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INFLUENCE OF URIC ACID ON PHOTOSTABILITY OF FD&C BLUE NO. 2

A. F. Asker* and A. Collier

ABSTRACT

The effect of uric acid as a photoprotective agent for various solutions of FD&C Blue No. 2 was investivated. Due to the poor aqueous solubility of uric acid, solutions were made in glycerin, triethanolamine and N/5 NaOH. Uric acid in glycerin or triethanolamine was found to enhance the photostability of the dye solutions. The higher the concentration of uric acid in triethanolamine, the greater was the photoprotective action of uric acid. Increasing the amount of glycerin in solution resulted in acceleration of the rate of fading of the color presumably due to dielectric constant effect. The photoprotective action of uric acid was found to be influenced by the pH of the medium and its buffer species.

*School of Pharmacy, Florida A&M University, Tallahassee, Florida, 32307

INTRODUCTION

Coloring agents have been used in pharmacy to achieve a more elegant appearance of the various dosage forms and to help in their rapid identification. FD&C Blue No. 2 is a common coloring material that has been widely used and is considered one of only three synthetic coloring agents that are almost universally accepted (1).

Several reports (2-8) have been published on the stability of certified dyes. Jones et al. (9), showed that fading of solutions of FD&C Blue No. 2, when exposed to ordinary diffuse laboratory light, was due almost entirely to the oxidation of the colorant to isatinsulfonic acid and finally to sulfonated anthranilic acid. Desai and Giles (10) have stated in their review that oxidative decomposition is more destructive to FD&C Blue No. 2 than reduction. Kuramoto et al. (11), on the other hand postulated that the decomposition of FD&C Blue No. 2 took place by reduction via a semiquinone formation to a colorless leuco compound. Inskeep and Kretlow (12) observed that although a common property of dye-stuffs is their ability to take up hydrogen with the formation of colorless compounds, the leuco compounds so formed from indigo dyes were oxidized by air. The rate of fading of various dyes including FE&C Blue No. 2 has been found to be influenced by nonionic surfactants (3).

One approach for improving the photostability of colorants has been the use of ultraviolet absorbing chemi-

cals. The influence of ultraviolet absorbers on color stability of tablets coated with certified colorants and exposed to light, has been studied (4, 13). Sunscreening agents or ultraviolet absorbers enjoy wide applications in the cosmetic industry in the manufacture of sun-screen preparations. Asker and Coworkers (14) found that incorporating an ultraviolet absorber in light-sensitive reserpine solutions had a significant affect in protecting reserpine against photodegradation.

Therefore, this study was conducted to investigate the influence of uric acid as an ultraviolet absorber on the photostability of solutions of FD&C Blue No. 2. The photosensitivity of this dye will cause its solution to fade and eventually turn colorless. Uric acid is considered non-toxic, non-sensitizing and non-irritating, and therefore, its use as an ultraviolet absorber for some pharmaceuticals would be acceptable.

EXPERIMENTAL

Materials: FD&C Blue No. 2 (indigo carmine), uric acid, glycerin, triethanolamine, sodium hydroxide, citric acid, hydrochloric acid and monobasic potassium phosphate were obtained from commercial sources in reagent or pharmaceutical grade and were used without further purification.

Equipment: The following were used: a light stability cabient equipped with an 18-inch 15-watt Westinghouse long wavelength ultraviolet "black light" tube emitting most of

its radiations at approximately 3660 A^{O} to serve as the light source; a Spectronic 20 spectrophotometer; Orion digital pH meter.

Exposure to Light: The spectrophotometer tubes containing the solutions to be exposed to light were kept 9 cm from the light source. The temperature inside the light stability cabient was maintained at $28^{\circ} \pm 0.5$ to serve as control.

Procedure: The typical experimental procedure was as follows: Volumes of solutions prepared with and without uric acid, each of 5 ml were placed in 10 x 100 mm spectrophotometer tubes covered with parafilm and exposed to longwave ultraviolet radiations in the light stability cabinet. Absorbance readings were made at various time intervals on the Spectronic 20 at 610 nm using appropriate blanks. The concentration of the colorant remaining in solution was calculated by dividing the absorbance reading by the slope obtained from the Beer's plot shown in Figure 1.

Because of the poor solubility of uric acid in water, solutions were made in glycerin, triethanolamine and N/5 sodium hydroxide solution. Solution of uric acid in glycerin was prepared by dissolving 50 mg of uric acid in 200 g of glycerin previously heated to 100^{O}. Solution in triethanolamine was effected by mixing 50 mg of uric acid with 100 g of triethanolamine previously heated to 75^{O}. Solution in N/5 NaOH was prepared by dissolving 250 mg of uric acid in sufficient N/5 NaOH solution to make 100 ml.

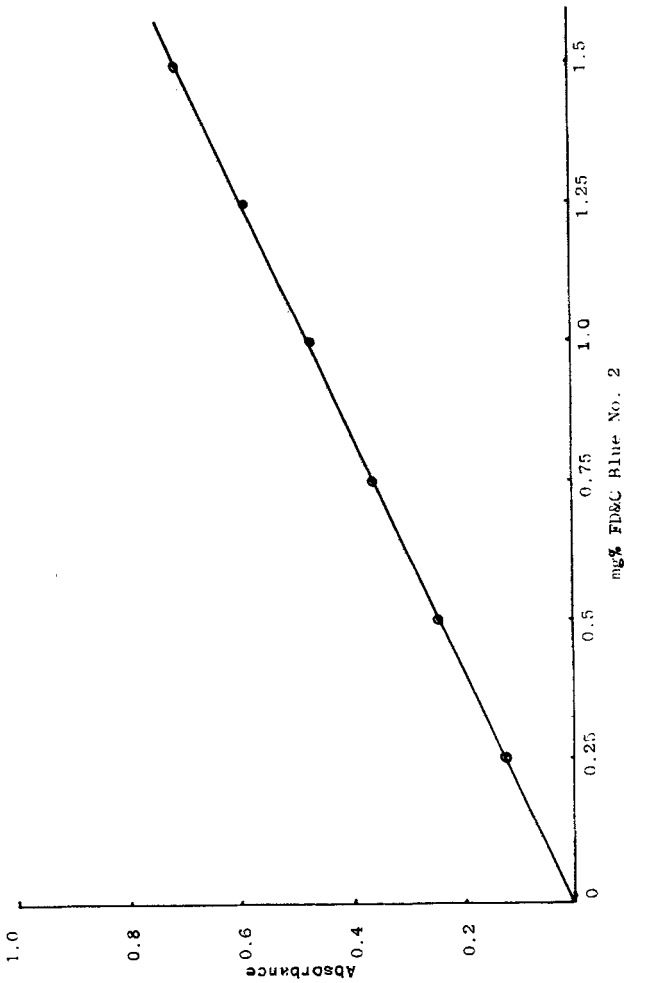

FIGURE 1. Beer's Plot For FD&C Blue No. 2 in Distilled Water

In studying the effect of variation of uric acid concentration on the photostability of FD&C Blue No. 2, various quantities of solutions of uric acid in glycerin or in triethanolamine were placed into 100-ml volumetric flasks. To each flask, 10 ml of 0.01% dye solution was introduced and the solution was made to volume with distilled water. Control solutions containing no uric acid were prepared in a similar fashion, but equal quantities of the solvents used to dissolve uric acid were placed into the flasks.

The effect of buffers on photodecomposition of FD&C Blue No. 2 was studied by placing a quantity of glycerin solution of uric acid containing 1.25 mg of uric acid into a 100-ml volumetric flask. 10 ml of 0.01% of the dye solution was then introduced into the flask. The solution was then made to volume by adding the buffer solution in question and mixing. Appropriate control solutions were similarly prepared without the incorporation of uric acid.

DISCUSSION OF RESULTS

Influence of Uric Acid on the Photosensitivity of FD&C Blue No. 2

Figure 2 shows that the incorporation of 1.25 mg% of uric acid dissolved in glycerin into a 1 mg% aqueous solution of the dye, produced a measurable protective action against photodegradation of the dye. Similarly, a 2.5 mg% uric acid dissolved in triethanolamine and incorporated into the dye solution demonstrated a pronounced photostabliizing effect of the dye as shown in Figure 3. On

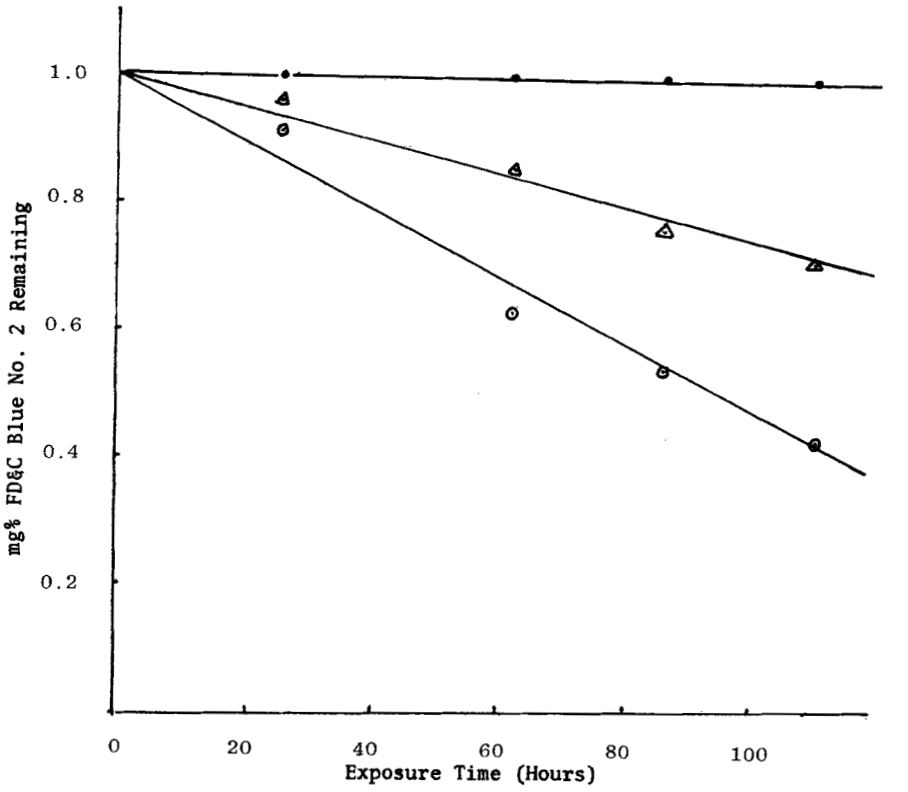

FIGURE 2. Effect of Light (Wavelength 3660 A^{O}) on Stability of FD&C Blue No. 2 Solution Containing 1.25 mg% Uric Acid Dissolved in Glycerin.

- ● Solution With or Without Uric Acid Stored in the Dark
- △ Solution With Uric Acid Exposed to Light
- ⊙ Solution Without Uric Acid Exposed to Light

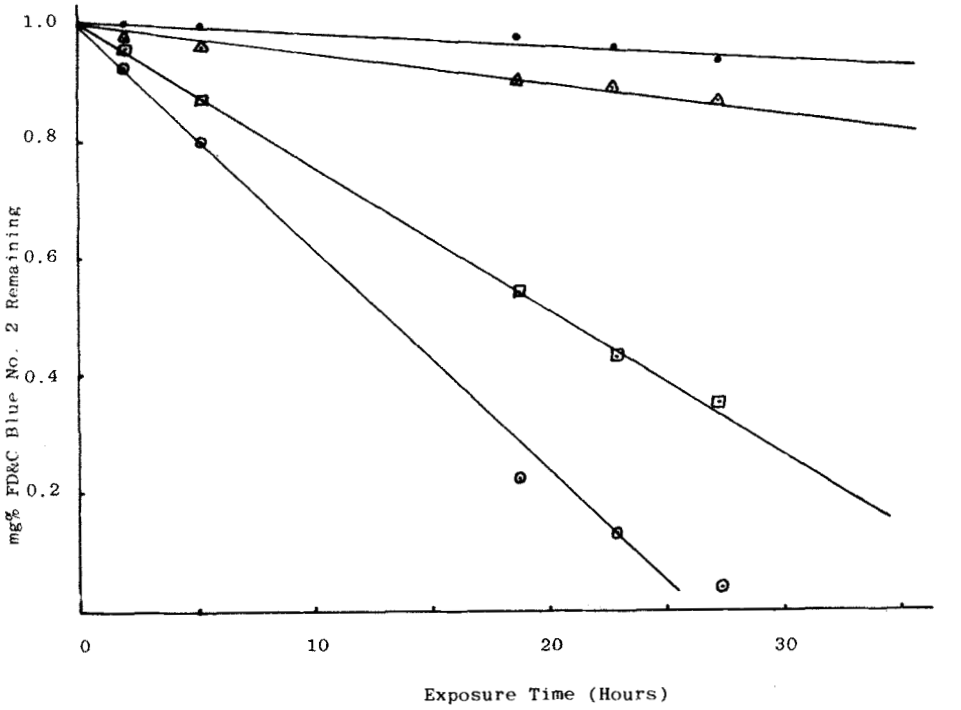

FIGURE 3. Effect of Light (Wavelength 3660 A^{O}) on Stability of FD&C Blue No. 2 Solution Containing 2.5 mg% Uric Acid Dissolved in Triethanolamine.

- ● Solution with Uric Acid Stored in the Dark
- △ Solution Without Uric Acid Stored in the Dark
- ⊡ Solution With Uric Acid Exposed to Light
- ⊙ Solution Without Uric Acid Exposed to Light

the other hand, solutions of uric acid in N/5 NaOH incorporated into the dye solution were found to accelerate the rate of color fading whether the solutions were kept in the dark or exposed to light as can be seen from Figure 4, 7 and 8. The color was visibly faded immediately after preparation of the samples. This may be attributed to the high alkalinity of the solution which has been reported to enhance decomposition of the dye (15). Moreover, Newburger et al. (16), in their study on photodecomposition of uric acid in presence of riboflavin, have found that the rate of photodegradation of uric acid increases as the pH increases within the range of 5.2-8.9. Therefore, at such high pH, uric acid would be considered an ineffective ultraviolet absorber.

From Figures 2 and 3, it is evident that the fading of the color appears to follow a zero-order rate. This would be expected from such reactions where the rate is determined by factors other than concentration of the reactant such as the absorption of light. Photodegradation of chlorpromazine hydrochloride (17), sodium nitroprusside (18), menadione (19) and reserpine (14) has been found to follow a zero-order rate.

Effect of Uric Acid Concentration:

For solutions of uric acid in glycerin, it would appear from Figure 5 that there is an optimum concentration of uric acid that would produce the maximum stabilizing effect. Such concentration is from 1.25-2.5 mg%. The reason for

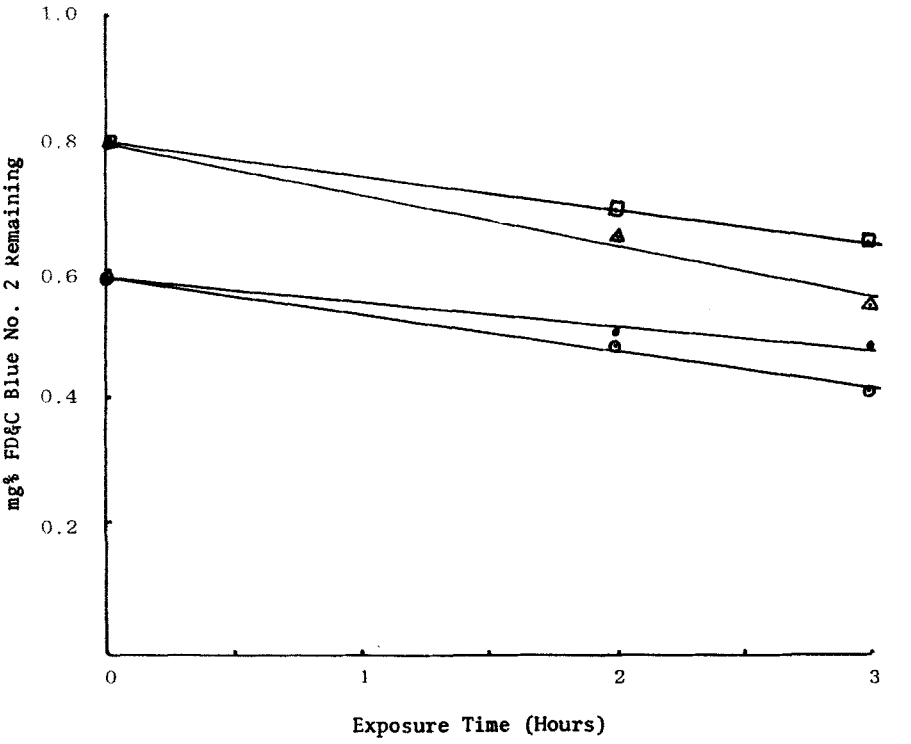

FIGURE 4. Effect of Light (Wavelength 3660 A^{O}) on Stability of FD&C Blue No. 2 Solution Containing 5 mg% Uric Acid Dissolved in N/5 NaOH.

- ● Solution With Uric Acid Stored in the Dark
- ◘ Solution Without Uric Acid Stored in the Dark
- ⊙ Solution With Uric Acid Exposed to Light
- ◬ Solution Without Uric Acid Exposed to Light

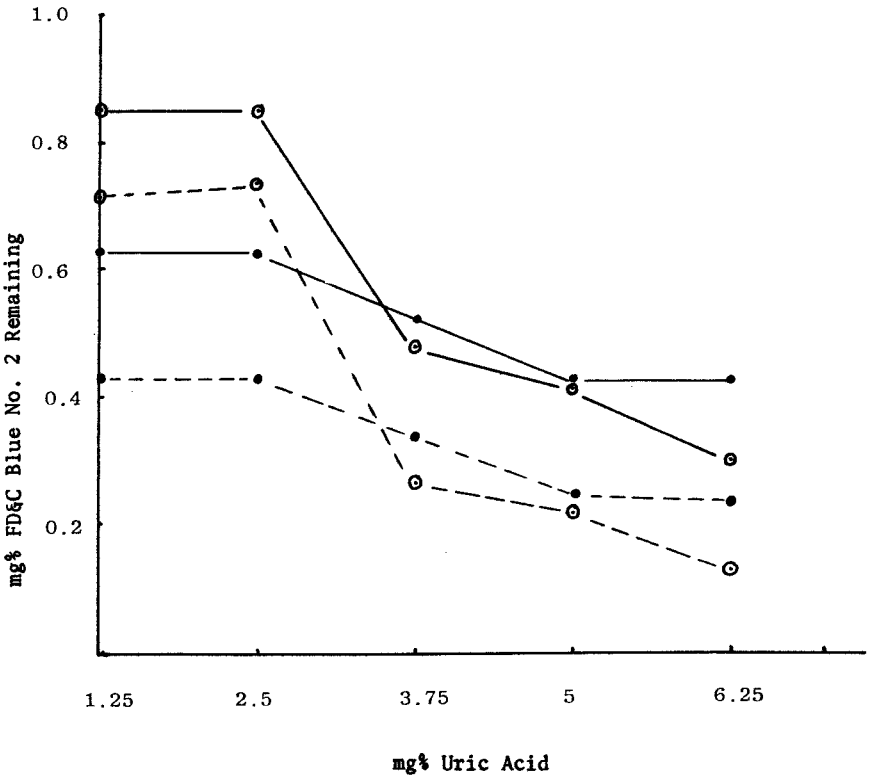

FIGURE 5. Effect of Uric Acid Concentration on Photostability of FD&C Blue No. 2 in Glycerin Solution.

⊙—● Solution With Uric Acid Exposed to Light For 63 Hours

●—● Solution Without Uric Acid Exposed to Light For 63 Hours

⊙···⊙ Solution With Uric Acid Exposed to Light For 111 Hours

●---● Solution Without Uric Acid Exposed to Light For 111 Hours

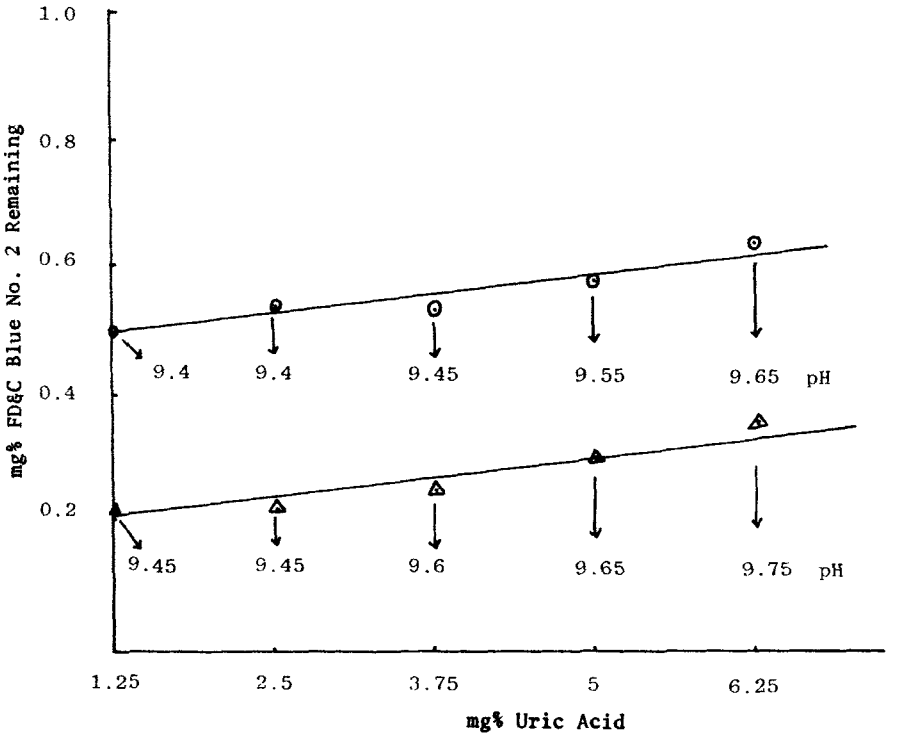

FIGURE 6. Effect of Uric Acid Concentration of Stability of FD&C Blue No. 2 in Triethanolamine Solution Exposed to Light For 18 Hours and 45 Minutes.

⊙ Solution With Uric Acid

△ Solution Without Uric Acid

the decrease in the stabilizing action of uric acid as its concentration increased beyond 2.5 mg% is that higher concentration of uric acid is associated with higher concentration of glycerin. The latter has been found in this study to enhance the photodecomposition of FD&C Blue No. 2 in the presence or absence of uric acid as will be discussed later.

In case of uric acid solutions in triethanolamine, it is evident from Figure 6 that the photostability of the dye increased as the concentration of uric acid increased within the concentration range studied.

On the othe hand, increasing the concentration of uric acid in solutions made with N/5 NaOH accelerated the rate of color fading of solutions exposed to light or kept in the dark as seen in Figures 7 and 8. This is due as stated before to the high alkalinity of the solutions which has a deleterious effect on the stability of dye as well as uric acid.

Effect of Glycerin Concentration:

Figures 9 and 10 show the effect of glycerin concentration on the fading of FD&C Blue No. 2 in presence and absence of uric acid respectively. It is evident that as the concentration of glycerin in the dye solution increases, the rate of color fading increases. The formation of the colorless leuco compounds in the degradation of dye-stuffs has been postulated by Inskeep and Kretlow (12) to be due to the ability of the anionic coloring agent to take up

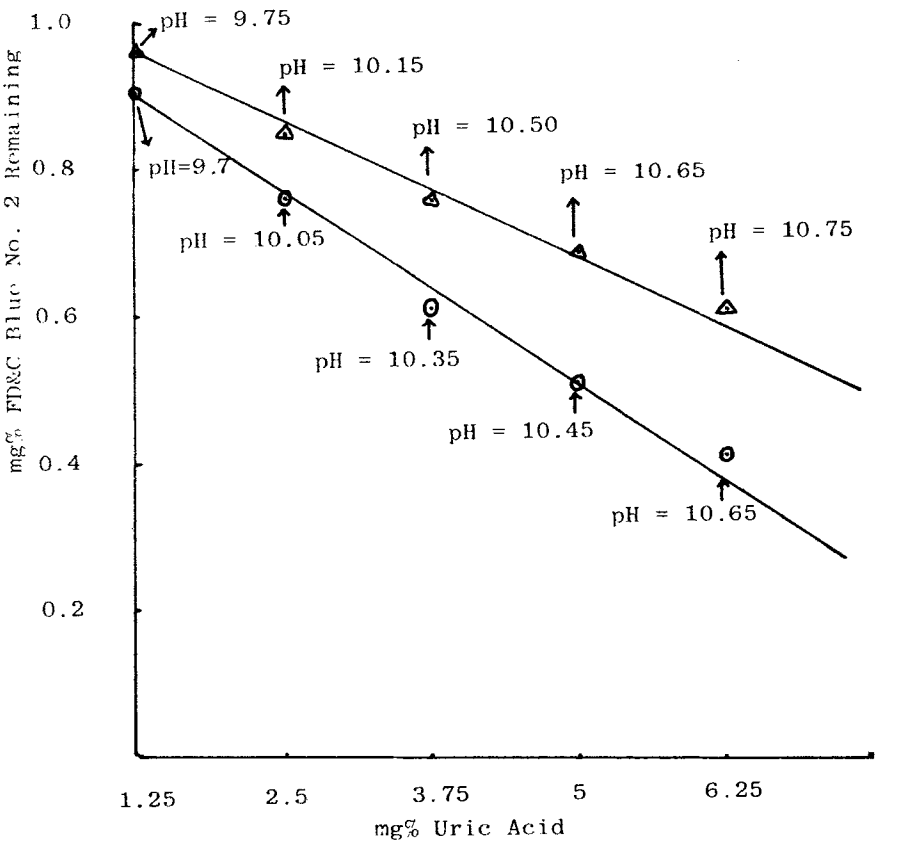

FIGURE 7. Effect of Uric Acid Concentration on Stability of FD&C Blue No. 2 in N/5 NaOH Solution Kept in the Dark For 2 Hours.

△ Solution Without Uric Acid

⊙ Solution With Uric Acid

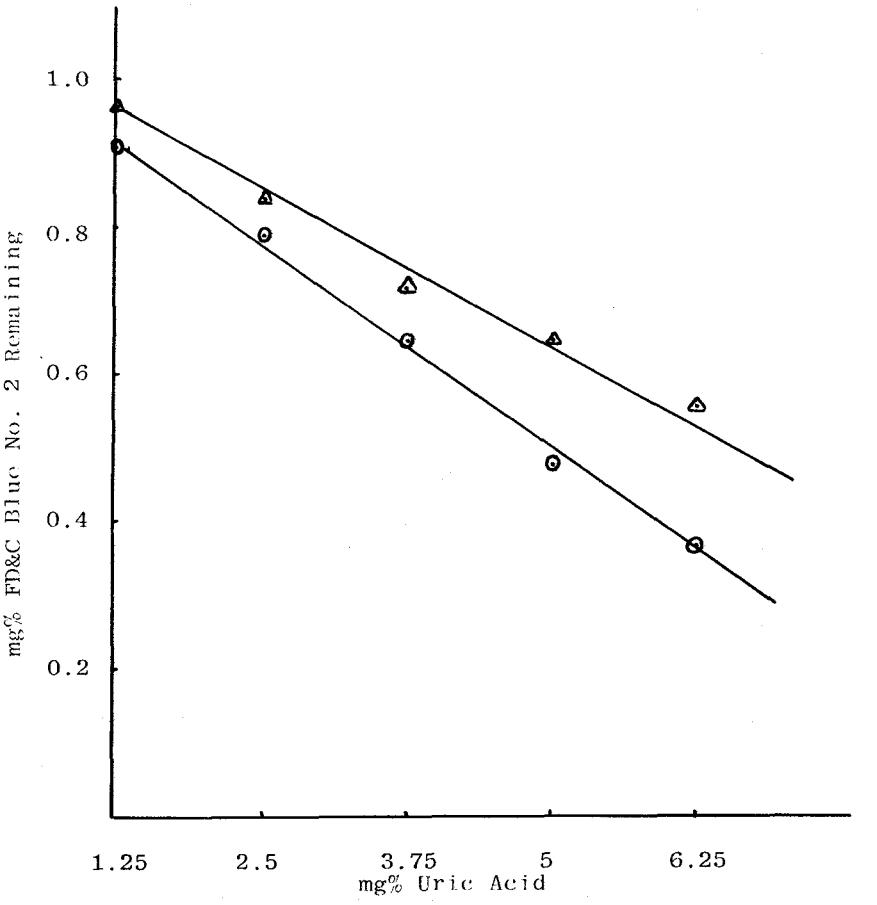

FIGURE 8. Effect of Uric Acid Concentration on Stability of FD&C Blue No. 2 in N/5 NaOH Solution Exposed to Light for 2 Hours

△ Solution Without Uric Acid

⊙ Solution With Uric Acid

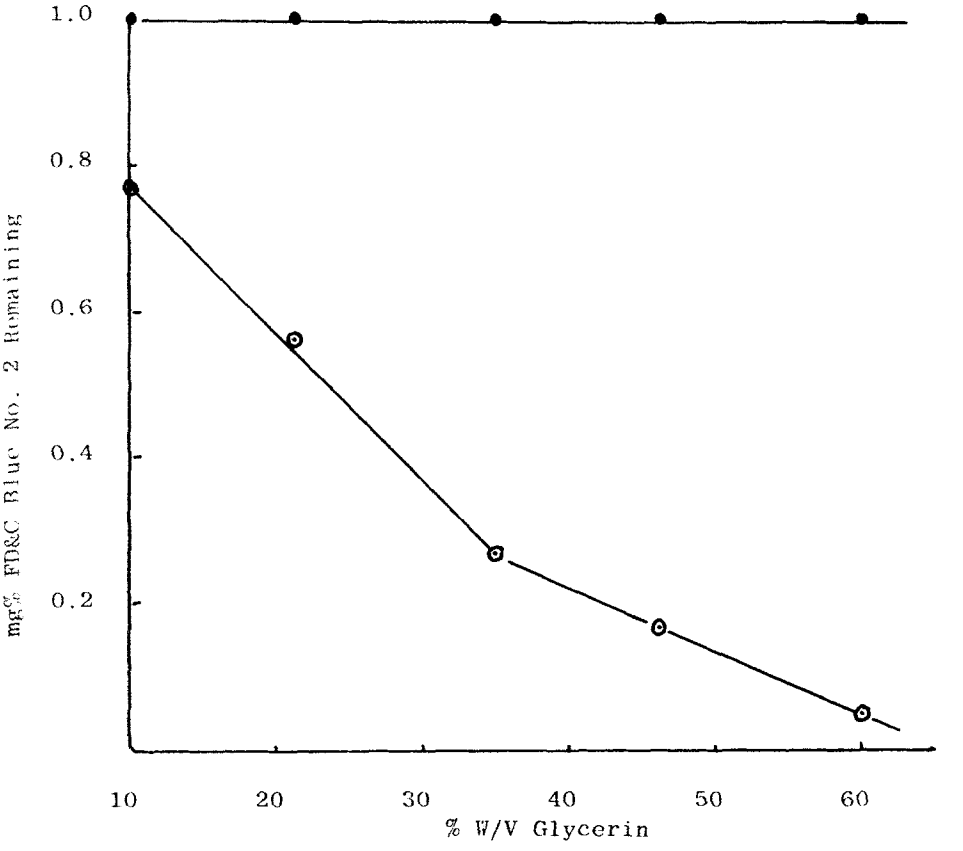

FIGURE 9. Effect of Glycerin Concentration on Photostability of FD&C Blue No. 2 Solution Containing 2.5 mg% Uric Acid.

● Solution Stored in the Dark for 64 Hours

⊙ Solution Exposed to Light for 64 Hours

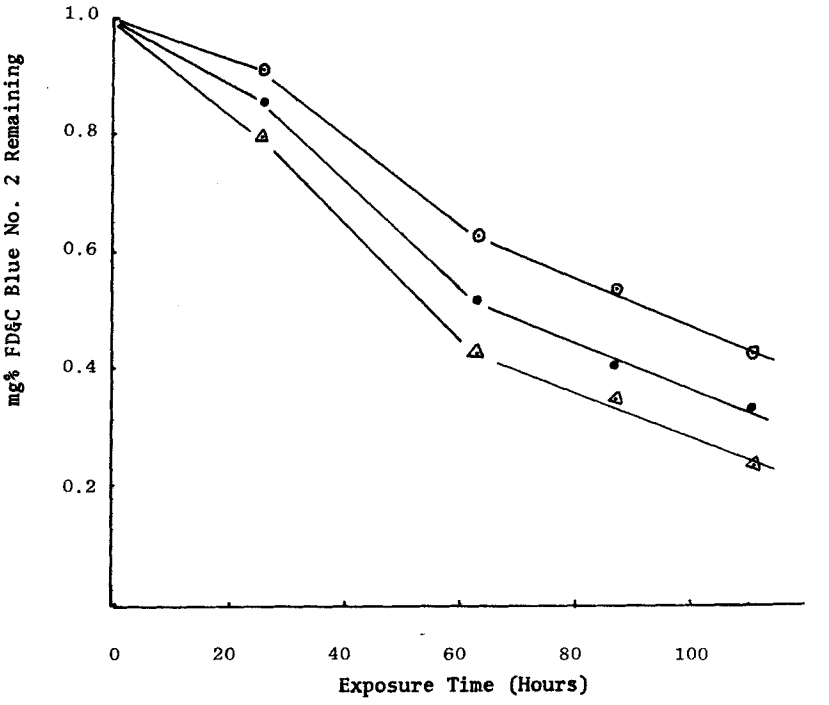

FIGURE 10. Effect of Light (Wavelength 366o A^{O}) on Stability of FD&C Blue No. 2 Solutions Containing No Uric Acid in Presence of Various Concentrations of Glycerin

⊙ 5% W/V Glycerin

● 15% W/V Glycerin

△ 25% W/V Glycerin

hydrogen. In such a reaction between oppositely charged species, a decrease in dielectric constant through increase of glycerin concentration is expected to accelerate the rate of reaction (20). Therefore, increasing glycerin concentration resulted in an acceleration of the rate of fading of dye in presence or absence of uric acid.

Effect of Buffers:

In order to eliminate the possibility that the rate of color fading was associated with variation in pH, the effect of uric acid as a photoprotective agent was studied in various buffered solutions.

It is evident from Figure 11 that in phosphate buffer of pH 7.2, uric acid demonstrated the greatest stabilizing effect which is comparable to that obtained by storing the dye solutions in the dark. In phosphate buffer of pH 8.15, uric acid produced some stabilizing effect as shown in Figure 12. However, in a phosphate buffer of pH 4.65, uric acid appeared to be an ineffective photoprotective agent as seen in Figure 13.

From Figure 14, it is evident that uric acid in citrate buffer of pH 4.65, was also an ineffective ultraviolet absorber and the rate of fading of the dye solution was substantially higher than that obtained with phosphate buffer of the same pH. About 58% of the dye degraded in the first 2 hours. It can be concluded therefore, that buffer species would also influence the photostability of the dye solution. Turi, et al. (21) found that citric acid

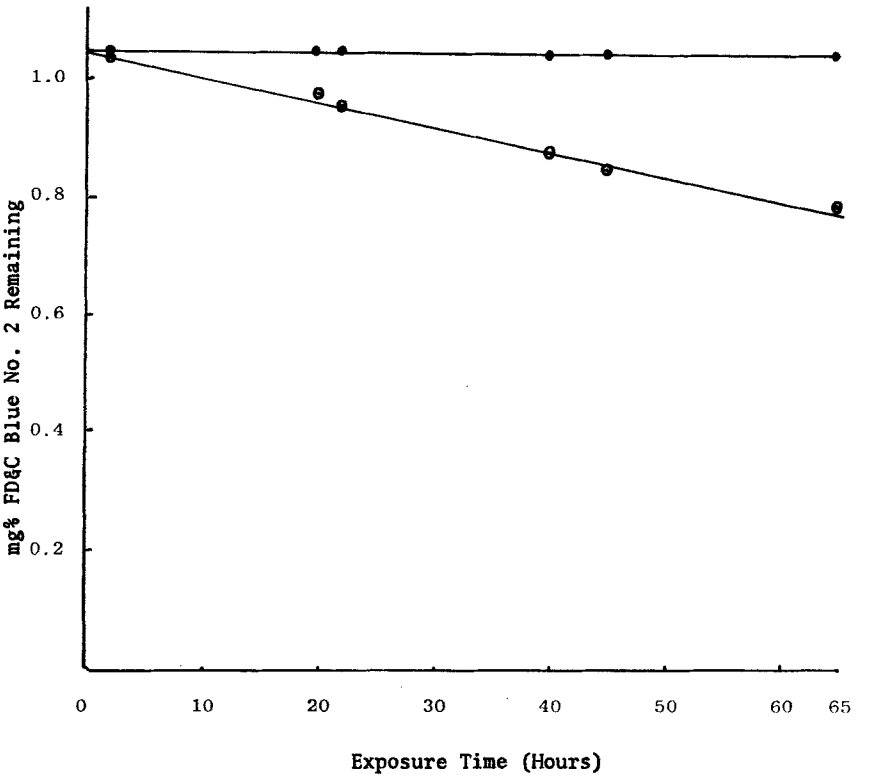

FIGURE 11. Effect of Phosphate Buffer, pH 7.2 on the Photoprotective Action of Uric Acid for Solutions of FD&C Blue No.2.

● Solutons With or Without Uric Acid Kept in the Dark or Solutions Containing Uric Acid and Exposed to Light

⊙ Solutions Without Uric Exposed to Light

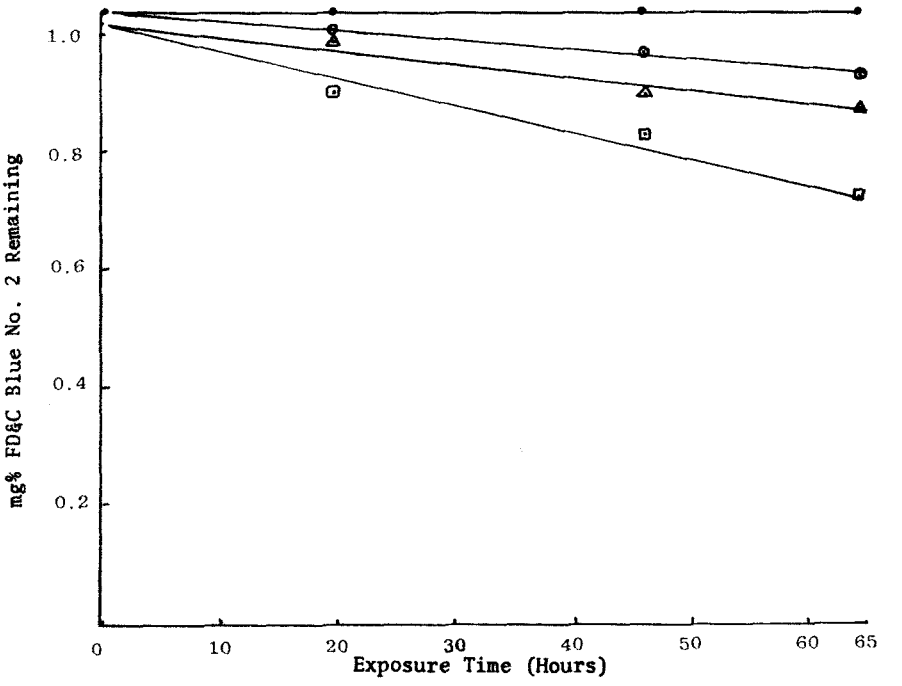

FIGURE 12. Effect of Phosphate Buffer, pH 8.15 on the Photoprotective Action of Uric Acid for Solution of FD&C Blue No. 2.

● Solutions With Uric Acid Kept in the Dark

△ Solutions With Uric Acid Kept in the Dark

⊙ Solutions With Uric Acid Exposed to Light

⊡ Solutions Without Uric Acid Exposed to Light

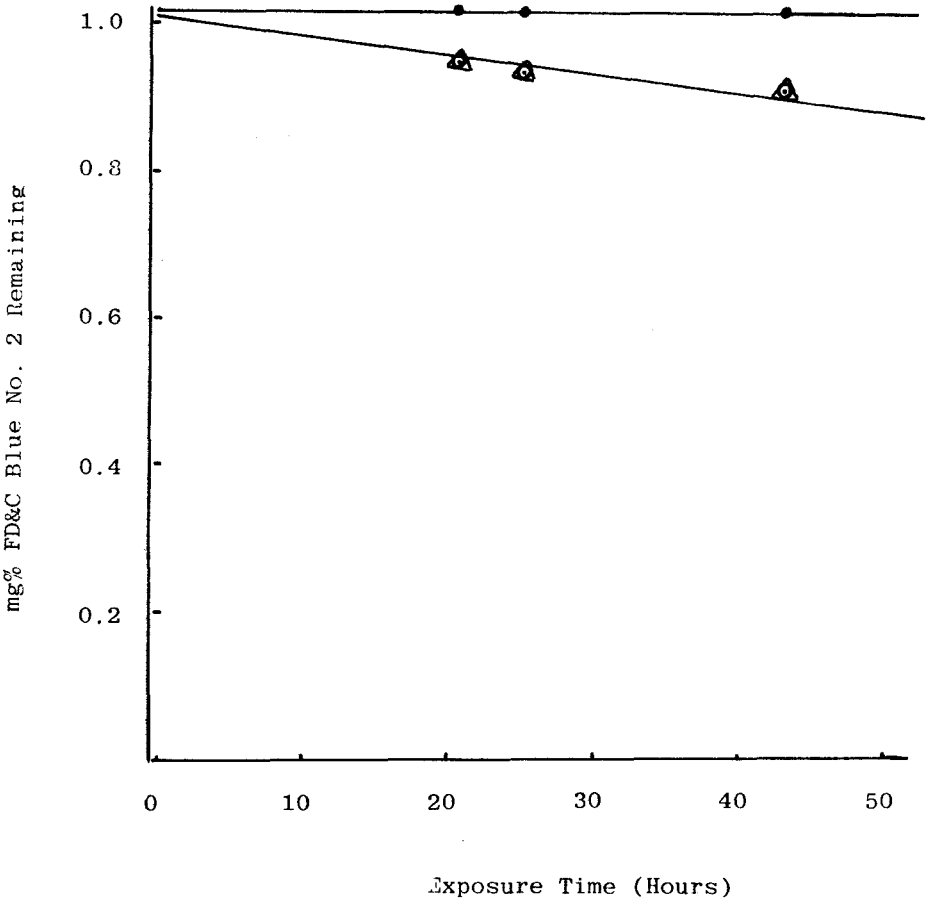

FIGURE 13. Effect of Phosphate Buffer, pH 4.65 on the Photoprotective Action of Uric Acid for Solutions of FD&C Blue No.2.

- ● Solutions With or Without Uric Acid Kept in the Dark
- ⊙ Solutions Without Uric Acid Exposed to Light
- △ Solutions With Uric Acid Exposed to Light

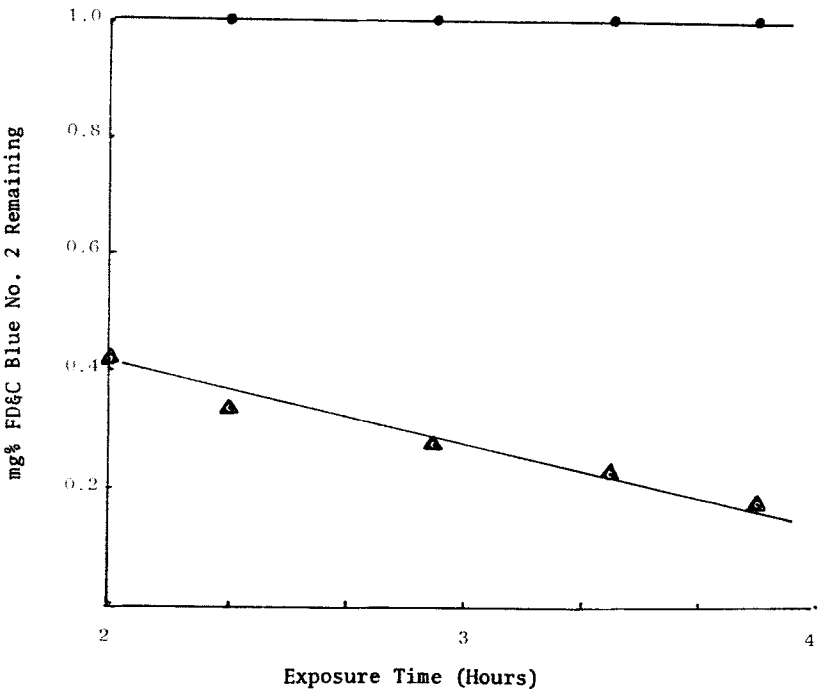

FIGURE 14. Effect of Citrate Buffer, pH 4.65 on the Photoprotective Action of Uric Acid for Solutions of FD&C Blue No. 2.

- ● Solutions With or Without Uric Acid Kept in the Dark
- ⊙ Solutions Without Uric Acid Exposed to Light
- △ Solutions With Uric Acid Exposed to Light

caused a rapid fading of the indigo carmine solution. Such a deterimental effect of citric acid to the dye appears to outweigh the photoprotective action of uric acid.

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