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Formation of Stable Free Radicals in Saccharides by Heating or by UV Irradiation

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The formation of stable free radicals was observed by means of ESR spectroscopy not only on heating but also on UV irradiation of powdered saccharides at room temperature in air. Among saccharides used here, the number of free radicals was largest in the powdered disaccharides. On the other hand, free radicals of glucose were obtained by UV irradiation, but not by heating. The number of free radicals was found to increase initially after heating or after irradiation, and then became almost constant. This constant value of the number of free radicals was found to increase with increase of the heating temperature or heating time, or with increase in the duration of irradiation. The ESR spectra of saccharides were not affected by oxygen, but were found to change gradually under conditions of high humidity. Moreover, a hysteresis effect was observed between the number of free radicals and the water content in UV-irradiated potato starch. Free radicals were found to be stable when suspended in benzene, but they were destroyed by kneading the saccharides in water or alcohols.

Keywords——ESR; free radical; saccharide; glucose; potato starch; incompatibility

Saccharides are believed to be among the most stable and the safest pharmaceutical powders. Accordingly, they are used very frequently in a variety of drugs as diluents, binders, disintegrants, coating materials, and so forth.[1] Further, a small amount (<1 wt %) of metal oxide is often added to a saccharide powder as a glidant in manufacturing drugs in order to alter the flow properties of the saccharide powder and to facilitate such operations as weighing, mixing, or transportation by decreasing friction.[2]

We reported previously the observation, by means of electron spin resonance (ESR) spectroscopy, of the formation of a small number of free radicals in saccharide powders merely by mixing them with 1 wt % of metal oxide and stirring.[3] On the other hand, appreciable amounts of free radicals were reported to be formed by the direct γ-ray irradiation of saccharide powders.[4—8] The spectral shapes and hyperfine splittings, as well as the amounts of free radicals, were discussed in some detail.

Recently we found that free radicals were also formed when saccharides alone were heated in air or irradiated with UV light. The number of free radicals thus formed was found to be far smaller than that in γ-irradiated saccharide. Consequently, analysis of the hyperfine structure of our ESR spectrum was difficult. Although it is also difficult to determine the number of free radicals, the information may be of fundamental significance in connection with the study of incompatibility, and may be helpful for improving the methods of preparation or preservation of drugs.

In this paper, we describe the formation of free radicals in saccharides by heating or by UV irradiation, and determine the number of free radicals by means of ESR spectroscopy in order to clarify the behavior of these free radicals.

Experimental

Materials——Commercial GR grade glucose, fructose, mannose, saccharose, lactose, α- and β-cyclodextrin, EP grade potato starch, soluble starch, and cellulose were used after being dried under a vacuum for an hour. Water-containing saccharides were obtained by allowing them to stand for 1—24 hr in a desiccator containing water at the bottom. The water contents of the saccharide powders were determined from the loss of weight upon vacuum drying.

ESR Measurement[9]——ESR spectra of the powders of saccharides were obtained in air with a JES-FE3X type spectrometer (X band, 100 kHz modulation) equipped with a JES-VT-3A2 variable temperature controller and a JES-UCT-2AX variable temperature adapter with the following instrumental settings: power, 20 mW; modulation amplitude, 6.3 G; scan rate, 31.25 G/min; gain, 5×1000; temperature, 25°. A signal having $g=2$ was observed at 3285 G with our machine. Amounts of free radicals were calculated with the aid of calibration curves obtained from the observed spectral intensites of saccharides impregnated with a standard radical (DPPH) and were converted to the numbers of free radicals of anhydrous saccharides. The lower limit of detection of free radicals was found to be 1×10^{12} spins/g.

Procedures——Saccharides were heated by standing them in the center of a heating bath for a suitable time (0.25—20 hr). A JEOL ES-05H type UV irradiation unit fitted with a 500 W mercury lamp was used for illumination.

As described later, time dependence of the intensity of the ESR spectrum was observed for the initial 20 hr after heating or after irradiation. Therefore, heated or irradiated saccharides were usually left to stand in air for 24 hr. If necessary, air was displaced by bubbling nitrogen gas for 30 min through a quartz ESR cell, which was then sealed and left for 24 hr.

Results and Discussion

Formation of Free Radicals in Saccharide Powders by Heating

The Effect of Heating Time——Table I shows the effect of heating time on the number of free radicals in powders of glucose, saccharose, lactose, potato starch, or cellulose at the same heating temperature (100°). Heating times were varied from 0.25—20 hours. As can be seen from this table, the number of free radicals was largest in the sacharose or lactose powder among saccharides used here. On the other hand, very little free radical formation was observed in glucose powder. The number of free radicals was found to increase almost linearly with increase in heating time for 1—3 hours at the same heating temperature (100°), but the increase was smaller with further increase in the heating time.

Table I. The Effect of Heating Time on the Number of Spins/g in Saccharide Powders at a Constant Temperature (100°)

Saccharide	Heating time (hr)					
	0.25	0.5	1	3	5	20
Glucose	0×10^{14}	0×10^{14}	0×10^{14}	0×10^{14}	0×10^{14}	0×10^{14}
Saccharose	7.2	14	29	79	118	151
Lactose	7.0	12	24	64	100	143
Potato starch	0.9	1.8	3.4	7.9	8.5	10
Cellulose	0.02	0.04	0.09	0.3	0.4	0.7

Table II. The Effect of Temperature on the Number of Spins/g in the Saccharide Powders for a Given Heating Time (1 hr)

Saccharide	Temperature (°C)				
	70	100	120	150	170
Glucose	0×10^{14}	0×10^{14}	0×10^{14}	0×10^{14}	0×10^{14}
Saccharose	3.1	29	365	5910	7770
Lactose	2.2	24	299	6320	8140
Potato starch	0.8	3.4	48	914	7910
Cellulose	0	0.09	1.4	141	5910

The Effect of Heating Temperature——Table II shows the effect of heating temperature on the number of free radicals in saccharide powders at a given heating time (1 hour). The heating temperature was varied from 70—170°. The number of free radicals increased with increase of the temperature at the same heating time. Below 150°, disaccharides such as saccharose and lactose produced the largest amounts of free radicals among the saccharides used here. Very little free radical formation was observed in glucose powder, even on heating it just below the decomposition point (140°) for 3 hours in air. At 170°, all saccharide powders turned a brown-black color due to decomposition. Even in this case, free radicals were not observed in glucose, although very large amounts of free radicals were formed in other saccharides.

TABLE III. The Effect of UV Irradiation at 25° on the Number of Spins/g in Saccharide Powders

Saccharide	UV Irradiation duration (hr)					
	0.25	0.5	1	3	5	20
Glucose	1.0×10^{14}	2.0×10^{14}	4.1×10^{14}	12×10^{14}	16×10^{14}	58×10^{14}
Saccharose	6.5	13	26	55	88	188
Lactose	6.0	12	22	54	87	184
Potato starch	3.5	7.0	14	35	56	128
Cellulose	1.7	3.2	6.7	20	31	77

Formation of Free Radicals in Saccharide Powders by UV Irradiation

Table III shows the effect of the duration of UV irradiation at 25° on the number of free radicals in the saccharide powders. In this case, in contrast to the results obtained by heating, free radical formation was observed even in the glucose powder. Although the numbers of free radicals were found to be largest in the disaccharide powders, they were comparable in order of magnitude with those of other saccharides. The number of free radicals was found to increase linearly with increase in the duration of UV irradiation up to 3—5 hours, but the increase was smaller with further increase in the duration of irradiation.

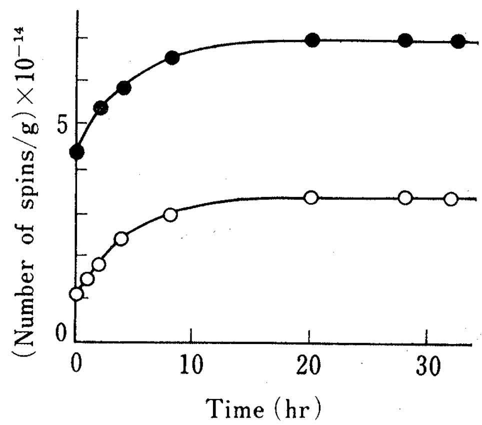

Fig. 1. Time Dependence of the Number of Free Radicals Generated in Potato Starch Powder after Heating or after UV Irradiation

—○—: heated at 100° for one hour.
—●—: irradiated for 30 minutes at 25°.

Time Dependence of the Number of Free Radicals

Fig. 1 depicts the time dependence of the number of free radicals generated in potato starch powder by heating (100°, 1 hour) or by UV irradiation (25°, 30 minutes). As can be seen from this figure, the number of free radicals tends to increase with the passage of time for the initial 20 hours after heating or after irradiation, and then becomes almost constant. Later, it begins to decrease very slowly: less than 5% after a week.

The Results for Other Saccharides

The ESR spectra of monosaccharides such as fructose and mannose were found to be almost the same as that of glucose. Moreover, free radicals generated in powder of α- or β-cyclodextrin, or soluble starch showed almost the same behavior as those generated in potato starch.

Effects of Environment

Free radicals showed almost the same behavior in air and in an atmosphere of nitrogen. That is to say, the number of free radicals in a saccharide and its type of time dependence were not affected by oxygen. On the other hand, the shapes and intensities of ESR spectra of heated or irradiated saccharides were found to change gradually even in an atmosphere of nitrogen under conditions of high humidity. This is probably because of chemical reaction between water molecules and free radicals of the saccharides, forming different kinds of free radicals. However, the decrease in the total number of free radicals with time was found to be very small. For example, about 20% of free radicals were destroyed in the case of potato starch containing about 20 wt % moisture at one week after UV irradiation.

Free radicals were very stable when suspended in benzene, even after the suspension had been vigorously stirred for 10 minutes. On the other hand, very small amounts of free radicals ($<1\times10^{12}$ spins/g) were observed when saccharide powder that had been dried under a vacuum was kneaded well with a polar liquid such as water, methanol, ethanol, or acetone. In this case, free radicals did not reappear even when the powder was again dried under a vacuum.

Effect of Water Content on the Number of Free Radicals in UV-Irradiated Potato Starch

A hysteresis effect was observed between water content and the number of free radicals in UV-irradiated potato starch. In the first place, the number of free radicals was found to decrease with increase in water content before irradiation. Free radical formation was not observed when potato starch powder containing 40 wt % water was illuminated at 25°. Next, when the potato starch powder was dried under a vacuum, UV-irradiated, and then forced to adsorb moisture by being left in a desiccator containing water at the bottom, the number of free radicals was found to decrease with increase in the amount of adsorbed water. However, free radicals did not disappear completely even in potato starch containing 70 wt % water.

Stability of Free Radicals

Free radicals were found to be very stable to oxygen and rather stable to moisture even at room temperature in air. The stability of these free radicals can be explained on the basis of intramolecular hydrogen bonding with OH groups, which exist abundantly in saccharides.[6]

Generally speaking, free radical formation in glucose powder was not observed on heating. This is explained by the fact that glucose is deliquescent, so that application of heat cannot remove water molecules from the surface of glucose particles. Free radical formation in glucose powder upon UV irradiation can be accounted for by the fact that the energy of the UV light is received directly by target glucose particles and is not influenced by water molecules adsorbed on the surface of the particles. Moreover, the following experimental evidence supports this explanation: free radicals of glucose could be observed if the powder was heated *in vacuo*, and free radicals of glucose thus formed were found to be very stable even at room temperature in air.

Application to the Study of Incompatibility

We have clarified that saccharides are not always stable and that free radicals are formed under conditions involving high temperature, exposure to light, and so on. These free radicals may react with other molecules, particularly with water, resulting in modification of the original saccharides, and possibly reducing the efficacy of medicines in which they are present. This type of chemical reaction can be described as incompatibility, and has not been considered previously. As a practical measure, saccharides for use in the manufacture of drugs should be dried under reduced pressure without heating.

References and Notes

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