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# Graft Photopolymerization of Styrene to Wheat Gluten Protein in Dimethyl Sulfoxide*

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## SUMMARY

To determine the suitability of dimethyl sulfoxide (DMSO) as a solvent for photopolymerization, solutions of wheat gluten protein (0.28-0.93% by weight) and styrene (4.13-12.65% by weight) in DMSO were irradiated by a 200-W high-pressure mercury arc lamp from 3 min up to 1 hr. Graft copolymers of gluten styrene resulted that contained styrene residues ranging from 2 to 23% by weight. When gluten protein was photolyzed in DMSO alone, a significant amount of sulfur from the solvent was incorporated; however, styrene successfully competed with the solvent for free radical sites. The rate of grafting was directly related to both the concentration of gluten and of styrene. Also, the ratio of grafted polystyrene to gluten in the graft polymer indicated that the grafts were composed of small units of polystyrene.

## INTRODUCTION

A previous study that involved the photochemical decomposition of wheat gluten protein in the nonaqueous solvent, dimethyl sulfoxide

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(DMSO) [1] showed that extensive photodestruction of certain amino acids occurred in this solvent. Presumably, a large number of free radical sites were formed on the protein during photolysis. We also assumed that these free radical sites would be available for grafting by $\alpha,\beta$-unsaturated monomers, such as styrene. However, during the photolysis of proteins in DMSO, sulfur became incorporated into the photoproduct. Since the most reasonable source of this sulfur is from a reaction of the solvent, DMSO, with the protein, there is possibly a competition here between the solvent and monomer for the free radical sites of the protein. Therefore, it was necessary to establish whether graft polymerization of vinyl monomers would occur in DMSO, and to what extent the solvent would compete with vinyl monomers for reactive sites on the protein. Specifically, we determined the suitability of DMSO as a solvent for the graft photopolymerization of styrene to wheat gluten protein. (Information on the structure and properties of this protein is contained in a number of previous publications from this Laboratory [2-5].) DMSO is particularly useful because it dissolves many proteins not soluble in other organic solvents and also because it is compatible with common vinyl monomers.

The grafting reaction is expected to proceed by light-initiated free-radical formation, followed by propagation of the polystyrene chain (Scheme 1).



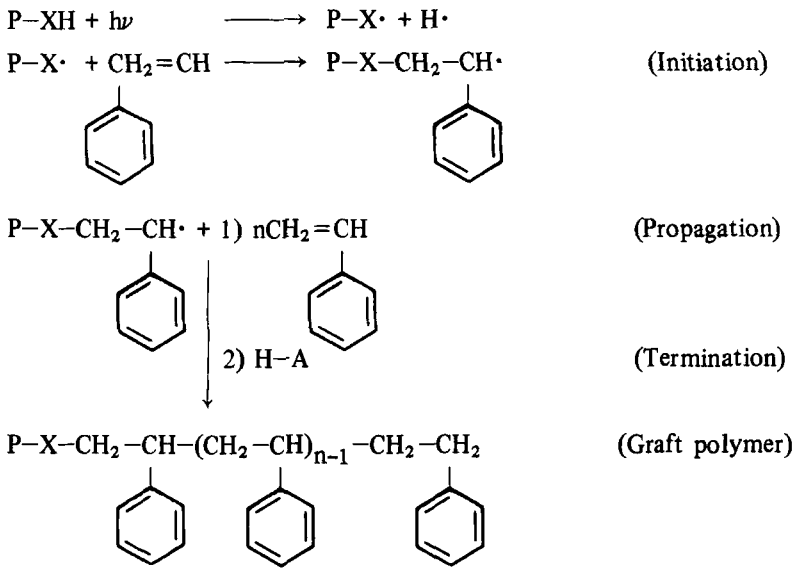
## EXPERIMENTAL

### Materials

Wheat gluten protein was isolated from Ponca wheat flour by the method of Jones et al. [4]. DMSO (Baker analyzed reagent) was dried with calcium hydride, distilled under vacuum, and stored under nitrogen until used. Styrene (Eastman red label) was distilled under vacuum at 22°C and used immediately.

### Photolysis Procedure

The protein and styrene for each solution were dissolved in DMSO to a total volume of 40 ml. The concentrations of protein varied from 0.28 to 0.93% w/w and the concentrations of styrene from 4.13 to 12.65% w/w. For photolysis, the solutions were placed in quartz test tubes (20 X 175 mm) and arranged around a central ultraviolet light source (Hanovia 654A36, 200-W high-pressure mercury arc). The tubes were held in place at a distance




P = Protein

X = Protein functional groups

Scheme I

of 4 cm (center of light to center of tube) by a circular Plexiglas frame, which did not obstruct the path of light to the solutions (Fig. 1). The 200-W lamp was cooled by circulating tap water, and the area surrounding the reaction tubes remained at room temperature. After photolysis, the reaction was quenched with hydroquinone and the solutions were dialyzed against 70% ethanol. This step was followed by exhaustive dialysis against 0.01 N acetic acid and then the solutions were lyophilized to a solid product. The reaction mixture was extracted with benzene to remove any homopolymer and then analyzed for carbon content.

## Extent of Grafting

The amount of styrene grafted to the protein was calculated from the carbon content by Eq. (1), assuming that the grafting reaction and the product recovered were homogeneous:

$$\text{Wt styrene, mg} = \frac{\text{Wt gluten, mg (\%C, graft polymer - \%C, gluten)}}{\text{(\%C, styrene - \%C, graft polymer)}} \quad (1)$$

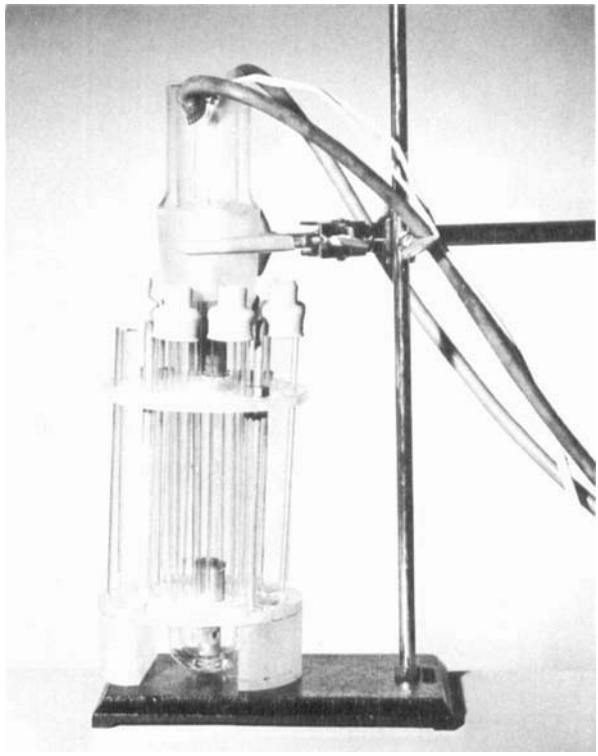


**Fig. 1.** Photolysis apparatus.

The per cent grafting was also estimated by a nuclear magnetic resonance (NMR) technique. The intensity of the signal near $\tau$ 2.7 associated with aromatic protons in the graft copolymer was about four times greater than the corresponding signal for gluten photolyzed in the absence of styrene. Since the enhanced signal in the NMR spectrum of the gluten-styrene copolymer must be due to the presence of additional aromatic residues, it is possible to calculate the per cent styrene in the copolymer from the known number of aromatic amino acid residues in gluten photolyzed in the absence of styrene. The calculated value of 28% agrees reasonably well with average values determined independently from carbon analyses.

The infrared (IR) spectrum of the gluten-styrene copolymer has a new strong peak at 690 $cm^{-1}$ associated with monosubstituted benzene derivatives [6]. This peak does not appear in the IR spectrum of gluten. The

IR data, therefore, qualitatively confirm the presence of styrene residues in the protein derivative.

### Amino Acid Analysis

Samples of graft polymer were hydrolyzed with constant boiling HCl by refluxing for 24 hr. After removal of HCl by rotary evaporation, the samples were diluted with 2.2 M citrate buffer to a concentration of approximately 1 mg of original sample per ml for analysis. The insoluble material was filtered, washed, and dried for further characterization. Each sample solution was then analyzed for milligrams of nitrogen per milliliter and the amino acid analysis data were calculated on this basis. Hydrolyzed samples were analyzed for amino acids in a Technicon automatic analyzer TSM and data automatically computed as described by Cavins and Friedman [7].



## RESULTS

Some of the properties of the graft polymer are shown in the left-hand column of Table 1. In the right-hand column are comparable properties of a sample blank of wheat gluten photolyzed under identical conditions except for the presence of styrene.

From the data given in Table 1, it is evident that the character of the graft polymer is different from that of a sample of gluten photolyzed without styrene. The most obvious changes are carbon content, solubility, and extent of acid hydrolysis. The melting points and amino acids analyses are not substantially different, and a low level of grafting is indicated.

Some of the variables that affect this grafting are the concentrations of protein and styrene and the time of photolysis.

Figure 2 shows the effect of gluten concentrations on the amount of styrene grafted during a 25-min photolysis in DMSO at a fixed styrene concentration of 12.6% w/w. At a gluten concentration of 0.28% w/w, the amount of styrene grafted represents only a small fraction (0.46%) of the total styrene. When gluten concentration is doubled to 0.56% w/w, the amount of styrene grafted is also doubled. Again this amount represents only a small percentage (0.80%) of the styrene available.

Figure 3 plots the effect of styrene concentration on the amount of styrene grafted at a fixed concentration of gluten (0.46% w/w) during a 1-hr photolysis. When styrene concentration is 4.13% w/w, 22 mg of

**Table 1.** Nature of Graft Polymer

| Gluten 0.93% w/w,[a] styrene 12.6% w/w | Gluten 0.93% w/w[a] |
|---|---|
| 59.1% Carbon | 47.5% Carbon |
| Insoluble[b] 0.1 N HOAc, 6M guanidine·HCl | Soluble[b] 0.1 N HOAc, 6M guanidine·HCl, DMSO, benzyl alcohol/HOAc (95/5) |
| Soluble DMSO, benzyl alcohol/HOAc (95/5) | |
| Partially hydrolyzed by 6 N HCl reflux, 24 hr | Completely hydrolyzed by 6 N HCl reflux, 24 hr |
| Amino acid analysis similar to blank | |
| Insoluble material from acid hydrolysis 84% C, 0.9% N not completely soluble in benzene | |
| Mp 275-295 dec. | Mp 275-295 dec. |

[a]Photolyzed 1 hr in DMSO under conditions previously described.
[b]Sample is concentration of 2 mg/ml.



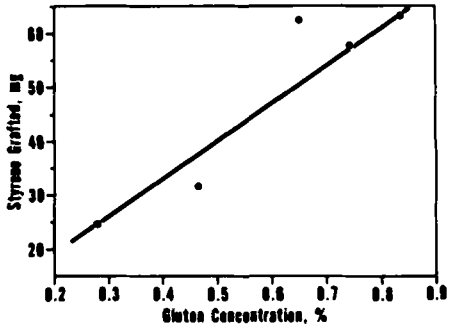



**Fig. 2.** Amount of styrene grafted as a function of gluten concentration in dimethyl sulfoxide (DMSO). Fixed styrene (12.65%) and gluten concentrations expressed as per cent by weight. Photolysis for 25 min with 200-W high-pressure mercury arc lamp.

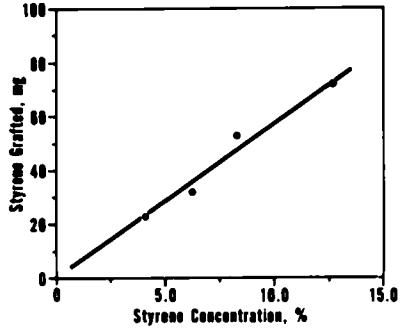



Fig. 3. Increase in amount of styrene grafted as styrene concentration increases. Fixed gluten (0.46%) and styrene concentrations expressed as per cent by weight in DMSO. Photolysis for 1 hr by 200-W high-pressure mercury arc lamp.



styrene is grafted to the 200 mg of gluten present. When styrene concentration is doubled to 8.26% w/w, the amount of styrene grafted also doubles to 46 mg. At a styrene concentration of 12.65% w/w, 72 mg of styrene is grafted. In each case a constant percentage (~1.25%) of the total styrene available is grafted. These facts are complemented by rate studies (Fig. 4) which show that the rate of grafting is directly related to styrene and gluten

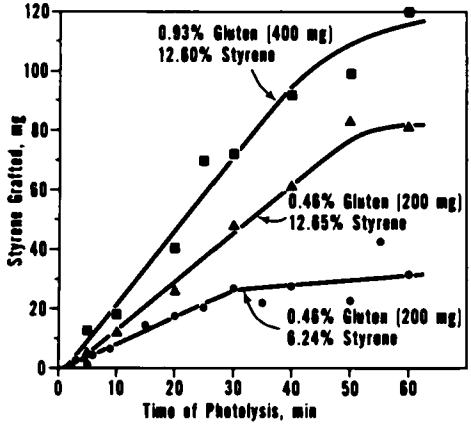



Fig. 4. Rate of grafting of styrene to gluten protein in DMSO. Protein and styrene concentrations expressed as per cent by weight. Photolysis with 200-W high-pressure mercury arc lamp.

concentrations. For a solution of styrene (6.24% w/w) and gluten (0.46% w/w) in DMSO the rate of grafting is approximately 0.75 mg/min and the reaction is complete in 30 min. Doubling the concentration of styrene to 12.65% w/w increases the rate to 1.5 mg/min and the reaction is complete in 50 min. Doubling both the gluten (0.93% w/w) and styrene (12.65% w/w) concentrations increases the rate of 2.6 mg/min and the reaction is almost over in 50 min.

## DISCUSSION

These results indicate that the product formed when gluten and styrene are photolyzed in DMSO is a graft polymer that has properties different from gluten or photolyzed gluten. Also, the amount and rate of grafting are direct functions of the concentration of gluten and styrene. The rate of grafting, therefore, can be represented by an expression involving the concentration of gluten and styrene each to the first power:

$$\text{Rate} = k[\text{Gluten}][\text{Styrene}] \quad (2)$$

where rate is rate of grafting to gluten and k is a constant.

At the highest level of grafting (1 hr, 0.93% gluten, 12.6% styrene) the styrene grafted amounts to 23% by weight of the product. Since the average molecular weight of an amino acid is 100 (styrene 104), roughly 23 molecules of styrene are present for every 100 molecules of graft polymer or abut three amino acids per styrene molecule.

A purely chemical average of the molecular weight of the graft can be determined by elemental analysis of that portion of the graft polymer not hydrolyzed by 6 N HCl. The percentage carbon and nitrogen of the grafts and their residual amino acids suggest that the average graft is 10-15 units long. Unfortunately, this estimate gives no idea of the actual distribution of molecular weights involved and thus no idea of the number of amino acids involved in grafting. Since amino acid composition changes little with increasing amounts of grafting, only a small portion of the amino acids is likely to be involved in grafting (5% or less). At any rate, the highest level of grafting uses only 2.2% of the total styrene available. Another 1% is used to form homopolymer of MW $>$ 10,000. Six or seven per cent more of the total available styrene is consumed by reactions other than these (Fig. 5), probably in formation of lower MW homopolymers. After 60 min of photolysis, only 10% of the styrene available

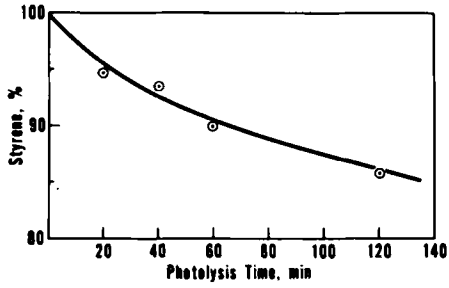



Fig. 5. Decrease in monomer as a function of time of photolysis.

has been utilized. This level indicates little reactivity under these conditions.

As reported previously, photolysis of gluten protein in DMSO without vinyl monomer significantly increased the sulfur content of the photoproduct; the most obvious source of this sulfur incorporation was the solvent. The percentage sulfur of gluten that has been photolyzed for 1 hr in DMSO without styrene is 1.90% (cf. 0.95% for unphotolyzed gluten). When styrene is added to a DMSO solution of gluten, the amount of sulfur incorporated into the gluten by photolysis is decreased (Fig. 6). Since the presence of styrene (~10% w/w) halts the incorporation of sulfur, the vinyl monomer effectively competes with solvent for free radical sites on the protein.

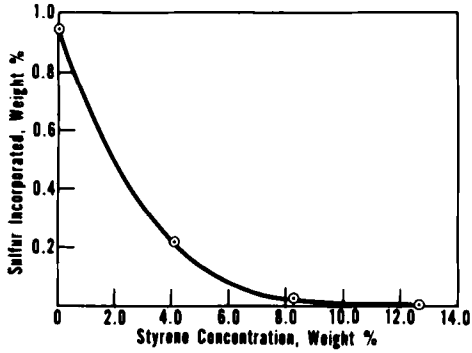



Fig. 6. Decrease in sulfur incorporated into wheat gluten during 1 hr photolysis in DMSO as function of styrene concentration.

We conclude that: (a) Grafting of styrene to gluten in DMSO does occur under photolytic conditions; however, the grafting reaction is not efficient under the conditions used. (b) A product is formed whose properties differ

from gluten or photolyzed gluten and which probably consists of small chains (10-15 units) of polystyrene grafted into a limited number of amino acids (less than 5.0%). (c) The amount and rate of grafting are direct functions of the concentration of gluten and styrene in the reaction mixture. (d) At a concentration of 10% w/w, styrene competes effectively with DMSO for reactive sites on the protein.

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