

Graft Copolymers of Starch. I. Copolymerization of Gelatinized Wheat Starch with Acrylonitrile. Fractionation of Copolymer and Effect of Solvent on Copolymer Composition*

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Synopsis

The starch-polyacrylonitrile graft copolymer prepared from gelatinized wheat starch with ceric ammonium nitrate as the initiator has been freed of ungrafted polyacrylonitrile and separated into fractions by extraction with dimethylformamide, γ -butyrolactone, and dimethyl sulfoxide. The copolymer fractions obtained differed appreciably in level of add-on, molecular weights of grafted chains, and grafting frequency. To determine the molecular weights of grafted chains, the starch part of the copolymer was effectively removed by hydrolysis with α -amylase. It was necessary to dissolve or swell the polymer in dimethyl sulfoxide and freshly precipitate it by addition to water before enzymatic hydrolysis. In studying the effect of reaction medium on copolymer composition, there was less ungrafted homopolymer formed and a higher yield of graft copolymer possessing more frequent grafts with water than with aqueous dimethylformamide or aqueous ethylene glycol. Polymer solubility, the results of control polymerizations of acrylonitrile in the absence of starch, and the detection of glucose endgroups on the polyacrylonitrile liberated from the fractionated polymer by hydrolysis are presented as evidence that the copolymers obtained are true grafts rather than intimate mixtures.

INTRODUCTION

A number of reports have appeared that describe graft copolymerization of acrylonitrile with starch in which redox systems, irradiation, and ozonization are used as means of initiation.¹⁻⁵ To our knowledge, however, no detailed fractionation of the starch-polyacrylonitrile copolymer has been carried out with the idea of examining the structures of fractions with different solubility properties.

A fractionating scheme was therefore developed that permits both the separation of starch-polyacrylonitrile copolymer from ungrafted homopolymer and the separation of graft copolymer into components having different solubilities, different grafting frequencies, and different molecular

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weight grafts. A method of hydrolysis is reported whereby starch can be removed from the copolymer under very mild conditions in order that the molecular weight of the grafted polyacrylonitrile (PAN) might be determined. A series of graft polymerizations is described in which these methods are used to show the effect of changes in reaction medium on copolymer composition. Finally, several lines of evidence are presented to show that a true graft copolymer has been formed, as opposed to an intimate mixture where no primary chemical bonds exist between starch and PAN. Because of the known efficiency of ceric ammonium nitrate in initiating graft polymerization to starch,^{2,4,5} we chose this initiator for our study.

EXPERIMENTAL

Spectra

Infrared spectra were recorded on a Perkin-Elmer Model 137 Infracord spectrophotometer. Ultraviolet spectra were obtained on a Perkin-Elmer Model 202 spectrophotometer.

Materials

Starch. The starch used was unmodified edible wheat starch (Huron Starbake, manufactured by the Hercules Powder Company) which contained 10–12% H₂O.

Monomer. Acrylonitrile (Eastman Kodak, practical grade) was fractionated at atmospheric pressure through a 15-in. Vigreux column. A center cut was collected and stored in amber glass at 5°C. until used.

Catalyst Solution. The catalyst solution was prepared by the dissolution of 13.9 g. of ceric ammonium nitrate (G. Frederick Smith Chemical Company) in 250 ml. of 1*N* nitric acid. Catalyst solutions which had been stored at room temperature for more than 3 weeks were discarded and fresh solutions prepared.

α -Amylase. The α -amylase used was obtained from British Drug Houses, Ltd. (distributed by the Ealing Corp.). The activity was not determined.

Other Reagents. The dimethylformamide (DMF) and ethylene glycol used in polymerization reactions were fractionated through a 15-in. Vigreux column, and center cuts were collected for use.

Graft Polymerization

In a typical reaction, 25.4 g. of wheat starch (containing 13.9% H₂O) and 500 ml. of the particular solvent selected were stirred and heated at 83–85°C. for 1 hr. while a slow stream of nitrogen was allowed to bubble through the reaction mixture. The mixture was cooled to room temperature and the nitrogen bubbler removed. Acrylonitrile (31.8 g.) was then added, followed after 5 min. by 7.5 ml. of ceric ammonium nitrate catalyst solution (to give a ceric ion concentration of 1.52×10^{-3} mole/l.). After

the mixture had stirred under a nitrogen atmosphere for a period of 3 hr. at room temperature, the reaction mass was poured into 1 liter of distilled water and the mixture centrifuged. The solid was washed with both water and ethanol, and then it was dried in a vacuum oven at 60°C.

The alcohol washing was discarded and the combined aqueous washings were distilled through a Vigreux column under reduced pressure until about 250 ml. of distillate was collected (b.p. 60°C./148 mm.). The amount of unreacted acrylonitrile in the distillate was then determined by the method of Beesing et al.⁶ (addition of *n*-dodecyl mercaptan and Triton B followed by titration of unreacted mercaptan with standard iodine solution).

The liquid remaining after removal of unreacted monomer was evaporated to dryness and the weight of residue determined. For all reactions, the material balance at this point was greater than 93%. The crude graft copolymer was then fractionated with DMF, dimethyl sulfoxide (DMSO), and γ -butyrolactone as described under Results and Discussion.

Hydrolysis of Graft Copolymer with α -Amylase

In a typical experiment, 0.8 g. of graft copolymer and 100 ml. of DMSO were stirred and heated on a steam bath until either a solution or a smooth dispersion was obtained. This mixture was added to water in a Waring Blendor and the mixture dispersed. The aqueous mixture was diluted with water to a volume of approximately 600 ml., and a solution of 0.20 g. of α -amylase in about 20 ml. of water was added. The resulting mixture was held at 50°C. for 4–5 hr. and then dialyzed against distilled water to remove DMSO and carbohydrate. The solid was then removed by filtration, washed with water and alcohol, and dried in a vacuum oven at 60°C. An infrared spectrum of this material (Nujol mull) showed little, if any, unhydrolyzed starch. The final product was further dried for 4 hr. under vacuum at 56°C. over phosphorus pentoxide and the intrinsic viscosity determined at 25°C. in DMF solution by using Cannon-Fenske viscometers (kinetic energy corrections were not applied). The molecular weight was then calculated from the equation:⁷

$$[\eta] = 3.92 \times 10^{-4} \bar{M}_n^{0.75}$$

From the molecular weight and the per cent nitrogen in the unhydrolyzed copolymer, the average number of anhydroglucose units (AGU) per grafted chain was calculated.

RESULTS AND DISCUSSION

Fractionation of Graft Copolymer

Acrylonitrile was allowed to copolymerize with starch as described in the Experimental section, and both unreacted monomer and water solubles were removed from the reaction product. The material which was insoluble in water was then fractionated as shown in Figure 1.

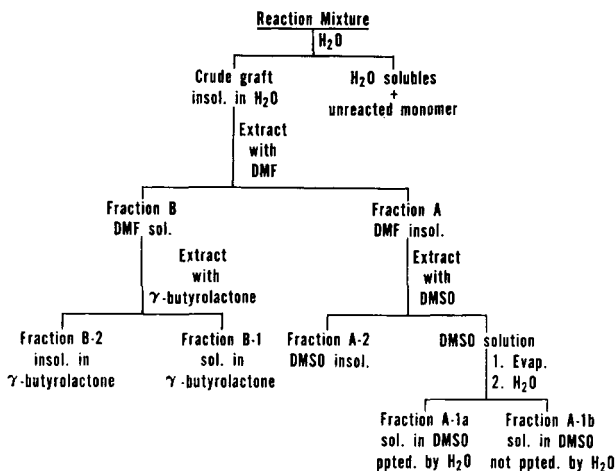


Fig. 1. Fractionation of starch-polyacrylonitrile graft copolymer.

To remove ungrafted PAN, the crude copolymer was extracted successively with fresh portions of DMF until evaporation of a few drops of the extract showed that a negligible amount of material was going into solution. The DMF-soluble fraction (B) usually contained at least 5% starch, as shown by the infrared spectrum. When fraction B contained more than about 15% starch, it was further fractionated according to its solubility in γ -butyrolactone, which is a good solvent for ungrafted PAN but a poorer solvent than DMF for starch-PAN copolymer. The lactone-insoluble fraction (B-2) was considered to be mainly graft copolymer. The lactone-soluble fraction (B-1) was mostly ungrafted PAN; however, this material contained 5–15% starch, as estimated from the infrared spectrum. This starch is probably grafted material which has been solubilized owing to a high level of add-on.

The DMF-insoluble fraction (A, Fig. 1) was extracted successively with fresh portions of DMSO until evaporation of the extract showed that a negligible amount of material was going into solution. The DMSO-insoluble fraction was a highly swollen gel. The DMSO extract, after concentration to a small volume, was added to water to precipitate a water-insoluble solid (fraction A-1a). The aqueous filtrate from the latter fraction was evaporated to a small volume, from which fraction A-1b was obtained by precipitation with ethanol. As expected, fraction A-1b contained mostly starch, as evidenced by its low nitrogen content.

The fractions containing starch-PAN graft are, therefore, B-2, A-2, and A-1a. Nitrogen analyses, molecular weights of the grafted chains, and the average number of AGU per grafted chain were determined for these fractions.

Since DMSO is known to dissolve both starch and PAN, it was thought that the insolubility of fraction A-2 might be due to crosslinking in the graft copolymer. Portions of the DMSO-insoluble fractions from two separate

experiments were, therefore, acetylated with acetic anhydride and pyridine. Although the infrared spectra of the isolated products still showed hydroxyl absorption, in both of the acetylation reactions more than 98% of the product was soluble in DMF. Consequently, there is probably little or no crosslinking in the DMSO-insoluble graft.

Hydrolysis of Graft Copolymers

To determine the molecular weight of the grafted chains, starch was removed from the copolymer through hydrolysis with α -amylase, and the intrinsic viscosity of the water-insoluble PAN (containing glucose end-groups) was determined as described in the Experimental section. It was found that if the starch copolymer was dissolved (or swollen) in DMSO and then was freshly precipitated by addition to water, the removal of carbohydrate from PAN was usually complete to within the limit of infrared detection in 4–5 hr. at 50°C. The presence of DMSO appeared to have no adverse effect on the enzyme. Progress of the hydrolysis could be followed conveniently in the infrared by observing the disappearance of absorption bands due to starch at 3370, 1075, and 1020 cm.^{-1} . Infrared spectra of synthetic mixtures of starch and PAN indicated that 5% starch in PAN could be detected. However, if the amount of starch in PAN dropped much below 5%, detection was difficult.

Hydrolysis with α -amylase without prior treatment with DMSO tended to give erratic results. In some experiments complete hydrolysis was obtained, whereas in other reactions 10–15% starch was detected (by infrared) in the hydrolyzed sample. Hydrolysis with mineral acids also proved unsatisfactory. Hydrolysis was incomplete when 0.5*N* hydrochloric acid was used at reflux temperature, and with 75% sulfuric acid at room temperature, extensive reaction of the nitrile groups was observed.

Composition of the Graft Copolymer and Influence of Reaction Medium

In Table I are collected the results of two graft copolymerization reactions. Reaction conditions for both experiments were the same except that run 1 was made in water and run 2 in 32% aqueous DMF. The procedures described above were used for both the fractionation of the copolymers and the hydrolysis of the respective fractions. Both reactions yielded fractions with a wide range of add-on, grafting frequency, and grafted chain lengths. In general, the molecular weights of grafted chains were high and the actual number of PAN chains grafted to a particular starch molecule was rather low.

Table II shows the results of three reactions in which 50% aqueous DMF (run 1), 68% aqueous DMF (run 2), and 50% aqueous ethylene glycol (run 3) were employed as reaction media. For these three reactions the amount of grafting was so slight, as evidenced by the low nitrogen content for fraction A, that the products were not carried through the complete separation procedure.

TABLE I
Effect of Solvent on Copolymer Composition

Fraction ^a	Run ^b	Wt., g.	PAN in fraction, %	MW of graft	AGU/graft
Recovered monomer	1	3.4			
	2	2.8			
Water solubles	1	2.6			
	2	2.7			
A-1a	1	17.8	50.3	620,000	3770
	2	7.1	42.4	662,000	5560
A-1b	1	3.2	8.7		
	2	2.9	3.6		
A-2	1	4.4	46.4	780,000	5570
	2	16.3	33.3	750,000	9370
B-2	1	13.8	65.9	266,000	850
	2	Negligible	—	—	—
B-1	1	1.7	(Contains about 5% starch)		
	2	17.4	(Contains 10–15% starch)		

^a Fraction numbers are those given in Fig. 1.

^b Run 1 made in H₂O; Run 2, in 32% dimethylformamide (DMF).

TABLE II
Effect of Solvent on Copolymer Composition

Fraction ^a	Run ^b	Wt., g.	PAN in fraction, %	MW of graft	AGU/graft
Recovered monomer	1	10.7			
	2	22.6			
	3	2.6			
Water solubles	1	4.3			
	2	1.6			
	3	2.5			
A	1	17.6	5.1	219,000	25,100
	2	22.6	3.7	—	—
	3	20.1	4.2	160,000	22,500
B	1	14.2	(Contains 5–10% starch)		
	2	4.0	(Contains less than 5% starch)		
	3	27.2	(Contains less than 5% starch)		

^a Fraction numbers are those given in Fig. 1.

^b Run 1 made in 50% aqueous dimethylformamide (DMF); run 2, in 68% aqueous DMF; run 3, in 50% aqueous ethylene glycol.

As seen in Tables I and II, there was less ungrafted homopolymer formed and a higher yield of graft copolymer possessing more frequent grafts with water as a reaction medium than with aqueous DMF or aqueous ethylene glycol. A similar detrimental effect of DMF has been observed elsewhere in the grafting of acrylonitrile to ungelatinized starch.⁸ The inferiority of aqueous ethylene glycol is best explained by a reaction between ethylene glycol and ceric ion to generate free radicals which initiate the polymeriza-

tion of acrylonitrile to give the observed high yield of ungrafted homopolymer.⁹ A similar reaction is possible for DMF.¹⁰

Proof of Grafting

There are several lines of evidence which indicate that fractions A-2, B-2, and A-1a (Fig. 1) are indeed grafts and not merely physical mixtures of starch and PAN. One indication that our products are true grafts is the solubility properties of these fractions, which were discussed earlier in connection with the fractionation procedure. Since PAN could be extracted nearly completely from a synthetic mixture of PAN and starch by either DMF or γ -butyrolactone, the presence of appreciable amounts of PAN in our reaction products after extraction is good evidence for grafting. That our material is not a mixture of intertwined chains of PAN and starch (owing to polymerization of monomer within the starch matrix) is indicated by an examination of the products described in Table II, where little actual grafting was observed, but from which ungrafted PAN could be readily extracted. The isolation of a fraction (A-2) which is insoluble in a solvent (DMSO) known to dissolve both PAN and starch also strongly suggests a copolymer of the two components, the size and shape of which preclude dissolution by the common solvent. Since a solution of pure wheat starch in DMSO is not precipitated on addition of water, fraction A-1a must also be mostly graft copolymer.

Additional proof that the isolated fractions are true grafts is that in aqueous solution in the presence of ceric ammonium nitrate, the conversion of acrylonitrile to polymer (MW 880,000) was only 16.5% in the absence of starch, as compared with an 89% conversion in the presence of starch. Since it is not likely that more ungrafted PAN would be formed in the presence of starch than in its absence, this 16.5% conversion constitutes an approximate upper limit for the amount of ungrafted PAN. In contrast, a 61% conversion of acrylonitrile to polymer (MW 306,000) was obtained in the absence of starch in a solvent system composed of equal volumes of DMF and water.

A final proof of grafting was obtained through the detection of glucose endgroups in the PAN resulting from α -amylase hydrolysis of the graft copolymer. Since the PAN obtained from hydrolysis showed little, if any, absorption in the infrared that could be attributed to glucose endgroups, this material was benzoylated, with benzoyl chloride and pyridine, and the benzoyl groups were detected in both the infrared and the ultraviolet. For comparison, a synthetic mixture of starch and PAN was hydrolyzed and benzoylated under identical conditions.

Infrared spectra (Nujol mull) of both benzoylated samples showed weak carbonyl absorption in the same position as found for a sample of benzoyl starch (1715 cm.^{-1}). However, the carbonyl absorption in the PAN sample derived from hydrolysis of the graft copolymer was much more intense than that observed for the sample from the synthetic mixture.

In the ultraviolet (DMF solution), PAN exhibited a broad band in the 265–290 $m\mu$ region; however, absorption at 275 and 283 $m\mu$ due to benzoylated endgroups was visible in the sample of PAN derived from graft copolymer. The PAN derived from the synthetic mixture exhibited the same spectrum as a sample of unbenzoylated PAN. Extinction coefficients were not calculated, since neither the number of glucose units on the end of each PAN chain nor the extent of benzoylation could be predicted with any degree of accuracy.

Currently, studies are in progress on the effects of a number of reaction conditions on both the grafting frequencies and molecular weights of the grafted chains for the copolymerization of gelatinized wheat starch with acrylonitrile initiated by ceric ion.

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Mention of trade names should not be construed as recommendation or endorsement by the U. S. Department of Agriculture over those not mentioned.

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Résumé

Le copolymère greffé à base d'amidon avec le polyacrylonitrile préparé au départ d'amidon de froment gélatinisé avec des nitrates ammonico-cériques comme initiateurs, a été séparé de tout polyacrylonitrile non-greffé et scindé en fractions par extraction au diméthylformamide, γ -butyrolactone, et diméthylsulfoxyde. Les fractions de copolymères obtenues différaient de façon appréciable en composition, en poids moléculaire de chaînes greffées et en fréquence de greffage. En vue de déterminer les poids moléculaires des chaînes greffées, la partie amidon du copolymère a été éloignée efficacement par hydrolyse à l' α -amylase. Il était nécessaire de dissoudre ou de gonfler le polymère dans le diméthylsulfoxyde et de le précipiter fraîchement par addition d'eau avant l'hydrolyse enzymatique. En étudiant l'effet du milieu de réaction sur la composition du copolymère, il y avait moins de homopolymères non-greffés formés et un plus grand rendement en copolymères greffés présentant des greffons plus fréquents en présence d'eau qu'en présence de diméthylformamide aqueux ou d'éthylène-glycol aqueux. La solubilité du polymère, les résultats des polymérisations de contrôle de l'acrylonitrile en absence d'amidon et la détection du glucose comme groupes terminaux dans l'acrylonitrile libéré au départ de polymères fractionnés par hydrolyse sont présentés comme preuve que les

copolymères obtenus sont de véritables polymères greffés plutôt que des mélanges intimes des deux polymères.

Zusammenfassung

Das aus gelatinierter Weizenstärke mit Cernitrat als Starter hergestellte Stärke-Polyacrylnitrilpfropfcopolymere wurde vom nichtsaufgepfropften Polyacrylnitril befreit und durch Extraktion mit Dimethylformamid, γ -Butyrolacton und Dimethylsulfoxyd in Fraktionen zerlegt. Die erhaltenen Copolymerfraktionen unterschieden sich beträchtlich im Aufpfropfungsgrad, im Molekulargewicht der aufgepfropften Ketten und in der Pfpfungshäufigkeit. Zur Bestimmung des Molekulargewichts der aufgepfropften Ketten wurde der Stärketeil des Copolymeren durch Hydrolyse mit α -Amylase entfernt. Vor der enzymatischen Hydrolyse war es notwendig, das Polymere in Dimethylsulfoxyd zu lösen oder zu quellen und durch Zusatz von Wasser auszufällen. Bei der Untersuchung des Einflusses des Reaktionsmediums auf die Copolymerzusammensetzung wurde in Wasser nichtaufgepfropftes Homopolymeres und eine höhere Ausbeute an Pfpfcopolymeren mit häufigerer Pfpfung gefunden als in wässrigem Dimethylformamid oder Äthylenglycol. Die Polymerlöslichkeit, die Ergebnisse von Kontrollversuchen an Acrylnitril in Abwesenheit von Stärke sowie der Nachweis von Glucoseendgruppen an aus den fraktionierten Polymeren durch Hydrolyse freigesetztem Polyacrylnitril werden als Beweis für die Bildung von wirklichen Pfpfcopolymeren und nicht von innigen Mischungen angeführt.

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