

the solutions of the complex exhibit the same form, irrespective of the concentration ratio between the reactants, this indicating the presence of only one complex.

*The method of continuous variation.* Curves of continuous variation showed a rounded maximum at the mole fraction 0.5, the curve branches had no inversion and the gradient at the end point was different from zero, these features pointing to the presence of only one complex with the composition SeDi. Detailed curves of continuous variation recorded in the low mole fraction range of selenium confirmed the above conclusions.

*The straight-line method.* Presupposing the presence of a complex of the form  $Se_mDi_n$ , the straight-line method of Asmus<sup>1</sup> with the modification introduced by Klausen and Langmyhr<sup>2</sup> gave the coefficients  $m = 1$  and  $n = 1$ , this confirming the composition SeDi.

*Stability constant.* With the experimental data from the method of continuous variation and from the straight-line method the stability constant for the complex SeDi was calculated to be  $2.8 \times 10^4$  and  $2.5 \times 10^4$ , respectively.

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Received November 22, 1966.

## Effect of Crystal Dimensions on the Yield of Radiation Induced Radicals in Organic Substances

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It is generally assumed that the chemical alterations observed when organic substances are exposed to ionizing radiation to a large extent are mediated by free radicals. Information about the formation and properties of radiation-induced free radicals

can be obtained by electron spin resonance (ESR) spectroscopy. The radical yield, expressed by the  $G$ -value (number of radicals observed per 100 eV absorbed radiation energy), has been determined for a large number of organic substances. The results presented in the literature usually refer to conditions such as high vacuum, a fixed temperature like, for example, room temperature, and extrapolation to zero dose or to doses below 1 megarad where linear "dose-effect" curves are obtained. It appears that even under well-defined conditions the radical yield for a substance may vary considerably from one laboratory to another.<sup>1</sup> These discrepancies may in part be attributed to factors involved in the ESR technique such as the reference system and the quantitative procedure used. However, the observed radical yield may also be influenced by the chemical and physical form of the organic compound. Thus, the hydrochloride of a substance very often exhibits a larger radical yield than the pure compound.<sup>2</sup> Some recent experiments carried out in this laboratory (to be published) demonstrate that the radical yield for a freeze-dried substance may vary with the pH of the solution prior to lyophilization.

In the present communication experimental data will be presented which indicate that the physical dimensions of the crystals in a sample may be of importance with regard to the observable yield of radiation induced radicals.

The amino acid glycine, the dipeptide glycylglycine, and the two enzymes trypsin and lysozyme were studied. Single crystals of glycine and glycylglycine, grown by slow evaporation from aqueous solutions, were compared with freeze-dried samples. The two enzymes were dissolved in water and recrystallized by slow evaporation at 0°C. In this way relatively large particles were obtained which probably consist of microcrystals held together by more amorphous regions. These enzyme particles were ground in a mortar and particles, more or less spherical in shape, were obtained. Particles with a diameter within a certain range were selected by a series of sieves with well-defined openings. In the present experiments the diameter varied from approximately 20  $\mu$  up to about 500  $\mu$ . For all substances the yield data were compared to those for freeze-dried samples which presumably consist of the smallest particles.

The samples were evacuated to a vacuum of about  $10^{-4}$  mm Hg, which is sufficient to

Table 1. Radical yield and crystal size.

Substance	Crystal dimension	Irradiation temperature	Yield (G-Value)	Ratio <sup>a</sup>
Glycine	Crystalline <sup>b</sup>	77°K	3.4	1.24
Glycine	Freeze-dried	77°K	4.2	
Glycine	Single crystal <sup>c</sup>	295°K	6.2	1.10
Glycine	Freeze-dried	295°K	6.8	
Glycyl-glycine	Crystalline	77°K	5.0	1.26
Glycyl-glycine	Freeze-dried	77°K	6.3	
Glycyl-glycine · HCl	Single crystal <sup>d</sup>	295°K	16.6	1.10
Glycyl-glycine · HCl	Freeze-dried	295°K	18.3	
Trypsin	500 $\mu < d - e$	295°K	3.6	1.61
Trypsin	297 $\mu < d < 500 \mu$	295°K	3.6	
Trypsin	149 $\mu < d < 297 \mu$	295°K	4.5	
Trypsin	53 $\mu < d < 149 \mu$	295°K	4.8	
Trypsin	— $d < 37 \mu$	295°K	5.1	
Trypsin	Freeze-dried	295°K	5.8	
Lysozyme	297 $\mu < d < 500 \mu$	295°K	4.1	1.44
Lysozyme	37 $\mu < d < 53 \mu$	295°K	5.0	
Lysozyme	Freeze-dried	295°K	5.9	

<sup>a</sup> The data given in this column refer to the ratio between the largest and the smallest yields within each set of comparable measurements.

<sup>b</sup> Polycrystalline samples were prepared from single crystals by grinding in a mortar.

<sup>c</sup> Single crystals were grown by slow evaporation from aqueous solution. The glycine crystals are monoclinic and all ESR measurements were carried out with the magnetic field in the *ab* plane.

<sup>d</sup> The crystals were grown by slow evaporation from aqueous solution. The crystals are monoclinic with the space group  $P2_1/c$ . All ESR measurements were carried out with the magnetic field in the *bc* plane.

<sup>e</sup> The crystals or particles are more or less spherical in shape. Particles with diameter (*d*) in a certain range were obtained by a series of sieves with well defined openings.

prevent any oxygen effect. The samples were irradiated with 220 kV X-rays to a dose of  $5 \times 10^5$  rad, which is low enough to ensure that we are on the linear part of the dose-effect curve. ESR spectra were recorded with an X-band spectrometer with 110 kc/sec modulation frequency. The first derivative spectra were integrated twice and compared with those for the reference system. Anthracite carbon powder, calibrated against DPPH freshly recrystallized from  $CS_2$ , was used as reference system. The absolute yield data may suffer from large systematic errors, but the relative values between the different samples are reproducible to within 5 %.

The results of the present experiments are given in Table 1. It appears that the

crystal or particle dimensions have a significant influence on the number of radicals trapped in a sample. Thus, the radical yield decreases with increasing particle diameter as revealed for the two enzymes. The data obtained with glycine indicate that the difference between freeze-dried and crystalline samples is larger when the samples are irradiated at liquid nitrogen temperature than at room temperature.

The present results are of interest with regard to the mechanisms for stabilization of radiation induced radicals. The initial events following the absorption of radiation energy result in a variety of species containing unpaired spins. Some of these

ESR centers disappear rapidly whereas others are trapped or become involved in secondary reactions leading to other types of free radicals.<sup>2</sup> The free radicals studied in most ESR experiments are those that for certain reasons are trapped and consequently have a sufficiently long lifetime. The present results demonstrate that the observed radical yield increases with decreasing particle dimensions. This observation suggests that the concentration of traps or sites for unpaired spins is not homogeneous, but increases towards the surface of the particle.

The nature and properties of the radical traps are largely unknown. It is reasonable to assume that lattice imperfections and disorder represent one type of sites or traps for unpaired spins. In some recent experiments Phillips *et al.*<sup>3</sup> observed that freeze-dried glucose was crystallographically indistinguishable from crystalline glucose. However, from electron microscopy it appeared that the freeze-dried crystals exhibited a textured solid where the imperfection and disorder in position reached such a degree that space lattice was no longer a useful representation. It is likely that the concentration of imperfections and disorder is larger on the surface compared to the interior of a crystal, and furthermore, larger on the surface of a freeze-dried sample compared to a more perfectly grown single crystal. Consequently the observation that freeze-dried samples exhibit a larger radical yield than polycrystalline samples and single crystals is in agreement with the hypothesis that the concentration of radical traps increases towards the surface of a particle.

Whereas the initial formation of unpaired spins presumably is uniform throughout the crystal when X- and  $\gamma$ -rays are used, the trapping of ESR centers seems to be more efficient on the surface. On the other hand, it is possible to produce free radicals only in a surface layer by bombardment with thermal hydrogen atoms. It seems therefore possible to study the distribution of radical traps in a crystal in more detail by a combination of hydrogen bombardment and X-rays. Work along these lines is now in progress.

This investigation was supported by *Division of Radiological Health, Bureau of State Service, U.S. Public Health*. The author is fellow of the Norwegian Cancer Society.

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Received November 23, 1966.