The Acetylation and Enzymatic Degradation of Starch Films

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SYNOPSIS

The preparation of films from starch and their degradation by amylase enzymes is described. Starch acetate was prepared by acetylation of starch with a pyridine/acetic anhydride mixture. The resulting polymer was cast into films from solutions of 90% formic acid. A series of films with a range of acetyl content were then exposed to buffered amylase solutions and the retained tensile strength measured. It was found that with a sufficient acetyl content the wet strength of the films was maintained in the aqueous solutions, but that the acetyl content was sufficiently low enough to permit degradation by a mixture of alpha and beta amylases within a period of 1 h. These films could be useful as membranes in bioreactors, which could be degraded at will by the addition of enzymes to the system. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Considerable effort has been devoted to the development of durable membranes. However, it may be advantageous to have a film or membrane that after having served its function of providing structural support or a separation can be conveniently and rapidly eliminated or substantially degraded. A use for such a membrane would be to facilitate the easy recovery of intact cells from a bioreactor.

Amylose and amylose triacetate are well known to be film formers. In fact, films of linear amylose acetate were reported to be comparable to those of cellulose triacetate.[1,2] More recently, the preparation of controlled cellulose/starch graft copolymers, which function as biodegradable thermoplastics, have been reported.[3,4] The objective of the work described in this article is to determine to what degree acetylated starch, in film form, could be enzymatically degraded. The hydrophobic character imparted by acetylation would allow the amylose to have an improved wet strength in an aqueous environment. However, upon exposure to amylase and the resultant hydrolysis of the polymer, a severely weakened film would be obtained that could easily be ruptured and washed away. A starch with a high content of linear amylose (70%) was selected as a practical material to study.

The acetylated starch would have several advantages as a structural fiber or film-forming polymer compared to native starch. The acetylation of starch is a well-known reaction and is a relatively easy derivative to synthesize.[5] As noted, starch acetate is considerably more hydrophobic than is starch and has been shown to have better tensile property retention in an aqueous environment. Another advantage is that starch acetate has an improved solubility compared to starch and is easily cast into films from simple solvents. The degree of acetylation is easily controlled by transesterification, allowing polymers to be produced with a range of hydrophobicity.

The results of this work indicate that it is possible to make films of starch acetate that are sufficiently hydrophobic to maintain their tensile strength in water but still be susceptible to enzymatic attach by amylases. We found that retained tensile strength after exposure to enzyme solutions was as low as 30% after 0.5 h of exposure to enzyme. The next step in this work is to determine optimum conditions for producing semipermeable membranes from these polymers.

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EXPERIMENTAL

Starch

The starch employed for this study was Amaizo I0535-107, obtained from Amaizo Corp. It is reported to be 70% w/w linear amylose and 30% w/w amylopectin, derived from a special hybrid corn.

Preparation of Starch Acetate

Following the method of Whistler,[6] 50 g of starch was added to 400 mL of pyridine and heated to 95°C for 2 h under N_2. Then, 165 mL of acetic anhydride was added dropwise, the temperature was lowered to 75°C, and the reaction continued for 22 h. The suspended and dissolved polymer was coagulated by adding 2 L of isopropanol with stirring. The white fibrillar mass that separated from the solution was filtered off and collected. This filtrate was washed thoroughly with methanol. The polymer was dried at room temperature under vacuum for 24 h. The reaction yielded 62.7 g of polymer with a percent acetyl value of 36.4.

Acetyl Content

A sample (1 g) of starch acetate was added to 30 mL of 0.4*N* sodium hydroxide to saponify the ester. The flask was kept under N_2 gas for 24 h to limit absorption of carbon dioxide from the atmosphere. The excess base was then determined by titration with 1*N* HCl and phenolphthalein indicator. To improve determination of the end point, an excess of 1*N* HCl was used and back-titrated with 0.1*N* NaOH. The following equation was used to determine the acetyl content (%):

$$\% \text{ Acetyl} = [(\text{mL of } 0.4N \text{ NaOH}) - (\text{mL of } 1.0N \text{ HCl}) + (\text{mL of } 0.1N \text{ NaOH})] \times (43 \text{ g/mol acetyl})(100)]$$

Preparation of Starch Acetate Films

Formic acid (90%) was chosen as a volatile solvent for the starch acetate. A casting solution was prepared by making up a 2% w/v solution that was kept in a refrigerator for 2 days in order to obtain a clear viscous solution. Films were cast onto a sheet of glass using a doctor knife. The gap between the doctor knife and the glass surface was 0.20 in. By washing the glass prior to casting, first with 50% aq NaOH and then with water, the films released more easily. The films turned opaque white upon air-drying. The films were washed in a methanol bath to rinse any last traces of formic acid and then allowed to air-dry.

Transesterification of the Starch Acetate Films

The films were transesterified in a 0.025*M* solution of sodium methoxide dissolved in methanol. The acetate films, approximately 50 by 200 mm, were placed in sealed jars containing the sodium methoxide solution, purged with N_2, and sealed. At the designated times, a piece of film was removed from the solution, rinsed with methanol, and air-dried.

Preparation and Use of Enzyme Solutions

Potassium phosphate buffer solution was chosen to control the pH of the enzyme solution. The amylase used was obtained from Sigma Chemical Co., Type VIII-A, from barley malt, and is a mixture of alpha and beta amylases. To make up the amylase solutions, 1 g of Type VIII-A amylase (equivalent to 2500 units of alpha amylase) was added to 10 mL of pH 6.9 phosphate buffer and then diluted to 50 mL with distilled water.

The larger film samples that had been transesterified were cut into pieces 25 by 50 mm. Each of these film samples were then cut lengthwise to give two pieces 12 by 50 mm. One piece was introduced into the enzyme solution and the other into the blank buffer solution at ambient temperatures. After removal from the buffer or enzyme solution, the films were washed first in water, then in methanol, and finally allowed to air-dry.

Tensile Testing of Samples

An Instron Tester Model 1123 was used to determine film-breaking strength. The film thickness and width were measured with a micrometer. Those films that failed by tearing slowly from an edge defect or those that broke at the jaw were discarded.

RESULTS AND DISCUSSION

Starch Acetate Polymer and Film

The preparation of starch acetate was tried at different temperatures and time intervals, on 1 and 2 g samples, according to the heterogeneous procedure of Whistler.[6] As a result, the best product, judged on the basis of sample color and the percent acetyl

content (% acetyl), occurred with a long acetylation time (22 h) at a mild temperature (75°C).

Following casting and air-drying, the films were transesterified to a lower degree of substitution to give a range of values. The transesterification reaction between sodium methoxide in methanol and starch acetate was used to obtain films with a lower content of ester groups than is obtained by direct esterification. The effect of the sodium methoxide on the percent acetyl content of the starch acetate film over time is shown in Figure 1. This reagent also has the advantage that it does not swell the film the way an aqueous sodium hydroxide solution would if saponification were to be employed to reduce the percent acetyl content.

The tensile properties of the control films, along with those films exposed to enzyme, are presented in Table I. A plot of the tensile strength of the control films as a function of transesterification time is shown Figure 2. These films were also exposed to the blank buffer solutions. Of concern was whether the exposure of the films to sodium methoxide solutions and the blank buffer solutions leads to substantial degradation of the tensile properties. The data of Figure 2 indicate that there is not a strength loss with transesterification. In fact, there may be a trend for the films to increase in tensile strength with deacetylation via transesterification. This is not surprising as the transesterification process in methanol may enhance recrystallization of the amylose and lead to an increase in tensile strength.

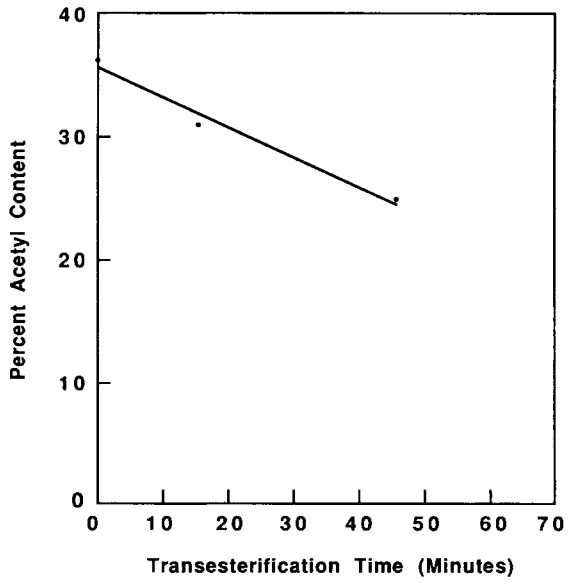

Figure 1 The degree of substitution for amylose acetate film after transesterification in 0.025 *M* $NaOCH_3$ in methanol, at room temperature.

Table I Amylose Acetate Film Data for Samples Exposed to Amylase for 1 h

Sample No. (min)	Time $NaOCH_3$ (min)	Tensile Strength (MPa)	Percent Retained Strength
1	15	2.49	44.5
2	15	5.60	Control
3	15	5.82	86.4
4	15	6.74	Control
5	15	4.30	75.3
6	15	5.71	Control
7	30	4.12	51.3
8	30	8.03	Control
9	30	6.11	78.0
10	30	7.83	Control
11	30	6.35	83.9
12	30	7.56	Control
13	45	3.97	46.1
14	45	8.61	Control
15	45	5.28	70.9
16	45	7.45	Control
17	45	4.11	51.6
18	45	7.96	Control
19	60	3.94	45.0
20	60	8.75	Control
21	60	4.35	67.9
22	60	6.40	Control
23	60	3.81	42.0
24	60	9.07	Control
25	60	3.27	35.3
26	60	9.24	Control
27	90	4.57	53.7
28	90	8.51	Control
29	90	4.43	46.0
30	90	9.63	Control
31	90	3.92	52.7
32	90	7.43	Control
33	90	3.54	44.0
34	90	8.04	Control
35	90	5.38	48.9
36	90	11.0	Control
37	90	3.98	51.5
38	90	7.73	Control

The Action of Amylase on Starch

It was previously known at the start of this work that a starch acetate polymer could be found that would have suitable regions of a low degree of substitution (DS) so as to be attacked by amylase.[7-9] These patents describe the hydrolysis of starch acetates to produce lower viscosity polymers. However, the overall DS of the polymer would have to be high enough to render the films of these polymers stable in an aqueous environment. These conditions are

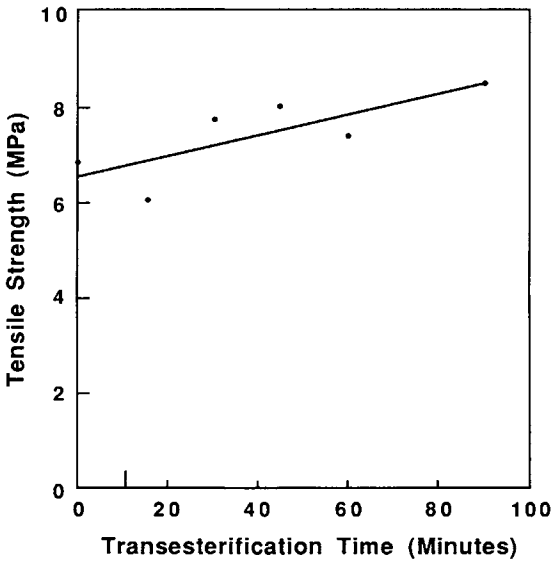

Figure 2 The tensile strength of control films vs. transesterification time in 0.025*M* $NaOCH_3$. Each sample was also exposed to blank buffer solution for 1 h.

necessary for this material to be useful as a membrane component in a bioreactor. The action of amylases on chemically modified starch and amyloses has been reviewed.[10]

There are primarily two different chemical forms of amylase, alpha-amylase and beta-amylase, which hydrolyze amylose in different ways.[10] To achieve the most rapid degradation of the films, a mixture of the two was employed. The amylose substrate can also have an effect on the performance of the enzymes. This study utilized a mixture of linear amylose and the branched polymer, amylopectin. Park and Rollings[11] recently showed the interrelationship of enzyme action and amylose substrate conformations. They suggest that it is the crystal type and not the degree of crystallinity that has the dominate effect in influencing reaction rates.

Alpha-amylases cause hydrolysis of the alpha-(1-4)-glucosidic bond by random attack on the amylose molecule, yielding fragments of low molecular weight dextrins. Although alpha-amylase cannot hydrolyze the alpha-1-6 bonds, it has the ability to bypass them to form a dextrin, or "limit" dextrin, of four to eight D-glucose units containing the original alpha-1-6 linkage of the amylopectin component. A beta-amylase attaches itself parallel and specifically to the nonreducing end of the amylose chain, thus liberating maltose by stepwise hydrolysis. Beta-amylase is a saccharifying enzyme as opposed to alpha-amylase as a dextrinizing enzyme.

Results of the Enzyme Treatments

Enzymatic hydrolysis of starch acetate that has been pretreated with sodium methoxide affords a convenient and rapid means for obtaining oligomers, which should lead to a rapid loss of tensile strength. To follow loss of tensile strength, the percent retained tensile strength of the enzyme-treated film was compared to an untreated control from an adjacent region of the original film. This accounts for the effect of local changes in the film strength from batch-to-batch production of film. From these data, a correlation of retained tensile strength as a function of DS upon treatment with enzyme was found. No sample showed greater than 100% retained strength. In Figure 3, the percent retained strength of the film, after exposure to enzyme for 1 h, is shown as a function of the transesterification time. These results from Table I are shown in Figure 3. The tensile strength of the enzyme-treated film decreased with respect to increased time of transesterification. This implies that enzyme more efficiently attacks film with low DS, as expected. Those samples that had been transesterified more than 120 min did not survive intact upon exposure to the aqueous enzyme solutions. In other words, these films were insufficiently hydrophobic to stay intact in water and they also were the films that were the most efficiently hydrolyzed by the enzyme.

The effect of extended exposure to amylase was

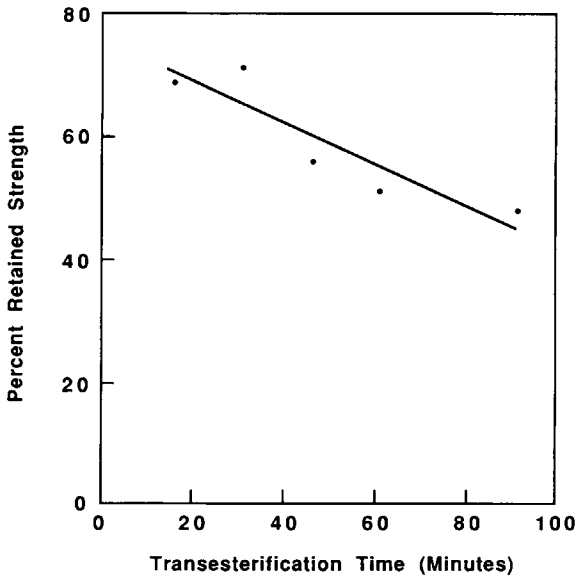

Figure 3 The percent of retained strength of amylose acetate film vs. transesterification in 0.025*M* $NaOCH_3$. All samples were treated with enzyme for 1 h.

Table II Amylose Acetate Film Data for Samples Transesterified to 22% Acetyl Content

Sample No.	Time Enzyme (min)	Tensile Strength (MPa)	Percent Retained Strength
1	45	5.48	94.2
2	—	5.82	Control
3	45	9.19	59.6
4	—	15.4	Control
5	90	4.45	34.8
6	—	12.8	Control
7	90	3.87	28.0
8	—	13.8	Control
9	180	3.22	31.2
10	—	10.3	Control
11	180	2.64	57.0
12	—	4.63	Control
13	180	3.08	53.8
14	—	5.72	Control
15	990	1.36	33.8
16	—	4.02	Control
17	990	5.58	82.7
18	—	6.75	Control

also examined for the starch films. From the behavior of the starch films observed in Figure 3, a transesterification time of 1 h was selected as optimum. This gives a percent acetyl of 22% for these films. In Table II, the retained tensile strength is shown as a function of amylase hydrolysis time. The tensile strength of each enzyme-treated film is much lower than that of its control. Interestingly, there is a trend in the percent retained strengths reported in Table II that suggests that there may be a leveling-off value of tensile strength after 90 min exposure to the enzyme. Since these films are not fully deacetylated, there may be regions in the films not attacked by the amylase mixture due to the presence of acetate groups.

In conclusion, it was found that it is possible to make starch acetate films that are sufficiently hydrophobic to preserve their tensile strength in water, but still be susceptible to rapid enzymatic hydrolysis when exposed to a mixture of alpha- and beta-amylases. The degree of substitution of the starch acetate films was successfully controlled by the sodium methoxide/methanol transesterification. The nonswelling characteristics of this reagent did not disturb the tensile properties of the film. Most significantly, the tensile strength of film with a fixed degree of substitution consistently decreased with increasing exposure time to the enzyme. Correspondingly, films with a lower degree of acetylation degraded more completely upon exposure to a fixed level of enzyme. It was demonstrated that the transesterification of the films or exposure of the films to only the buffer solution did not degrade the film strength.

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REFERENCES

1. R. L. Whistler and G. E. Hilbert, *Ind. Eng. Chem.*, **36,** 796 (1944).
2. I. A. Wolff et al., *Ind. Eng. Chem.*, **43,** 915 (1951).
3. R. Narayan, Z. L. Lu, Z. X. Chen, and N. Stacy, *Polym. Prepr., Am. Chem. Soc. Div. Polym. Chem.* **29**(2), 106 (1988).
4. R. Narayan, N. Stacy, and Z. L. Lu, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.*, **30**(1), 105 (1989).
5. M. Rutenberg and D. Solarek, in *Starch: Chemistry and Technology,* R. L. Whistler, J. Bemiller, and E. Paschall, Eds., Academic Press, New York, 1984, p. 311.
6. R. L. Whistler, *Adv. Carbohydr. Chem.*, **1,** 279 (1945).
7. F. Verbanac, U.S. Pat. 4,052,226 (1977).
8. R. Hayashi et al., Jpn. Pat. 1,252,296 (*Chem. Abstr.*, **112,** 137563m) (1988).
9. J. Nakahara, Jpn. Pat. 1,162,894 (*Chem. Abstr.*, **111,** 235450w) (1989).
10. J. Robyt, in *Starch: Chemistry and Technology,* R. L. Whistler, J. Bemiller, and E. Paschall, Eds., Academic Press, New York, 1984, p. 87.
11. J. T. Park and J. E. Rollings, *Enzyme Microb. Technol.*, **11,** 334 (1989).

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