COMMUNICATIONS TO THE EDITOR

DETECTION OF POLYMERIZATION RADICALS BY PARAMAGNETIC RESONANCE[1]

Sir:

We have directly verified the presence of free radicals formed during vinyl polymerization by means of paramagnetic resonance absorption spectroscopy. Cross-linked systems such as glycol dimethacrylate (GDM) in which relatively high concentrations of long-lived radicals may be expected have been employed.

GDM polymers were formed by heating monomer and an initiator at 60–100° in sealed glass tubes until gelation occurred. They were then cooled and the spectra of the partially polymerized samples observed at room temperature.[2] Identical spectra, except for intensity, were observed using one (and sometimes ten) weight per cent. of several initiators: benzoyl peroxide, azobisisobutyronitrile, *t*-butyl perbenzoate, and di-*t*-butyl peroxide. Irradiation of the gelled samples at room temperature with a Hanovia type 7420, 500-watt, mercury-vapor lamp increased the intensity of spectra, again without change in form. The radical concentration is estimated to be as high as 10^{-3} to 10^{-4} molar in some samples, and radicals are still observable in the sealed tubes after three months storage.

When GDM is diluted with methyl methacrylate (MM), we observe identical spectra except for a possible decrease in intensity, and with pure MM, spectra are only observable in a highly polymerized sample after intense irradiation.

The spectrum of GDM and MM polymers consists of five symmetrically located absorption peaks with four subsidiary peaks interspersed between the main lines. It spreads over a region of about 100 gauss centered about a value of the magnetic field close to the position expected for a free electron, and is similar in its essential details to the spectrum obtained from X-ray irradiated poly-(MM) by Schneider, Day, and Stein.[3] These authors attributed this complex spectrum to interactions between electrons trapped in holes at more or less regular intervals in the polymer matrix, but our work indicates that it represents the actual free radicals involved in the polymerization process (or, in their case, perhaps depolymerization) immobilized in the gelled or glassy system. The complex fine structure observed may be due to interactions between the unpaired electrons and the magnetic moment of neighboring protons, but we have been unable to devise a satisfactory splitting pattern from the presumed structure of the radical species believed to be present.

Spectra also have been observed in gels of vinyl methacrylate, divinylbenzene (a 55% technical material), and glycol diacrylate. Vinyl methacrylate gives a spectrum similar to GDM except that individual peaks are broadened and subsidiary peaks have disappeared. The spectrum from divinylbenzene at the level of microwave power normally used shows a single peak, but when the level is reduced, it splits into three partially resolved lines spread over 47 gauss between extreme points of maximum slope, indicating that saturation of the spin system is occurring. The spectrum from glycol diacrylate, which was only observed after moderate irradiation, is similar to that from divinyl benzene at low power levels, but the lines are not as well resolved.

This variation of spectrum with monomer structure is further evidence that we are actually observing the radicals involved in the polymerization process, and shows the utility of the method in both detecting and identifying the intermediates in free radical processes.

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[1] Supported in part by the Squier Signal Laboratory, U. S. Army Signal Corps.

[2] A preliminary account of our instrument, which employs a type 2K25 klystron at a wave length of 3.2 cm., has been published: J. M. Hirshon, R. L. White, and G. K. Fraenkel, *Rev. Sci. Instr.*, **23**, 772 (1952). A detailed account has been submitted for publication by J. M. Hirshon and G. K. Fraenkel to *Rev. Sci. Instr.*

[3] E. E. Schneider, M. J. Day and G. Stein, *Nature, Lond.*, **168**, 645 (1951).

A TECHNIQUE FOR STEPWISE DEGRADATION OF PROTEINS FROM THE AMINO-END[1]

Sir:

The techniques used for stepwise degradation of peptides by the phenyl isothiocyanate method[2] cannot be applied to proteins, since the phenylthiocarbamyl proteins are insoluble in the acid media needed for formation of phenylthiohydantoins (PTH). Saturation of the solution with guanidine hydrochloride has overcome this difficulty with insulin.[3]

A technique has now been developed which is considerably simpler and permits the stepwise release of PTH's from a number of proteins. The principal feature is the use of small strips of filter paper as carriers for the protein throughout the procedure. The distribution of the protein over a big surface renders it accessible to the reagents and prevents precipitation by acid.[4]

Aliquots of a protein solution (0.05 or 0.1 ml., 1–3 mg.) are applied evenly to about four-fifths of the area of 2–12 small strips (1 × 5 cm.) of Whatman No. 1, held suspended by chromatographic clamps and marked at one end. The papers are allowed to dry, then wetted with 0.05 ml. of 20%

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[1] Aided by a grant from the National Foundation for Infantile Paralysis.

[2] P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950); M. Ottesen and A. Wollenberger, *Compt. rend. trav. lab. Carlsberg, Ser. chim.*, **28**, 463 (1953); B. Dahlerup-Petersen, K. Linderstrøm-Lang and M. Ottesen, *Acta Chem. Scand.*, **6**, 1135 (1952).

[3] H. Fraenkel-Conrat and J. Fraenkel-Conrat, *ibid.*, **5**, 1409 (1951).

[4] The use of paper strips was suggested by the work of J. Leonis (*Bull. Soc. Chim. Belges*, **61**, 524 (1952)), but it fulfills here a different and more crucial role.