

## Note on Free Radicals in Starch Derivatives<sup>1</sup>

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Previous investigations<sup>3</sup> revealed the existence of long-lived free radicals, to the extent of  $10^{16}$  unpaired electrons per g. of material, in starch which had been cross-linked by treatment with gaseous hydrogen chloride. On the basis of subsequent findings about the reaction, a possible mechanism for their formation was suggested by Thomson (1). The main reaction of starch and HCl is one of dehydration. It was suggested that the free radicals originate whenever intermolecular elimination of water takes place from hydroxyl groups and carbon-bound hydrogen atoms on neighboring glucose units whose distances are not favorable to the production of carbon-carbon bonds. Their long lives were attributed to the immobility of the polymeric chains that are further restricted by cross-linking. Species bearing unpaired electrons are presumably trapped in a complex matrix of the polysaccharide or its fragments.

### MATERIALS AND METHODS

Potato starch, supplied by the Idaho Potato Starch Company in Blackfoot, Idaho, was used in its natural granular condition. Its only pretreatment consisted of drying under vacuum for 2 hr. The hydrogen chloride and chlorine were both regular grade from Matheson Chemical Company.

The suggested mechanism was tested by a kinetics study in which the build-up of free radicals was compared with the progress of the dehydration reaction. A 15-g. sample of dried starch was allowed to react under a constant pressure of HCl gas at 70°C., according to the method described in an earlier article (2). Samples (0.5 g.) were removed at various reaction times. These were dried and degassed under vacuum at 70°C. for several hours; elementary analyses were performed and electron spin resonance spectra recorded.<sup>4</sup>

A similar group of samples was taken from a reaction of dried granular starch with liquid chlorine for reasons explained below. This reaction was conducted in a Hastelloy-C pressure reactor at 70°C. as described earlier (3,4).

Electron spin resonance work was done by the Analytical Instrument Division of Varian Associates in Palo Alto, Calif., with a Varian model E-3, 9.5 Gc EPR spectrometer. Concentrations of unpaired electrons in the samples were calculated; their spectra were compared with a standard sample

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<sup>3</sup>Unpublished results of analyses of HCl-starch products by Dr. Cornelius Steelink of the University of Arizona, Sept. 25, 1964. The existence of stable free radicals was suspected earlier by H. N. Barham. See reference 4.

<sup>4</sup>ESR measurements are based on the fact that any system containing unpaired electrons, if placed in the appropriate magnetic field and irradiated with the frequency equal to the frequency of the processing electrons, will display an absorption spectrum. The integrated area under the differentiated absorption curve is proportional to the number of unpaired electrons available for observation. The method is nondestructive with a minimum sensitivity of  $5 \times 10^{10}$  unpaired electrons per g. It might be noted that ordinary starch shows no absorption, hence contains no free radicals.

containing a known amount of unpaired electrons. The accuracy of the method is  $\pm 10\%$ .

### RESULTS AND DISCUSSION

Results of the HCl reaction are shown plotted in Fig. 1. All data have been reduced to atoms of hydrogen or oxygen or number of unpaired electrons (free radicals) per glucose unit. For convenience, these have been plotted against a logarithmic time scale.

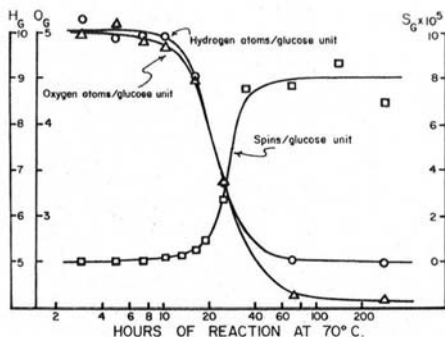


Fig. 1. Change in hydrogen, oxygen, and free radical content with time of reaction. All data have been reduced to a per-glucose-unit basis. Scales for hydrogen and oxygen are shown at left, for free radicals at right.

These curves are not inconsistent with the proposed mechanism in that free radical formation coincides with dehydration. The EPR spectra of the solid products consist of a single absorption line without any resolvable hyperfine structure. No interpretation of the spectra is possible at this time, but a similarity to those obtained by Bennett (5) with pyrolyzed carbohydrates is noted. The peak-to-peak line width of 7.2 gauss in our samples compares significantly with the 8-gauss width reported by Bennett. Both our spectra and those of Bennett have similar  $G$  values<sup>5</sup> of 2.0030. The spin lattice relaxation of these samples, a matter of seconds, is rather long, which implies a complex molecular structure, as might be expected with cross-linking. Successive measurement of free radical concentration over a period of 3 weeks indicated no apparent change. By EPR standards they may therefore be considered quite stable.

In an earlier study of the reaction of starch with liquid chlorine (3,4), a black product was obtained during the long induction period of over 8 hr. (at 70°C.) that preceded chlorination. This product closely resembled the products obtained with hydrogen chloride. Further chlorination, however,

<sup>5</sup>The  $G$ -value or "spectroscopic splitting factor" describes the interaction of an unpaired electron with an externally applied magnetic field; specifically, it is the ratio of total electron magnetic moment to its spin value. For a free electron  $G = 2.00229$ . Ordinarily the  $G$  value corresponds to the center of the spectrum. When the electron makes contact with surrounding atoms whose nuclear spin  $I \neq 0$ , a  $G$ -value shift will occur. This parameter is important, since the magnitude and direction of this shift will depend on the "kind" of atoms surrounding the electron and on the degree of electron delocalization.

converted the black product into a bright-yellow, chlorinated derivative of approximately 32% chlorine content, corresponding to two chlorine atoms per glucose unit. It therefore seemed appropriate to compare EPR measurements on products at various stages of the chlorination reaction with those of the HCl reaction. If free radicals also occurred in this reaction, they might be compared.

Three samples were chosen: one early in the induction period, one taken at the black stage near the end of the induction period, and one yellow product corresponding to the dichloride. The first two yielded a single absorption line at the magnetic field identical to that of the HCl products, with total first magnetic moments (intensities) corresponding to  $9 \times 10^{13}$  and  $2 \times 10^{15}$  electronic spins per g. respectively. These are equivalent to  $2.4 \times 10^{-8}$  and  $5.6 \times 10^{-7}$  electronic spins per glucose unit.

The yellow, postinduction period product (the dichloride) yielded two surprises. Instead of an expected decrease in free radicals, on the basis of our thinking that these radicals would be consumed in triggering of the chlorination reaction at the end of the induction period, there was an increase in this same peak to  $4 \times 10^{15}$  spins per g. ( $1.5 \times 10^{-6}$  spins per glucose unit). In addition there was a new absorption line, 110 gauss up-field from  $G = 2.0030$  (toward lower field strength), with some partially resolved hyperfine structure. This new line was estimated to represent  $1.0 \times 10^{16}$  spins per g., corresponding to  $3.8 \times 10^{-6}$  spins per glucose unit.

On the basis of these data it is hypothesized that (a) free radicals in the HCl-reaction are formed during the dehydration-crosslinking reaction; (b) a formation of similar free radicals occurs during the induction period of the reaction of starch with liquid chlorine. The HCl for this is obtained as a by-product of chlorination; (c) chlorination is not triggered by these free radicals; (d) in addition to the type of free radical found in the HCl reaction, a second different free radical is formed after the induction period of the chlorination reaction; and (e) it is possible that these free radicals (of both types) are not part of the main reactions, but rather are by-products resulting from structural features of the starch granule.

Although the significance of these free radicals is still unclear, their amount and long-lived nature warrants their further study.

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