[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Photoreduction of Dyes in Rigid Media. I. Triphenylmethane Dyes[1]

BY GERALD OSTER, JACQUES JOUSSOT-DUBIEN[2] AND BARRET BROYDE

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Certain triphenylmethane dyes can be photoreduced when incorporated in glucose glasses, whereas the same dyes in aqueous solutions of glucose are unaffected by light. All triphenylmethane dyes become increasingly more fluorescent the greater the viscosity of the medium. In the case of the dyes which are photoreducible in glucose glass, however, superposed on the room temperature fluorescence is an α-phosphorescence (delayed fluorescence). In the more rigid glasses the phosphorescence lifetime was found to decrease with increasing temperature but to be practically independent of viscosity. The rate of photoreduction of acid fuchsin in glucose as a function of temperature is maximal at about 60°. The kinetic and spectral data suggest that three processes must be considered: (1) the suppression of internal conversion of the single excited state to the ground state by increasing the viscosity of the medium, (2) the transitions from the metastable state to the ground state, (3) the reaction between dye molecules in the metastable state and glucose. The first process is favored by lowering the temperature, whereas the reverse is the case for the latter two processes.

Introduction

It has been established that the photoreduction of dyes in aqueous media containing mild reducing agents proceeds *via* a long-lived intermediate state.[3-8] This state has a lifetime in water of the order of 10^{-4} sec. compared with about 10^{-9} sec. for the lifetime of the first singlet electronically excited state. It was further found that those dyes which are photoreducible exhibit a prolonged phosphorescence in glycerol at low temperatures.[3-8] Such phosphorescence is associated with transitions from a metastable state of energy intermediate between that of the first singlet excited state and the ground state.[9] The metastable state, at least for the case of fluorescein in boric acid glass, has been identified as the triplet state.[10-12]

In order to undergo transition to the long lived excited state, triphenylmethane dyes must be held in some fixed configuration when excited.[5] These dyes in water do not undergo appreciable photoreduction unless they are bound to high polymers. In addition, the lifetime of the first excited state of these dyes is prolonged as shown by the appearance of fluorescence when polymer is added to the solution.[5] This fluorescence phenomenon also occurs for diphenylmethane dyes and for stilbene derivatives,[13,14] that is, for those dyes where the molecules are capable of internal rotation. The fluorescence of such dyes is also enhanced by increasing the microscopic viscosity of the medium.[15]

By suppressing internal conversion from the excited singlet state to the ground state, the number of transitions to the metastable state should increase. It would follow, therefore, that for molecules capable of internal rotation, such as the triphenylmethane dyes, the rate of photoreduction should increase with increasing viscosity. However, photoreduction involves binary encounters between the light excited dye molecules and the electron donor molecules. Such encounters are diffusion-controlled and hence their number per unit time varies inversely as the viscosity of the medium. Therefore, increasing the viscosity has two opposing effects on the rate of photoreduction.[16]

If the medium itself is the reducing agent then the photoreduction does not depend on binary diffusion encounters. In this case, every collision of an excited dye molecule with its nearest neighbors is an encounter between the reaction species and the frequency should be independent of viscosity. Indeed, this appears to be the case when glucose glass is used as the medium. We have found that despite the enormous viscosity of the glass photoreduction of certain triphenylmethane dyes does occur in this medium. It is the purpose of the present paper to describe and interpret our results for the photoreduction of triphenylmethane dyes, particularly acid fuchsin, in various glucose glasses.

Experimental

Materials.—The dyes used were histological grade obtained from National Aniline Division of Allied Chemical and Dye Corporation. Glucose was Fisher reagent grade.

Preparation of Glucose Glasses.—The properties of glucose glasses depend considerably on the mode of preparation. Two methods were used in the course of the present work. The first method yields a yellowish glass from which all the water has not been completely eliminated and in which the dye can be dissolved. The second method gives a practically colorless glass containing no water but in which the dye is only poorly soluble. For the first type glass the viscosity, as measured at 85° with a Brookfield viscometer (Model HBF) is seven times less than that of the second type glass.

The first type of glass is made by adding water to glucose, or when the dye is to be incorporated into the glass by adding an aqueous solution of the dye to glucose and stirring. When the mixture is homogeneous it is heated at 80° under vacuum until most of the hydrated water has been eliminated. The temperature of the system is raised to 160° and maintained there until all the crystals are melted, and then the system is rapidly cooled. The second type of glass is made by melting anhydrous glucose crystals to which may be added a very small amount of dye. This is carried out in an oil-bath kept at 210°. The melt is rapidly stirred and then cooled.

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Glasses containing a known amount of water were also made. The procedure followed is similar to that for the second type glass.

Optical Measurements.—The concentration of the dyes in the glass were determined colorimetrically. The glasses were dissolved in water and the transmissions were measured in a Bausch and Lomb Spectronic 20 colorimeter and compared with dye solutions of known concentrations. Beer's law is obeyed in the concentration range employed. The absorption spectra of the dye in water, glucose glass and other media were determined in a Beckman DU spectrophotometer.

The fluorescence spectra of the dyes in glucose glasses were obtained by using the recording spectrophotometer described elsewhere.[17] The specimens were illuminated with light from a 500 watt tungsten lamp TDC slide projector using interference filters whose wave length of maximum transmission corresponds to the maximum in absorption of the dye. The red sensitive RCA 1P22 multiplier phototube was employed as the detector.

The intensity and lifetime of phosphorescence were measured in a phosphoroscope in which the sample is illuminated through a slit in a revolving cylinder.[18] The intensity of the phosphorescence at different speeds of the revolving cylinder was determined using an Aminco photometer unit.[19]

The rates of photobleaching were measured optically at a fixed wave length using the spectrophotometer and projector mentioned above. The transmission of the colored glass was recorded as a function of time of irradiation. The rates were calculated from the initial slopes of the absorbance *versus* time curves.

The entire visible spectrum of the projector was employed since the rate of the photoreaction is small. Care was taken to maintain the sample at constant temperature. When the photoreaction was studied as a function of temperature, the same specimen was used throughout the temperature range since exact reproduction of a given glass is very difficult. The procedure was to illuminate a small portion of the glass at one temperature.

Results

The viscosities of the glasses at elevated temperatures made by the two methods are of the same order of magnitude as those obtained by Parks, *et al.*[20] Thus, at 85° the viscosities of the first and second type glass were 1.5×10^3 and 10.0×10^3 poises, respectively, compared with 2.5×10^3 poises for the glass of Parks, *et al.* Since the agreement is considered good for measurements on glasses, we have accepted the temperature dependence of the viscosity as reported by Parks, *et al.*, and have used these data in the Discussion.

There is appreciable change in the spectra of triphenylmethane dyes from those in water to those in glucose glass (Fig. 1). This is apparently not a viscosity effect since other hydroxy solvents (*e.g.*, methanol, glycerine, etc.) also cause a shift. Furthermore, the absorption spectra of the dyes in a glucose glass at room temperature, where the viscosity is of the order of 10^{13} poises, and at 100° where the viscosity is about 3×10^2 poises, are practically identical. Crystal violet and ethyl violet exhibit absorption spectra in glucose glass very similar to those exhibited by the dyes in aqueous solution containing a polymer to which they bind (*e.g.*, polymethacrylic acid).

Visual shifts in color of acid fuchsin dyes in glucose with alterations in temperature are due to the superposition of a red fluorescence which becomes more pronounced the lower the temperature. The fluorescence spectra of this dye in glucose glass at room temperature is illustrated in Fig. 1. The shoulder of the absorption spectra is not reproduced in the fluorescence spectra, at least as far as one can determine with the phototube employed.

All of the triphenylmethane dyes which we examined (acid fuchsin (CI 692), crystal violet (CI 681), ethyl violet (CI 682), malachite green (CI 657), rosaniline (CI 676), brilliant green (CI 662), Victoria blue (CI 690), ethyl green (CI 685), patent blue (CI 712) and wool green S (CI 737) when illuminated with white light fluoresce red in media with viscosities greater than about one poise. All the dyes which appear green in glucose glass (malachite green, ethyl green, patent blue, brilliant green and wool green S) also exhibit in glucose glass a green fluorescence when illuminated with near ultraviolet light.

The intensity of fluorescence of acid fuchsin in glucose glass is strongly temperature dependent as is seen in Fig. 2. At a given temperature the intensity decreases markedly when water is incorporated into the glucose glass until 35% water is present where the fluorescence is no longer discernible.

Of all the triphenylmethane dyes examined only acid fuchsin, crystal violet, rosanilin and Victoria blue are red phosphorescent in glucose glass at room temperature. This phosphorescence is of the α type, *i.e.*, delayed fluorescence. In the case of acid fuchsin the phosphorescence lifetime is 0.020 sec. at 25°, 0.015 sec. at 45° and 0.012 sec. at 50°. The intensity of phosphorescence decreases with temperature as depicted in Fig. 3. There is only a minor decrease in the intensity of phosphorescence at room temperature when small amounts of water are added to the glass but the phosphorescence becomes unobservable for water contents above 10%. The lifetime in a water-moistened glass of relatively low viscosity (about 10^8 poises) is 40% that for the hard glass (viscosity about 10^{13} poises).

Only crystal violet, rosanilin and especially acid fuchsin, of all the triphenylmethane dyes examined, were susceptible to photoreduction in glucose glass. Under the illuminating conditions employed (white light from the 500 watt tungsten lamp projector bleached to half its concentration in about 1 hr. In Fig. 4 are plotted the relative rates of photoreduction as a function of temperature. There is an optimal rate at 50° for the first type of glucose glass and at 57° for the second type of glucose glass. At room temperature the rate of photobleaching decreases as the water content of the glucose media increases and the rate is negligible at water contents above about 10%.

Discussion

The fluorescence spectra of acid fuchsin in glucose glass (Fig. 1) is not a mirror image of the absorption spectra. This suggests that the shoulder on the short wave length side of the absorption maximum is either a higher electronic excited state than that corresponding to the maximum or an electronic excited state of a new band system. The former suggestion seems unlikely since the shoulder is nearly the same wave length as the maximum. It is im-

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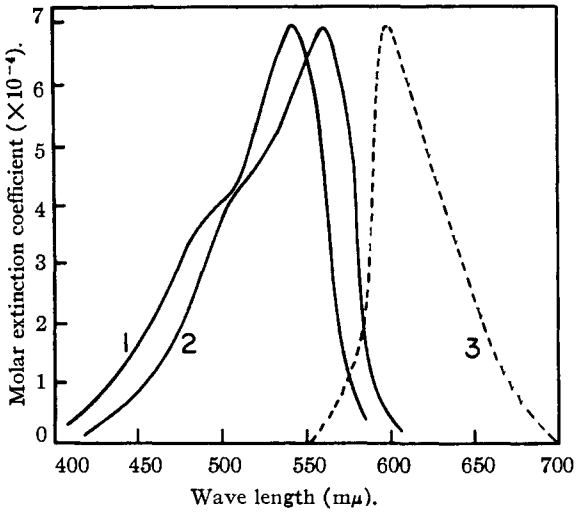

Fig. 1.—Absorption and fluorescence spectra of acid fuchsin curve 1, absorption in water; curve 2, absorption in glucose glass; curve 3, fluorescence (relative intensity) in glucose glass.

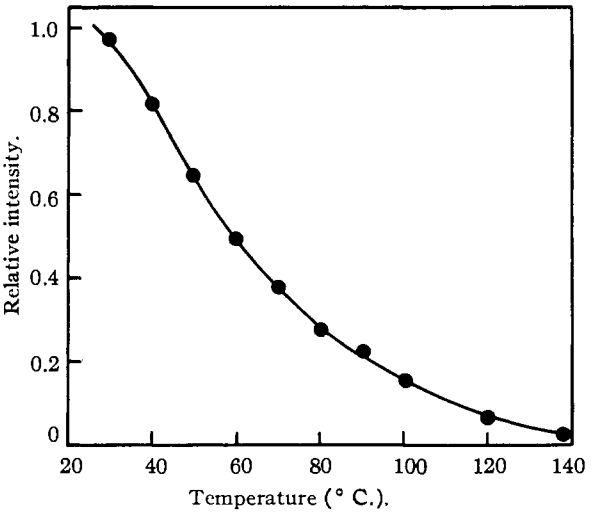

Fig. 2.—Fluorescence intensity of acid fuchsin in glucose as a function of temperature.

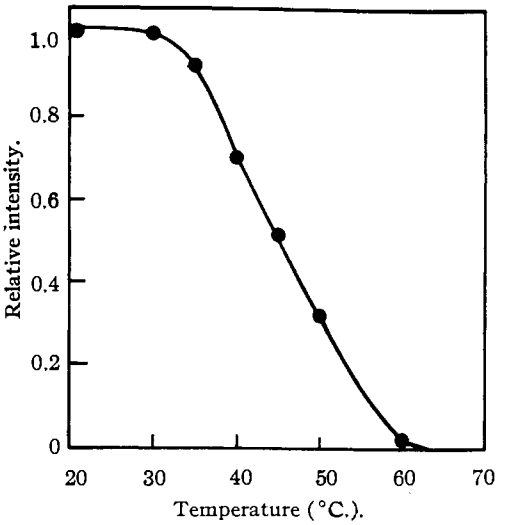

Fig. 3.—Phosphorescence (α-type) intensity of acid fuchsin in glucose as a function of temperature. Speed of revolving drum of phosphoroscope, 1400 r.p.m. Observation made at 140° from direction of incident beam.

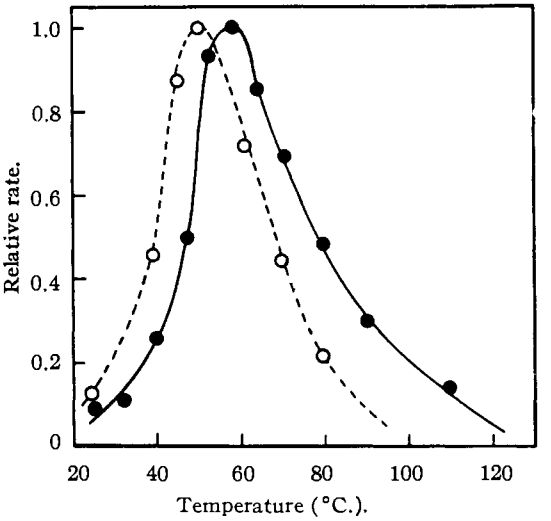

Fig. 4.—Relative rate of photoreduction of acid fuchsin in glucose as a function of temperature. Dotted curve, first type glass; continuous curve, second type glass.

teresting that of all the triphenylmethane dyes studied all the molecules containing the same substituents on the three rings have a single absorption maximum in the visible with sometimes a shoulder on the short wave length side. With those triphenylmethane dyes with substituents which make the molecule trigonally unsymmetric, however, the spectra consists of two absorption maxima at either end of the visible region and hence the dyes appear green. The two bands probably arise from two separate electronic excitation systems corresponding to the two dichroic axes of the dye molecule. The fact that for green dyes in glucose glass excitation in the short wave length band gives a green fluorescence while excitation in the long wave length band gives a red fluorescence implies the existence of two electronic excitation systems. The argument[11] that malachite green shows this phenomenon in low temperature glasses because it is claimed to be a mixture of the carbinol and normal forms and that the former fluoresce green seems to us to be irrelevant. The carbinol forms of other triphenylmethane dyes not showing the two absorption bands also fluoresce green.

The variation in the fluorescence intensity of the triphenylmethane dyes with temperature (Fig. 2) is governed largely by the viscosity of the medium rather than by the temperature *per se*. In the region where η/T is smaller than 0.1 (η is the viscosity in poises and T is the absolute temperature) the intensity of fluorescence F of acid fuchsin is given by

$$F \sim \frac{\eta/T}{A + \eta/T} \qquad (1)$$

where $A = 0.152$. This result is similar to that published[15] for auramine O but where $A = 0.047$ and the expression is valid for η/T smaller than about 5×10^{-2}. The triphenylmethane dye exhibits a greater dependence of its fluorescence on vis-

cosity than does the diphenylmethane dye. It has been proposed[15] that for such molecules, increasing the viscosity of the medium restricts internal rotational diffusion and thereby diminishes internal conversion from the excited singlet state to the ground state. With the case of acid fuchsin it is unfortunately not possible to calculate the probability of this transition because the absolute quantum yield of fluorescence is not known.

Where η/T is greater than about 0.1 (corresponding to about 70° in Fig. 2) the luminescence is greater than that calculated from eq. 1. This excess in intensity arises from a superposition of the α-phosphorescence (delayed fluorescence) on the normal fluorescence. The α-phosphorescence becomes prominent only when the viscosity of the glass is very high (Fig. 3). But, as also shown from the room temperature experiments on moistened glasses, the phosphorescence intensity is much less dependent on viscosity than is the fluorescence. The lifetime of the triplet state of anthracene was found by high intensity flash spectroscopy to be roughly proportional to the square root of the viscosity of the solvent.[21] For acid fuchsin in glucose glass, on the other hand, the lifetime of the metastable state is practically independent of viscosity of the extremely viscous medium. Thus we found a lowering of only 40% in the lifetime in going from 25 to 50° whereas the viscosity changed by a factor of 10^5. Furthermore, at room temperature the lifetime of phosphorescence does not change appreciably when the viscosity of the glucose glass is lowered by the addition of small amounts of water. If we assume that the lifetime is controlled entirely by the temperature then, using the Arrhenius expression, the activation energy is calculated to be 4 kcal./mole. This should equal the difference in energy between the first excited singlet state and the metastable state. As seen in Fig. 3 lowering the temperature below about 30° does not enhance the α-phosphorescence. We would expect that if the temperature is lowered below about 0°, the α-phosphorescence would decrease, and by about $-100°$ β-phosphorescence should predominate. This is the case for auramine O but is not measurable by our techniques for acid fuchsin since the β-phosphorescence lies too far in the red, about 700 mμ according to our calculations.

The fact that α-phosphorescence is associated with photoreducibility suggests that photoreduction involves the long lived metastable state. The conditions for enhancement of fluorescence favors, for certain dyes, their photoreducibility, but the appearance of the α-phosphorescence is the necessary criterion. A high quantum yield of fluorescence implies small internal conversion and hence a large percentage of the excited molecules are available for conversion to the metastable state. It has been stated that the role of rigid media in the enhancement of lifetimes of the metastable state is to suppress deactivating collisions by the medium.[22] We believe that for condensed media collisions with the surroundings are independent of the viscosity and the requirement of extremely high viscosity for phosphorescence of dyes is to hinder internal rotation of the molecule in the triplet state and thereby retard conversion to the ground singlet state. For rigid glasses the effect is independent of viscosity but is strongly temperature dependent.

The rate of photoreduction of acid fuchsin in glucose glass as a function of temperature (Fig. 4) is governed by three temperature dependent factors. Firstly, there is the number of molecules which are converted to the metastable state. The greater the viscosity of the medium (and hence the lower the temperature) the less internal conversion from the singlet state to the ground state occurs, and therefore the greater are the number of molecules which undergo transitions to the metastable state. Secondly, the lifetime of the metastable state is increased when the temperature is lowered. These two effects are opposed by the contribution of the activation energy barrier necessary to be overcome if the dye in the metastable state is to react with glucose. The combination of these three factors leads to an optimum rate as shown in Fig. 4. Calculation shows that the activation energy is 20 kcal./mole.

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