree of substitution;
synthesis.

INTRODUCTION

The importance of cellulose as the principal renewable resource is indisputable at present. It is considered that cellulosic materials, including also modified cellulosics, represent about half of the polymers consumed today in the world [1].

One of the problems, which focused the interest of the researchers in this field during the last decades is the biodegradability of cellulose and its derivatives, especially of water soluble derivatives. Some information has been obtained regarding the

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Table 1. The synthetic conditions and the characteristics of cellulose acetates.

Sample	Synthesis conditions				DS	DS_6/DS	DS_2/DS	DS_3/DS	η_{red1}	η_{red2}	$(\eta_{red\ 1} - \eta_{red\ 2}) / \eta_{red\ 1}$
	t (h)	toluene/ benzene (%)	water (%)	acetic acid (%)							
CA	24	0	10	90	2.50	-	-	-	1.233	1.094	0.113
TAC12/1	8	20	13	67	2.45	0.29	0.38	0.32	2.181	1.985	0.090
BAC1/1	8	(20)	8	72	2.38	0.30	0.35	0.35	1.880	1.634	0.131
TAC9/2	16	20	8	72	2.06	0.33	0.35	0.31	1.713	1.509	0.119
TAC12/3	24	20	13	67	2.02	0.30	0.38	0.32	1.562	1.411	0.097
AC/4	32	0	8	92	2.00	0.38	0.34	0.28	1.044	0.932	0.107
TAC12/4	32	20	13	67	1.77	0.38	0.35	0.27	1.252	1.158	0.075
BAC1/4	32	(20)	8	72	1.69	0.39	0.34	0.27	0.892	0.626	0.298
TAC14/3	24	34	8	58	1.65	0.42	0.35	0.23	0.360	0.261	0.275
TAC14/4	32	34	8	58	1.34	0.46	0.32	0.22	0.214	0.164	0.234

influence of the substituents and the degree of substitution (DS) on the enzymatic hydrolysis of cellulose derivatives [2 - 5], and more recently, the role of the substituent distribution within the anhydroglucose unit as well as along the cellulose chain has been discussed [6 - 8].

With respect to cellulose acetate, the most widely used ester of cellulose, most of the work published so far was performed with water soluble polymer and it is considered that this derivative is biodegradable up to degree of substitution (DS) = 1 [9]. However, recent papers found that cellulose acetates with DS values between 1.7 and 2.5 can be biodegraded by mixed culture systems [10].

This paper deals with a study concerning the susceptibility of cellulose acetates (CA) with different degrees of substitution to enzymatic attack in homogeneous and heterogeneous systems. The change of the viscosity of polymer solutions in adequate solvents is discussed and the molecular weight distribution, as revealed by gel permeation chromatography (GPC), is comparatively analyzed for CA samples obtained in different reaction systems.

EXPERIMENTAL

Cellulose Acetate Synthesis

The synthesis of cellulose acetates with different degrees of substitution was performed by the hydrolysis of high-acetyl cellulose acetate in aqueous acetic acid solution [11], as well as in the unconventional hydrolysis systems containing toluene or benzene, previously reported by the authors [12, 13].

Cellulose (cotton linters) was activated and acetylated with acetic anhydride in acetic acid medium, following the known procedure [14]. The highly substituted product obtained was first pre-hydrolyzed, without separation, in 90% aqueous acetic acid at 40°C for 10 h, then hydrolyzed at 60°C in different media containing acetic acid, water and toluene (or benzene), according to the procedure described in our previous papers [12,13]. The experimental conditions employed and the characteristics of the hydrolyzed cellulose acetates are presented in Table 1.

The water soluble cellulose acetates were obtained starting from a sample with DS = 2.40, which was hydrolyzed in different systems.

Characterization of Cellulose Acetate Samples

The degree of substitution of cellulose acetates was determined by the usual alkali saponification method [15].

The distribution of acetyl groups within the anhydroglucose unit was evaluated by ^1H NMR spectroscopy, using a JEOL spectrometer operating at 60 MHz and at ambient probe temperature. From

^{1}H NMR spectra, the distribution of the substituents at C_6, C_2 and C_3 positions was calculated by the method of Goodlett et al. [16] and expressed as fractions of total DS (DS_6/DS, DS_2/DS and DS_3/DS, respectively) (Table 1).

Enzymatic Treatment

A purified cellulase produced by Trichoderma reesei was used for enzymatic treatment of cellulose acetates.

The samples with DS ranging from 1.34 to 2.50 were suspended in aqueous solution of sodium acetate 0.05 M (0.5 g acetate to 100 mL solution) and 0.5 mL of the enzyme solution (6 g/100 mL) were added. This corresponds to a concentration of 30 mg enzyme to 100 mL of polymer solution. The treatment was allowed for 14 days at 25°C, then the suspensions were heated at 100°C for 10 min in order to destroy the enzyme and the samples were removed and purified by several washings with distilled water.

The enzymatic hydrolysis of water soluble cellulose acetates was carried out in homogeneous systems (0.5 g polymer dissolved in 100 mL sodium acetate solution) at 25°C for different periods of time (pH = 5), using the same solution of cellulase.

Viscometric Determinations

Cellulose acetates with DS = 1.34-2.50 were dissolved in adequate solvents, namely dimethylsulphoxide, for samples BAC 1/4 and TAC 14/4, and glacial acetic acid, for the others (Table 1) at a concentration of 0.0952 g/100 mL. The reduced viscosities of these solutions were measured at 25°C using an Ubbelohde viscometer. The measurements were performed for the initial acetates (η_{red1}), and after the enzymatic treatment (η_{red2}), of the samples.

For cellulose acetates hydrolyzed in solution, the specific viscosities of the solutions after the treatment were determined in the same conditions.

GPC Determinations

Samples hydrolyzed in aqueous suspensions, after purification were dissolved in dimethylformamide (0.004 g / 2 mL solvent) and injected into a GPC apparatus consisting of a column PLgel 5 m Mixed-C, at a flow rate of 0.7 mL/min.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis of Cellulose Acetates with DS = 1.34-2.50

The synthetic conditions and the characteristics of cellulose acetates are presented in Table 1. The reduced viscosities of polymers solutions before and after the enzymatic treatments are denoted as η_{red1} and η_{red2}, respectively.

The reduced viscosities decrease after enzymatic treatment for all the samples and the decrease is more pronounced for acetates synthesized in benzene-acetic acid-water system (sample BAC 1/4, DS = 1.69) and in toluene-acetic acid-water systems with higher amounts of toluene in the mixture (samples TAC 14/3, DS = 1.65, and TAC 14/4, DS = 1.34).

It can also be seen that, though there is no rigorous correlation between the relative decrease of the reduced viscosity (the ratio $(\eta_{red1} - \eta_{red2})/\eta_{red1}$, Table 1) and the degree of substitution, this decrease is generally more pronounced for DS values lower than 2.0 (Figure 1). This behaviour may be explained by the presence of more unsubstituted anhydroglucose units at DS < 2.0 which favour the formation of the complex enzyme-substrate. An exception is the sample TAC 12/4, synthesized with higher amount of water in the hydrolysis medium, which seems to favour a more uniform distribution of acetyl groups along the chains.

The data in Table 1 also indicate that no direct cor-

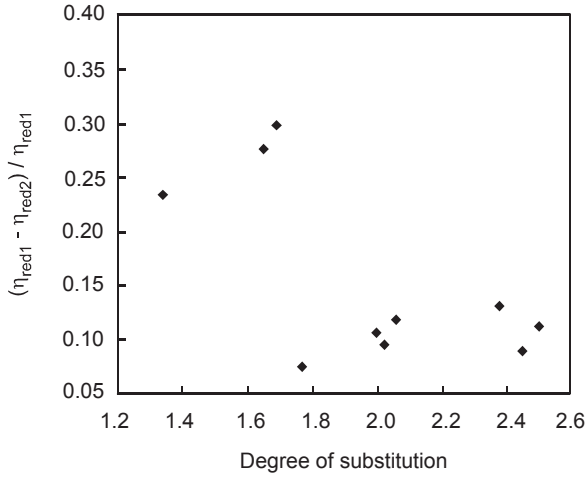

Figure 1. The relative decrease of reduced viscosity of cellulose acetates after enzymatic treatment in heterogeneous medium, as a function of DS.

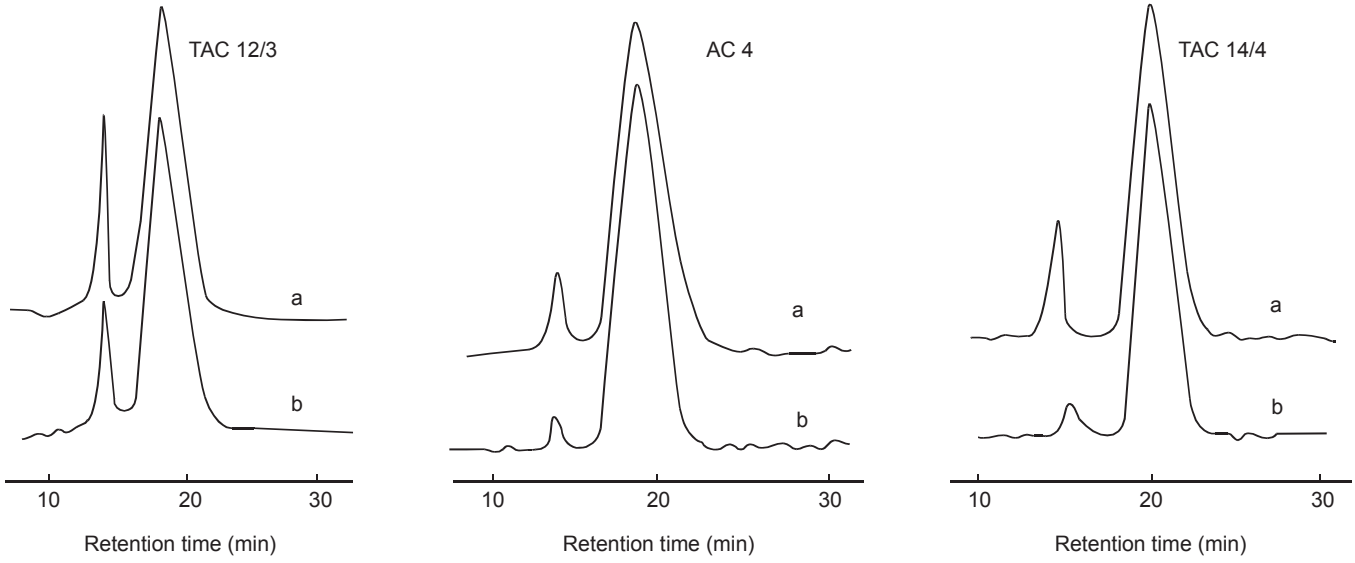

Figure 2. Gel permeation chromatograms of the samples TAC 12/3, AC 4 and TAC 14/4, before (a) and after (b) the enzymatic treatment.

relation can be established between the decrease of viscosity and the position of the acetyl groups within the anhydroglucose unit. However, the scattering of the experimental data plotted in Figure 1 suggests that the uniformity of substitution along the cellulose chain plays an important part in these heterogeneous degradation processes.

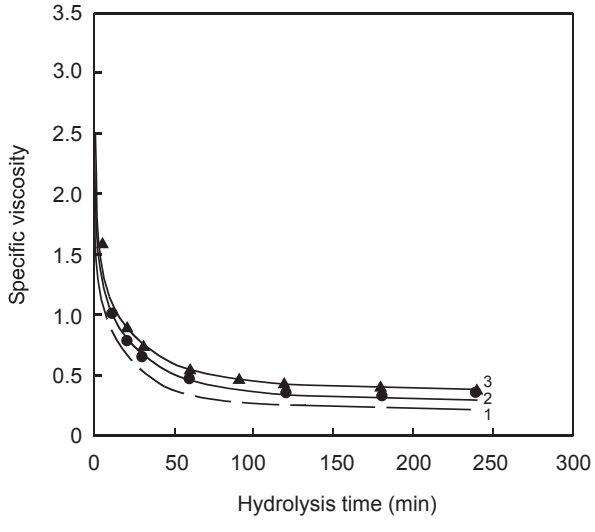

Figure 3. Enzymatic hydrolysis of water soluble cellulose acetates synthesized in different media: (1) benzene-acetic acid-water, DS = 0.47; (2) toluene-acetic acid-water, DS = 0.64; (3) acetic acid-water, DS = 0.80.

Distribution of the Molecular Weight for the Hydrolyzed Cellulose Acetates with DS = 1.34 - 2.50

Figure 2 presents the gel permeation chromatograms of some cellulose acetates before and after the enzymatic treatment.

The presence of a peak at lower retention time, corresponding to an apparent high molecular weight fraction of polymer, can be observed for all the samples investigated. The surface of this peak decreases after the enzymatic treatment. This apparent high molecular weight can be due to some associations of macromolecules by hydrogen bonds at OH groups of unsubstituted sequences of monomer units, which are preferentially attacked by the enzyme. The chromatograms suggest a correlation between the surface of these peaks and the degree of substitution at the primary carbon atom, C_6. Thus, the sample TAC 12/3 (having the most free OH groups at the primary position) exhibits the most pronounced association of macromolecules.

These associations also attest the uneven substitution along the polymer chain, so we may conclude that the more uneven samples are more susceptible to enzymatic attack in a heterogeneous medium.

Enzymatic Hydrolysis of Water Soluble Cellulose Acetates

Water soluble cellulose acetates could be obtained in

Table 2. The synthesis conditions and the characteristics of water soluble cellulose acetates (reaction temperature: 40°C).

Sample	The initial composition of the hydrolysis system (%)				Total reaction time (h)	Degree of substitution	Specific viscosity*
	benzene	toluene	acetic acid	water			
1	35	0	57	8	40	0.47	2.313
2	0	20	72	8	48	0.64	2.327
3	0	0	75	25	60	0.80	3.204

(*) represents the specific viscosity of aqueous solution of concentration 1.8 g / 100 mL.

unconventional hydrolysis media containing toluene or benzene in a shorter reaction time than that required in the known system acetic acid-water and without further addition of water during the process.

Table 2 summarizes the synthesis conditions and the characteristics of water soluble cellulose acetates obtained in three reaction media. The starting material was a cellulose acetate with DS = 2.40.

For these low DS values, the degradation of cellulosic chains during the hydrolysis process is more pronounced in systems containing toluene or benzene (sample 3 has higher specific viscosity).

Similar behaviour to enzymatic attack in aqueous solutions is observed for these three cellulose acetate samples with low DS values. After 4 h of enzymatic treatment, the major part of the polymers has been degraded. Though the initial specific viscosity of sample 3 (synthesized in system free of hydrocarbon) is higher (Table 2), after 4 h of enzymatic hydrolysis these values are close enough and the degradation curves are identical (Figure 3). This fact proves that cellulose acetate samples obtained in different systems do not differentiate from the point of view of their susceptibility to enzymatic attack in aqueous solutions.

As for the case of carboxymethylcellulose (which is an anionic polymer) [17], a first rapid hydrolysis step is observed, with a high rate of splitting of the glucosidic linkages, followed by a much slower degradation process.

CONCLUSION

- Cellulose acetates with DS between 1.34 and 2.50 are susceptible to enzymatic attack in a heterogeneous medium (aqueous suspension) in dependence, especially, of their degree of substitution. The presence of the organic solvent in the reaction medium does not influence significantly the susceptibility of acetates to enzymatic attack.

- For DS lower than 2, higher proportions of water in the reaction system used for acetate synthesis, seem to increase the resistance of polymers to enzymatic degradation.

- Water soluble cellulose acetates obtained in different media in the same conditions show similar behaviour to enzymatic attack in homogeneous medium (aqueous solution): a pronounced decrease of the specific viscosity of their solutions has been observed during the first 4 h of enzymatic treatment.

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