DETERMINATION OF THE EXTENT OF REACTION BETWEEN EPICHLOROHYDRIN AND STARCH[1]

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ABSTRACT

The rate and extent of cross-linking of starch with epichlorohydrin was investigated by determining the quantity of unreacted epichlorohydrin in reaction filtrates. The unreacted epichlorohydrin was converted to glycerol by hot alkali. Periodate oxidation of the glycerol solutions yielded formaldehyde, which was estimated colorimetrically using the chromotropic acid color reaction.

Under the reaction conditions described a maximum extent of reaction of 90% was obtained. A blank correction was required to account for alkali-solubilized material from starch, which under the conditions of analysis yielded an apparent value for epichlorohydrin. It was required, for valid application of these analyses, that the epichlorohydrin-starch reaction be conducted in a gas-tight system. The quantities of epichlorohydrin reacted, as indicated by the analyses, are not necessarily exclusively involved in cross-linking. It was found that with molar ratios of anhydroglucose units to epichlorohydrin as low as 1,200 to 1, marked inhibition of the granule to swelling in hot water resulted.

Epichlorohydrin has been extensively used to produce inhibited or cross-linked starches (1,6). In its reaction with hydroxyl groups of starch, mono- and diethers are formed, the diethers being either inter- or intramolecular. From this complex mixture the exact amount of cross-linking achieved by intermolecular diether formation would be difficult to ascertain. Generally, the extent of cross-linking is calculated on the basis of total epichlorohydrin introduced into an alkaline aqueous starch slurry, or it is qualitatively determined from the physical behavior of the pasted products (5). A more reliable estimate of the degree of cross-linking, based on the epichlorohydrin that has reacted with the starch, would be of practical interest to the starch industry.

The reaction of epichlorohydrin with starch was investigated in order to define more reliably the extent of cross-linking. This study entailed developing a procedure for accurately determining unreacted epichlorohydrin in the reaction mixtures.

Under the alkaline conditions required for the cross-linking of starch, partial hydrolysis of epichlorohydrin to glycerol occurred. The result was to preclude applying the method of Daniel and Gage (3) which is specific for epichlorohydrin. The rate and extent of reaction

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of epichlorohydrin with starch were determined by converting excess epichlorohydrin to glycerol, which was estimated spectrophotometrically by the chromotropic acid method (7) after periodate oxidation to formaldehyde (8).

Materials and Methods

Reagents. The commercial pearl corn starch that was used contained by analysis: methanol extractables, 0.63%; ash, 0.08%; nitrogen, 0.05%; and moisture, 9 to 12%.

Stock alkali solution was prepared by dissolving 0.66 g. of reagent grade sodium hydroxide and 16.66 g. of reagent grade anhydrous sodium sulfate in 100 ml. of distilled water.

Epichlorohydrin obtained from the Shell Chemical Corporation[2] was redistilled before use (b.p. 114.5°–116.5°C.). A solution was made by dissolving 0.196 ml., measured from a calibrated microburet, in 50 ml. of the stock alkali solution. A 5-ml. aliquot of this solution was diluted to 200 ml. with distilled water and contained 116 μg. of epichlorohydrin per ml.

Glycerol (Eastman White Label) had a purity of 95.0% by specific gravity. A solution was prepared by dissolving approximately 250 mg. in 50 ml. of the stock alkali solution. A 5-ml. aliquot of this solution was diluted to 200 ml. with distilled water and contained approximately 125 μg. of glycerol per ml.

The following standard solutions were prepared with analytical or C.P.-grade reagents: 0.1*M* sodium metaperiodate, 1.0*M* sodium arsenite, and 10*N* sulfuric acid.

Chromotropic acid (disodium 4,5-dihydroxy-2,7-naphthalenedisulfonate) in the amount of 1.13 g. was dissolved in 100 ml. distilled water and then filtered. To this were added 450 ml. of a sulfuric acid solution prepared by adding 300 ml. of C.P. sulfuric acid to 150 ml. distilled water. The chromotropic acid should be prepared fresh every 2 or 3 weeks. Exposure of the solution to direct sunlight should be avoided.

Anthrone reagents were prepared by dissolving 0.1 g. (Eastman White Label) in 50 ml. of concentrated C.P. sulfuric acid.

Reaction of Epichlorohydrin with Starch. Reaction conditions were essentially those described by Caldwell (2), with the exception that a gas-tight system was employed to prevent escape of volatile epichlorohydrin. One hundred grams (dry basis) of starch were suspended in 150 ml. of stock alkali solution by mechanical stirring. The

[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

desired quantity of epichlorohydrin (20–900 mg.) was then freshly dissolved in 50 ml. of stock alkali solution and added dropwise to the starch mixture in 3-5 minutes. After 18 hours at 25°C. the slurry was neutralized to pH 6.0 with 6*N* sulfuric acid and filtered. The starch was resuspended in distilled water (20% starch by weight), then filtered, and the two filtrates were combined and analyzed for unreacted epichlorohydrin. Two more washes, used in the isolation of cross-linked starches, had been shown to contain only 1.8% of the total recoverable epichlorohydrin and were therefore routinely omitted from the analysis.

Determination of Unreacted Epichlorohydrin. An aliquot (5–25 ml.) of the combined filtrates containing 500–750 μg. epichlorohydrin was pipetted into a 50-ml. volumetric flask and 1 ml. of 2*N* sodium hydroxide was added. An epichlorohydrin standard and a reagent blank were prepared in a similar manner. The stoppered flasks were heated on a steam bath for 1 hour, then cooled, and 1 ml. of 10*N* sulfuric acid was added, followed by 5 ml. of 0.1*M* sodium periodate. The flasks were placed in the dark for 10 minutes, after which 5 ml. of 1.0*M* sodium arsenite were added and the solutions diluted to 50 ml. with distilled water. One-milliliter aliquots were then pipetted into matched spectrophotometer tubes. Ten milliliters of chromotropic acid reagent were added rapidly with mixing to each of the tubes, which were then heated in a boiling-water bath for 30 minutes. The tubes were removed and cooled to room temperature, and the absorbance was read at wave length of 570 mμ in a Coleman Jr. Spectrophotometer using a water blank set at zero absorbance. Concentrations of the samples were calculated using absorbance and the instrument constant. Absorbance of the solutions obeyed the Beer-Lambert Law.

Solubility of Cross-Linked Starches. Solubility of the cross-linked starches in hot water was determined in the following manner: 0.800 g. (dry basis) of the reacted starch was weighed into a graduated 40-ml. centrifuge tube, and distilled water was added to the 40-ml. mark. The mixture was heated in a boiling-water bath for 30 minutes with occasional stirring as required to prevent settling. After cooling to room temperature the stirring rod was removed, distilled water was added to bring the volume to 40 ml., and the mixture was centrifuged at 3,000 r.p.m. for 10 minutes. Total solids content of the supernatant was determined by the anthrone method for carbohydrate material (4).

Results and Discussion

Blank Correction. The small quantity of soluble carbohydrate

material (approximately 0.04% by weight) in the filtrates from the reaction mixtures interfered with the epichlorohydrin analysis by producing formaldehyde on treatment with periodate. The correction for soluble carbohydrate was determined by treating corn starch in the same manner as the reacted starch but in the absence of epichlorohydrin. For one lot of corn starch a correction equivalent to 16.3 mg. apparent epichlorohydrin for each 100 g. of starch was obtained with a coefficient of variation of 2.65% in ten separate determinations. A different lot of starch, however, was found to have an average value of 21.5 mg. of apparent epichlorohydrin per 100 g. of starch. Appropriate correction was made for each lot of starch reacted.

Experimental evidence for the validity of the blank correction was obtained in the following manner: 200 g. of corn starch were dispersed in 300 ml. of the stock alkali solution by mechanical stirring for 48 hours at 25°C. The starch was filtered, washed with distilled water, and divided into two equal portions. One portion, approximately equivalent to 100 g. of starch, was reacted with epichlorohydrin in the normal procedure for 18 hours. A parallel trial without epichlorohydrin was made with the other portion. The blank correction was 2.5 mg. of apparent epichlorohydrin per 100 g. of starch. The percentage epichlorohydrin which had reacted with the starch, however, was identical with that found in a comparable experiment with the same starch not pretreated with alkali. The correction for the untreated starch was 16.3 mg. of apparent epichlorohydrin per 100 g. of starch. Similar results were obtained with various mole ratios of starch and epichlorohydrin.

Rate of Reaction. The rate and extent of reaction of epichlorohydrin and starch were determined by removing aliquots from the reaction mixture at various intervals of time. The supernatants were

TABLE I

RATE OF REACTION OF STARCH WITH EPICHLOROHYDRIN AT 25°C.

Reaction Time	Unreacted Epichlorohydrin			Epichlorohydrin Reacted [a]	Reacted	Calculated Cross-Linking
	Total	Blank	Corrected			
hours	*g*	*g*	*g*	*g*	%	*AGU/C.L.*
0.5	0.377	0.050	0.327	0.135	29.2	2,050
1.0	.375	.053	.322	.140	30.3	1,980
2.0	.338	.063	.275	.187	40.5	1,480
4.0	.305	.070	.235	.227	49.1	1,220
8.0	.249	.085	.164	.298	64.5	930
18.0	.186	.106	.080	.382	82.7	725
32.0	.174	.119	.055	.407	88.1	680
45.0	0.166	0.120	0.046	0.416	90.0	665

[a] Initially 0.462 g. of epichlorohydrin was added to 485 g. of starch under reaction conditions described earlier.

analyzed for unreacted epichlorohydrin and the isolated samples were pasted, as described previously, to determine changes in solubility. Experimental results from a reaction in which sufficient epichlorohydrin had been added to produce a calculated value of 600 anhydroglucose units per cross-link are shown in Table I. To establish the required blank correction, a similar preparation with no epichlorohydrin added was sampled at the same time intervals. The rate of reaction is shown by the plot of percentage epichlorohydrin reacted at various intervals of time (Fig. 1). The heterogeneous nature of the reaction system and surface adsorption effects would complicate a quantitative treatment of the kinetics of this reaction.

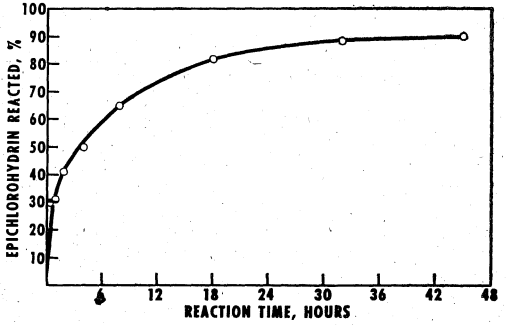

Fig. 1. Rate of reaction of epichlorohydrin with starch at 25°C.

At levels of reaction ranging from 2,500 to 2,550 anhydroglucose units per cross-link it was found that an average of 78.2% (coefficient of variation of 2.2%) of the quantity of epichlorohydrin initially added reacted with the starch in 18 hours. Representative data are given in Table II.

TABLE II

REACTION OF EPICHLOROHYDRIN WITH 100 GRAMS OF STARCH FOR 18 HOURS AT 25°C.

THEORETICAL CROSS-LINKING	EPICHLOROHYDRIN ADDED	UNREACTED EPICHLOROHYDRIN			EPICHLOROHYDRIN REACTED	REACTED
		Total	Blank	Corrected		
AGU/C.L.	*g*	*g*	*g*	*g*	*g*	%
50	1.140	0.288	0.0214	0.267	0.873	76.6
100	0.588	.143	.0215	.122	.466	79.3
247	.231	.0717	.0165	.0552	.1758	76.1
482	.1180	.0397	.0168	.0229	.0951	80.6
800	.0714	.0382	.0220	.0162	.0552	77.3
960	.0595	.0273	.0163	.0110	.0485	81.5
1,600	.0357	.0237	.0160	.0077	.0280	78.4
2,360	0.0241	0.0212	0.0163	0.0049	0.0192	79.7

Hot Water-Solubles. A near linear relationship exists between the quantity of hot water-solubles and the amount of epichlorohydrin that had reacted with the starch expressed in terms of anhydroglucose units per cross-link. Figure 2 shows that hot water-solubles can be a

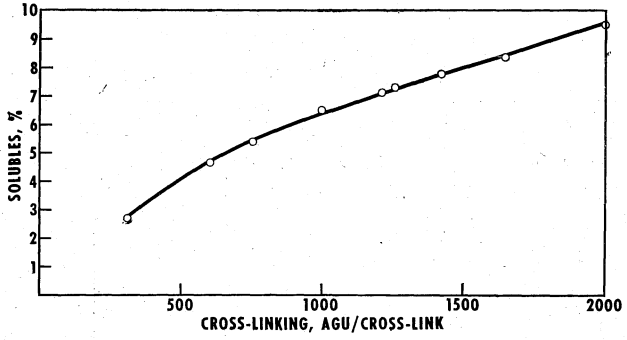

Fig. 2. Relation of solubles to cross-linking in epichlorohydrin-reacted starch.

dependable measure of the relative extent of cross-linking achieved with epichlorohydrin.

The results presented indicate that in a closed system the extent of reaction of epichlorohydrin with starch can be more accurately determined than in the past. Assuming that the percentage of reacted epichlorohydrin that produces cross-linking in starch is essentially constant throughout the reaction, the method as developed measures the relative extent of cross-linking achieved. Reacted epichlorohydrin and the amount of hot water-solubles in the products are directly related so that the solubles may be used to determine the relative cross-linking effected. A procedure for the determination of epichlorohydrin through conversion to glycerol is described.

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