of 2.10 Å as being typical of neutral sp^3-hybridized nitrogen atoms in macrocyclic high-spin nickel complexes (see ref 7 and 11), then an optimum hole size of ~4.25 Å is predicted for nickel(II). This is larger than that of 4.13 Å determined by X-ray for free $15\text{-}O_2N_2$ in a flat conformation (McPartlin, M.; Tasker, P. A.; Trotter, J., unpublished results) and supports the postulate that the hole size of $16\text{-}O_2N_2$ is the one of best fit for the present series of ligands.

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Photooxygenations in Aqueous Solution with a Hydrophilic Polymer-Immobilized Photosensitizer
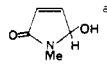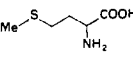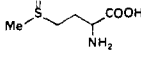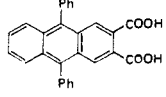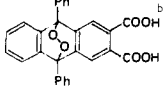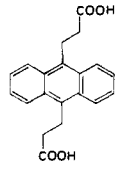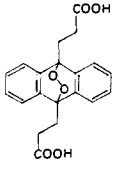
Sir:

In 1973 the synthesis and use of the first example of a heterogeneous sensitizer for singlet oxygen formation in organic solvents was reported.[1] This polymer-immobilized reagent, Ⓟ-Rose Bengal, consists of a photosensitizing dye, Rose Bengal, covalently bound to an insoluble poly(styrene–divinylbenzene) matrix. Photooxygenations employing this heterogeneous sensitizer, in place of a soluble dye, have been reported to be synthetically convenient and mechanistically less complicated.[2] Ⓟ-Rose Bengal has proved particularly useful in the isolation of unstable primary photooxygenation products such as 1,2-dioxetanes.[3]

Heterogeneous sensitizers permit the design of experiments not possible with soluble dyes. An example is Foote's recent three-phase test for the intermediacy of singlet oxygen in photooxidation.[4] Use of a polymer-bound substrate and Ⓟ-Rose Bengal allowed for more definitive conclusions than possible with Kautsky's early experiments.[5] Ⓟ-Rose Bengal has also been used in a similar way to show that photodynamic inactivation of *E. Coli* requires diffusion of singlet oxygen into the cell.[6] In addition to these unique features, heterogeneous sensitizers have a number of practical advantages over soluble sensitizers. These include (1) increased photostability of the dye on the polymer; (2) decreased secondary interaction of the photosensitizer with substrates or products; (3) utility in solvents in which the free dye is insoluble; (4) reuse of the sensitizer; (5) removal by simple filtration or sedimentation. The last feature implies, for example, that a continuous photooxidation process should be feasible, and that photooxygenations may be followed spectroscopically in regions normally masked by dye absorption.

Although Ⓟ-Rose Bengal has been demonstrated to be useful in most organic solvents, we have found it to be a poor photosensitizer in aqueous systems. The reason for this limited effectiveness is related to the observations that the hydrophobic polymer is not wetted by water, is difficult to suspend in aqueous media, and does not swell in water. A water-compatible, covalently immobilized sensitizer would extend the utility of the method to biological media. In view of the wide interest in singlet oxygen in biochemical systems, for instance regarding its role in photodynamic action,[7] and because of its use as a tool to probe protein structure,[8] we now report the preparation of such a sensitizer, suitable for use in water.
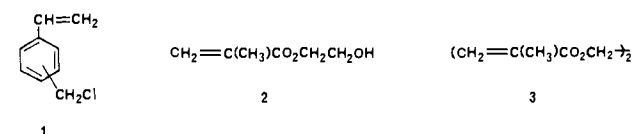
A hydrophilic polymer was prepared by copolymerization of chloromethylstyrene (60:40 mixture of meta and para isomers) (**1**) and the monomethacrylate ester of ethylene glycol (**2**) with the bis methacrylate ester of ethylene glycol (**3**) as

Table I. Photooxygenations with Ⓗⓟ-Rose Bengal

substrate	solvent	product	isolated yield	ref
	H_2O, pH 7	[a]	36%	16
	H_2O		85%	17
	H_2O, pH 9	[b]	89%	18
	H_2O, pH 8		95%	19
PhSMe	MeOH	PhSOMe [c]	99%	20

[a] With soluble Rose Bengal, purification of this product was significantly more troublesome. [b] This product was quantitatively converted back to starting material upon heating. [c] Overoxidation to the sulfone was not observed.
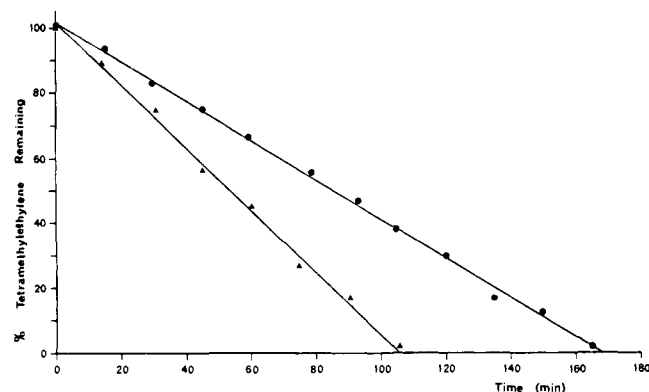
cross-linking agent. The resulting polymer was obtained in the form of beads (38–75 μ) and was heated with Rose Bengal in

dry dimethylformamide at 60 °C for 3 days to yield the hydrophilic sensitizer, Ⓗⓟ-Rose Bengal.[9] After careful extraction with several solvents to remove occluded dye,[10] the bright red beads were analyzed for bound Rose Bengal. The iodine content of 4.61% indicated that the heterogeneous sensitizer contained 0.091 mmol of Rose Bengal/g. The Ⓗⓟ-Rose Bengal was found to be wetted by water and to swell by 40% in this solvent.

The effectiveness of Ⓗⓟ-Rose Bengal in sensitizing singlet oxygen formation in water is illustrated by the successful photooxygenation of a number of substrates known to react with singlet oxygen in water.[11] Typically, photooxidations were conducted with 0.01–0.1 M substrate[12] in distilled water. Sodium hydroxide or hydrochloric acid were used to adjust pH where necessary. Suspensions of 5 to 20 mg/mL of Ⓗⓟ-Rose Bengal were generally used, except in large-scale reactions where as little as 0.3 mg/mL was found adequate.[13] Experiments have shown that a 1-cm depth of a stirred suspension containing 10 mg/mL of Ⓗⓟ-Rose Bengal absorbs ~93% of the incident light at 550 nm. Both 500-W quartz–halogen lamps with a UV-cutoff filter and a 300-W xenon lamp coupled with a 0.25 m Jarrell–Ash monochromator were used as light sources. Recently we have found that a 400-W sodium lamp (General Electric Lucalox) is particularly suited to this sensitizer. The bulk of the emission is at wavelengths absorbed by Ⓗⓟ-Rose Bengal, and the absence of a significant UV component obviates the need for filters. Further, the lamp is energy efficient, has a long lifetime, and requires little cooling.

The results of the photooxidation experiments are presented in Table I. The last entry indicates that the sensitizer is also synthetically useful in alcoholic solvents. In each case, the products of the reaction using Ⓗⓟ-Rose Bengal were compared with those obtained using soluble dye. UV–visible spectroscopy of filtered reaction solutions did not show any

Figure 1. Quantum yield studies with (HP)-Rose Bengal. Photooxidation of tetramethylethylene using a 400-W General Electric high-pressure sodium lamp: (▲) reaction solution with 0.07 M tetramethylethylene and 4.91×10^{-5} M soluble Rose Bengal in methanol; (●) reaction solution with 0.07 M tetramethylethylene and 10 mg/mL of (HP)-Rose Bengal in methanol. Identical apparatus was used in both cases, and controls showed that the path length was sufficient to ensure absorption of all of the incident light.

detectable absorption due to free Rose Bengal indicating that (HP)-Rose Bengal functions as a true heterogeneous sensitizer and is stable to photooxidative conditions. Indeed, it proved possible to reuse the sensitizer repeatedly with no apparent loss of efficiency.

In view of the satisfactory behavior of (HP)-Rose Bengal in water, we were prompted to compare the effectiveness of (HP)-Rose Bengal with that of (P)-Rose Bengal in a variety of solvents. Photooxidation of tetramethylethylene using standard suspensions of the two sensitizers showed that both sensitizers behaved very similarly in polar solvents such as acetone, dichloromethane, or methanol.[14] The quantum yield for singlet oxygen formation was determined for (HP)-Rose Bengal in methanol by comparison of the zero-order rates of photooxygenation of tetramethylethylene using this sensitizer and soluble Rose Bengal (Figure 1). The assumption of a steady-state concentration of singlet oxygen[1b] and the known quantum yield of 0.76 for its formation from soluble Rose Bengal in methanol[15] lead to a value of 0.48 for the quantum yield of singlet oxygen formation from (HP)-Rose Bengal in methanol. This figure compares very favorably with the value of 0.43 determined for (P)-Rose Bengal in dichloromethane.[1b] In contrast to (P)-Rose Bengal, however, we have found that (HP)-Rose Bengal is not useful as a sensitizer in nonpolar media such as dioxane, toluene, or octane, in accord with its hydrophilic nature.

A comparison of the relative effectiveness of the two heterogeneous sensitizers in water has been made. We have found that adequate suspensions of (P)-Rose Bengal result if 0.05% TWEEN 80 is added to permit wetting of this polymer. In the presence of TWEEN 80, using 10-mg/mL suspensions of both polymers, 9,10-anthracenedipropionic acid sodium salt (10^{-4} M) undergoes 63% reaction in 1 min with (HP)-Rose Bengal, whereas only 4% reaction occurs with (P)-Rose Bengal. Clearly the hydrophilic polymer-bound sensitizer is superior under these conditions and should provide a valuable alternative for photooxygenations in aqueous media.

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4a-Hydroperoxyflavin N-Oxidation of Tertiary Amines

Sir:

Xenobiotic substances are oxidatively metabolized in the hepatic tissue by microsomal monooxygenases of the cytochrome P-450 class and by flavomonooxygenases. The N-oxidation of amines in animals is a function of the hepatic flavomonooxygenases. Hepatic monooxygenase activities toward amines have become of particular concern[1][3] owing to the fact that people are increasingly subjected to numerous pharmacologically active nitrogen compounds (nicotine, tranquilizers, antihistamines, narcotics, hallucinogens, tropic alkaloids, ephedrine and derivatives, etc.) and the N-oxidation of arylamines and arylamides is a prerequisite in the conversion of these agents into their ultimate carcinogenic derivatives.[4]