Preliminary Report on the Comparative Stability of Certified Colorants with Lactose in Aqueous Solution

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The influence of conventional lactose and spray-processed lactose and their primary hydrolysis products (*d*-glucose and *d*-galactose) on the stability of various certified colorants has been investigated. Spectrophotometric and paper partition chromatographic examination indicated that in aqueous buffered solutions (pH 6.6 to 6.8), FD&C Red No. 4, FD&C Yellow No. 5, FD&C Green No. 3, and FD&C Blue No. 1 are relatively stable when exposed to exaggerated lighting and temperature in the presence of these sugars. FD&C Blue No. 2 was found to be very unstable, particularly when exposed to light, and the sugars significantly accelerate the decomposition of this color. The decomposition of FD&C Blue No. 2 appears to proceed by reduction to a semiquinone followed by oxidation. There appears to be some evidence to indicate molecular changes in the lactose when stored at high temperature. These changes appear to interfere with the spectrophotometric analysis of colorants which absorb between 228 mμ and 284 mμ.

THE INTRODUCTION of spray-drying procedures for the manufacture of alpha lactose monohydrate and the subsequent acceptance of this sugar by the official compendia afforded the pharmaceutical tablet developer with a pharmaceutical excipient exhibiting a number of advantages. Among these are flow and compaction properties unknown with conventionally manufactured lactose. The use of spray-dried lactose permits the economical production of tablets by direct compaction, a process which consists of only two steps—powder blending and compression. Through this method of preparation it is possible to formulate tablets of heat and moisture-sensitive ingredients in a simple manner and with enhanced stability.

Efforts to produce colored tablets by direct compaction through the use of blended dye triturates, lakes, or precolored disintegrants or lubricants with spray-processed lactose were not completely successful and were rather costly. Through the use of colored spray-dried lactose (the lactose being colored during the spray-drying operation), the preparation of colored tablets by direct compaction was made possible. The colored spray-dried lactose exhibits similar flow and compaction properties as the uncolored material. The preparation of colored spray-dried lactose was initiated in 1958 by Brownley and Reger (1).

The initial batches were manufactured with certified FD&C Blue No. 1, FD&C Yellow No. 5, FD&C Green No. 3, FD&C Orange No. 3, and the formerly certified FD&C Red No. 1. The quantities of colorants used varied from 0.008 to 0.05%. Preliminary investigation indicated that fading and color changes occurred in some of these lactoses. As examples, the colored lactose containing 0.05% FD&C Yellow No. 5 changed during normal ambient storage from a golden yellow to a dull yellowish-tan. The product containing 0.008% FD&C Red No. 1 varied in color from light to dark pink within a particular batch of lactose. These adverse changes, however, were not evident in colored lactose granules prepared with conventional lactose. These granules had been prepared by customary wet granulating techniques in the same dye concentration as in the colored spray-dried lactoses.

A possible explanation for the difference in color stability for the spray-dried and conventional lactose can be the presence of higher concentrations of lactose's hydrolysis products in the spray-dried lactose, as compared to the conventional lactose. It has been shown by Jenness and Patton (2), Weisberg (3), and Whittier (4) that heat differences during manufacture can cause appreciable difference in the extent of lactose hydrolysis. When analyzing the two types of lactose for other sugars according to U.S.P. methods, results indicated that some batches of spray-dried lactose contained two to four times as much other sugars as found in conventional lactose. The other sugars were identified as being the primary hydrolysis products of lactose, namely, D-glucose and D-galactose. These monosaccharides possess 30% greater reducing power than pure lactose (5). Therefore, it would be expected for the spray-dried

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lactose to have a more deleterious effect on colorants susceptible to reduction reactions than conventional lactose.

Although there has been an increase in recent years in the number of investigations concerned with stability of colorants used in pharmaceutical dosage forms (6–10), these studies did not consider the relative stability of spray-dried and conventionally processed lactose with certified dyes. Since the oxidation–reduction interreactions between dyes and pharmaceutical agents are a serious problem for the pharmaceutical industry, it seemed desirable to evaluate the relative stability of spray-dried lactose *vs.* conventional lactose with several commonly used certified colors. In addition, it was desirable to discern the exact influence of lactose's primary hydrolysis products on the stability of these colorants.

In this investigation, the chemical stability of dye-sugar mixtures was examined in aqueous solutions. Five dyes representing three of the major chemical categories were selected for study. The dyes and sugars were studied at 0.15 and 5% concentrations, respectively, in aqueous solutions buffered at pH 6.6 to 6.8. This buffer system was chosen in order to maintain a constant hydrogen ion concentration as well as to approximate the pH of a 0.15% aqueous solution of the majority of dyes.

EXPERIMENTAL

Materials Used.—Conventionally processed lactose U.S.P. (Avoset Co.); spray-processed lactose U.S.P. (Western Condensing Co.); dextrose U.S.P. (D-glucose); D-galactose (Matheson Coleman and Bell); FD&C Red No. 4 (monoazo); FD&C Blue No. 1 and FD&C Green No. 3 (triphenylmethane); FD&C Yellow No. 5 (pyrazolone); FD&C Blue No. 2 (indigoid) (H. Kohnstamn Co.); ethyl acetate and pyridine (Baker Chemical Co.); benzidine reagent (Matheson Coleman and Bell); Sorensen phosphate buffer solution at pH 6.6 to 6.8.

Equipment.—Beckman recording spectrophotometer, model DK-1; Beckman Zeromatic pH meter; light stability cabinet exhibiting intensified illumination as described in a previous publication (11); precision scientific oven maintained at 60° ± 1°; Whatman's No. 1 chromatographic paper supplied in sheets 18.25 inches × 22.5 inches; microliter pipets; chromatography jars, 18 inches high × 12 inches in diameter, including complete setup for descending development; spray bottles; ultraviolet lamp, long wave (3660 Å).

Procedure for Spectrophotometric Analysis.—Aqueous buffer solutions containing 0.15% dye, 0.15% dye + lactose, 0.15% dye + D-glucose and 0.15% dye + D-galactose were prepared and investigated for colorant stability. These solutions were filtered through sintered glass funnels and filled into 10-ml. clear neutraglas color-break ampuls. The ampuls were then sealed on the Popper HI laboratory ampul sealer. Each batch of ampuls was divided into three groups and stored as follows: Group one was stored at room temperature in light-resistant cardboard containers; group two was stored under intensified illumination in the light stability cabinet; group three was stored in light-resistant cardboard containers at 60° ± 1°. At designated time intervals, individual ampuls were removed and assayed spectrophotometrically by running a complete absorption spectra in the visible and ultraviolet regions. Absorbance was measured at the wavelengths indicated in Table I.

TABLE I.—TEST CONDITIONS USED FOR SPECTROPHOTOMETRIC ANALYSIS

	Absorption Maximum[a]		
	Visible mμ	Uultraviolet mμ	pH of Solution[b]
FD&C Red No. 4	505	303	6.65
FD&C Yellow No. 5	430	257	6.65
FD&C Green No. 3	628	302	6.70
FD&C Blue No. 1	625	307	6.80
FD&C Blue No. 2	617	285	6.72

[a] Beckman recording spectrophotometer, model DK-1.
[b] Beckman Zeromatic pH meter.

Procedure for Paper Partition Chromatography.—The solvent system was prepared by mixing 400 ml. of ethylacetate, 200 ml. of pyridine, and 400 ml. of water in a separatory funnel, shaken and allowed to separate. The lower phase was used to saturate the chamber. The upper phase was used to develop the chromatograms.

The chromatographic jars, used for descending chromatography, were lined with filter paper. The lower phase was placed in the bottom of the chamber with the liner dipping into the solvent. The upper phase was placed in the solvent troughs. The chamber was then allowed to equilibrate for 16 hours.

Points for the application of the sample were marked 3 cm. apart on a line 6.5 cm. from the top of Whatman's No. 1 paper cut to 46-cm. lengths. The samples and standards were dissolved in an appropriate solvent and the samples were spotted on the chromatograms in concentrations of 100 to 500 mcg. The size of the spot should be approximately 5 mm. in diameter. This was accomplished by repeated spotting and drying of the solvent. The chromats were placed into the troughs and developed for approximately 7 hours. The developed chromats were then air dried and sprayed with a benzidine reagent prepared according to Bacon and Edelman (12).

The chromats were then heated in an oven for 15 min. at 90°. The reducing sugars appeared as

TABLE II.—R_f VALUES OF DYES AND SUGARS

Compound	R_f Values[a]
Lactose	0.25
D-Glucose	0.35
D-Galactose	0.35
FD&C Red No. 4	0.55
FD&C Yellow No. 5	0.20
FD&C Blue No. 1	0.60
FD&C Blue No. 2	0.50
FD&C Green No. 3	0.47

[a] Solvent: ethyl acetate-pyridine-water 2:1:2 v/v.

brown spots. The sensitivity was increased by viewing under an ultraviolet lamp. Table II shows the R_f values for the sugars and dyes used in this study.

The use of this ethyl acetate-pyridine-water system for paper partition chromatography of colorants and sugars has the advantage over other previously reported systems for use with carbohydrates (22) in that good separations can be obtained in a shorter period of time.

RESULTS AND DISCUSSION

The influence of conventional lactose and spray-processed lactose and their primary hydrolysis products (D-glucose and D-galactose) on the stability of FD&C Red No. 4, FD&C Yellow No. 5, FD&C Green No. 3 and FD&C Blue No. 1 and No. 2 in solution has been investigated at several storage conditions.

FD&C Red No. 4.—The data presented in Tables III and IV indicate that FD&C Red No. 4 is reasonably stable equally in the presence of spray-processed lactose, conventionally processed lactose and lactose's primary hydrolysis products, D-glucose and D-galactose, at these conditions of testing. A change occurred in the absorption maximum for the buffered dye solutions but no change was seen in the dye-sugar solutions. This change was more pronounced in the visible than the ultraviolet range. The presence of the reducing sugars showed an apparent stabilizing effect on the dye as evidenced particularly by the data showing the influence of light. This improved stability is believed to be due to preferential reduction of the lactose instead of the dye.

TABLE III.—THE EFFECT OF LACTOSE, *d*-GLUCOSE, AND *d*-GALACTOSE ON THE ABSORBANCE OF FD&C RED NO. 4 AT 505 Mμ

FD&C Red No. 4–0.15% in	Zero Time	126 Days RT	28 Days 60°	49 Days E.L.[a]
0.15 *M* Phosphate buffer solution(pH 6.6)	0.470	0.465	0.460	0.422
5% Lactose U.S.P. (regular) solution	0.455	0.458	0.465	0.450
5% Lactose U.S.P. (spray-processed) solution	0.455	0.455	0.460	0.445
5% D-Glucose solution	0.470	0.459	0.465	0.460
5% D-Galactose solution	0.455	0.460	0.465	0.455

[a] Exaggerated lighting.

TABLE IV.—THE EFFECT OF LACTOSE, *d*-GLUCOSE AND *d*-GALACTOSE ON THE ABSORBANCE OF FD&C RED NO. 4 AT 303 Mμ

FD&C Red No. 4–0.15% in	Zero Time	126 Days RT	28 Days 60°	49 Days E.L.[a]
0.15 *M* Phosphate buffer solution (pH 6.6)	0.328	0.319	0.322	0.310
5% Lactose U.S.P. (regular) solution	0.319	0.315	0.323	0.320
5% Lactose U.S.P. (spray-processed) solution	0.322	0.313	0.320	0.323
5% D-Glucose solution	0.321	0.316	0.323	0.323
5% D-Galactose solution	0.320	0.318	0.323	0.325

[a] Exaggerated lighting.

This apparent stability of the dye-sugar solution was further shown by paper partition chromatography examination of the FD&C Red No. 4–sugar solutions after storage, as evidenced by no development of R_f values different from those obtained initially.

These results are in keeping with the recent findings of Swartz, *et al.* (13), in which tablets buffered to pH 7 containing FD&C Red No. 4 exhibited excellent chemical stability after storage for 20 days at 80°.

FD&C Yellow No. 5.—The effect of various sugars on the thermal and light stability of FD&C Yellow No. 5 in solution is summarized in Tables V and VI. The absorbance values indicate apparent stability of FD&C Yellow No. 5 with spray-processed lactose, conventionally processed lactose, and the hydrolysis products, D-glucose and D-galactose.

TABLE V.—THE EFFECT OF LACTOSE, D-GLUCOSE, AND D-GALACTOSE ON THE ABSORBANCE OF FD&C YELLOW NO. 5 AT 430 Mμ

FD&C Yellow No. 5–0.15% in	Zero Time	105 Days RT	105 Days 60°	105 Days E.L.[a]
Distilled water	0.370	0.350	0.345	0.352
0.15 *M* Phosphate buffer solution (pH 6.6)	0.365	0.354	0.353	0.350
5% Lactose U.S.P. (regular) solution	0.360	0.350	0.346	0.342
5% Lactose U.S.P. (spray-processed) solution	0.365	0.348	0.341	0.335
5% D-Glucose solution	0.370	0.350	0.348	0.342
5% D-Galactose solution	0.370	0.350	0.348	0.342

[a] Exaggerated lighting.

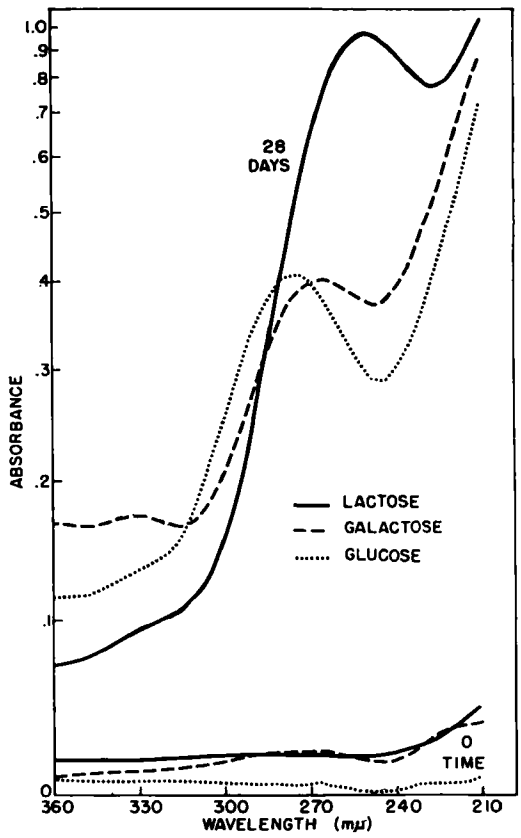

Fig. 1.—A plot of the ultraviolet absorption spectra of 5% solutions of lactose, D-glucose, and D-galactose indicating a change in the spectra after 28 days storage at 60°.

TABLE VI.—THE EFFECT OF LACTOSE, D-GLUCOSE, AND D-GALACTOSE ON THE ABSORBANCE OF FD&C YELLOW No. 5 AT 257 mμ

FD&C Yellow No. 5–0.15% in	Zero Time	105 Days RT	105 Days 60°	105 Days E.L.[a]
Distilled water	0.358	0.342	0.338	0.340
0.15 M Phosphate buffer solution (pH 6.6)	0.359	0.342	0.345	0.335
5% Lactose U.S.P. (regular) solution	0.350	0.335	0.349	0.330
5% Lactose U.S.P. (spray-processed) solution	0.355	0.335	0.348	0.340
5% D-Glucose solution	0.358	0.338	0.360	0.343
5% D-Galactose solution	0.358	0.338	0.360	0.343

[a] Exaggerated lighting.

A diminution in absorbance is seen for the dye-sugar solutions exposed to light. This is in keeping with the report by Lachman, *et al.* (14), who have shown that a flattening as well as a hypsochromic shift of the absorption maximum occurs for FD&C Yellow No. 5 when this colorant is exposed to exaggerated illumination in tablet formulations.

The dye-sugar solutions, after storage at 60°, failed to show the decrease in absorbance at 257 mμ as seen for the room temperature and light samples stored for the same period of time. This may be explained by the changes which take place in the sugar molecule when stored at 60° resulting in high absorption tendencies in the wavelength region of 228–284 mμ as shown in Fig. 1. Since the analysis for FD&C Yellow No. 5 was performed within this range, the results obtained would be expected to be higher than the true values because the absorbance due to the sugars is now playing a role. The tendency of the sugars to appear to develop absorption properties in this wavelength region is being further investigated.

Upon examining the paper chromatograms of the solutions of dye and dye-plus-sugars after storage, no change in R_f values was noticed. This further illustrates the apparent stability of FD&C Yellow No. 5 under the conditions of study. Exposure to exaggerated lighting, however, should be avoided.

FD&C Green No. 3.—The data presented in Tables VII and VIII show FD&C Green No. 3 to be essentially stable in the presence of lactose and its hydrolysis products. Paper partition chromatographic examination of these solutions stored for the same periods of time substantiated this effect. From the paper chromatograms shown in Fig. 2, it is indicated that four impurities (R_f values 0.15, 0.35, 0.65, and 0.75), initially present in the dye, subsequently disappeared upon storage. A possible explanation for this is that the impurities were over-

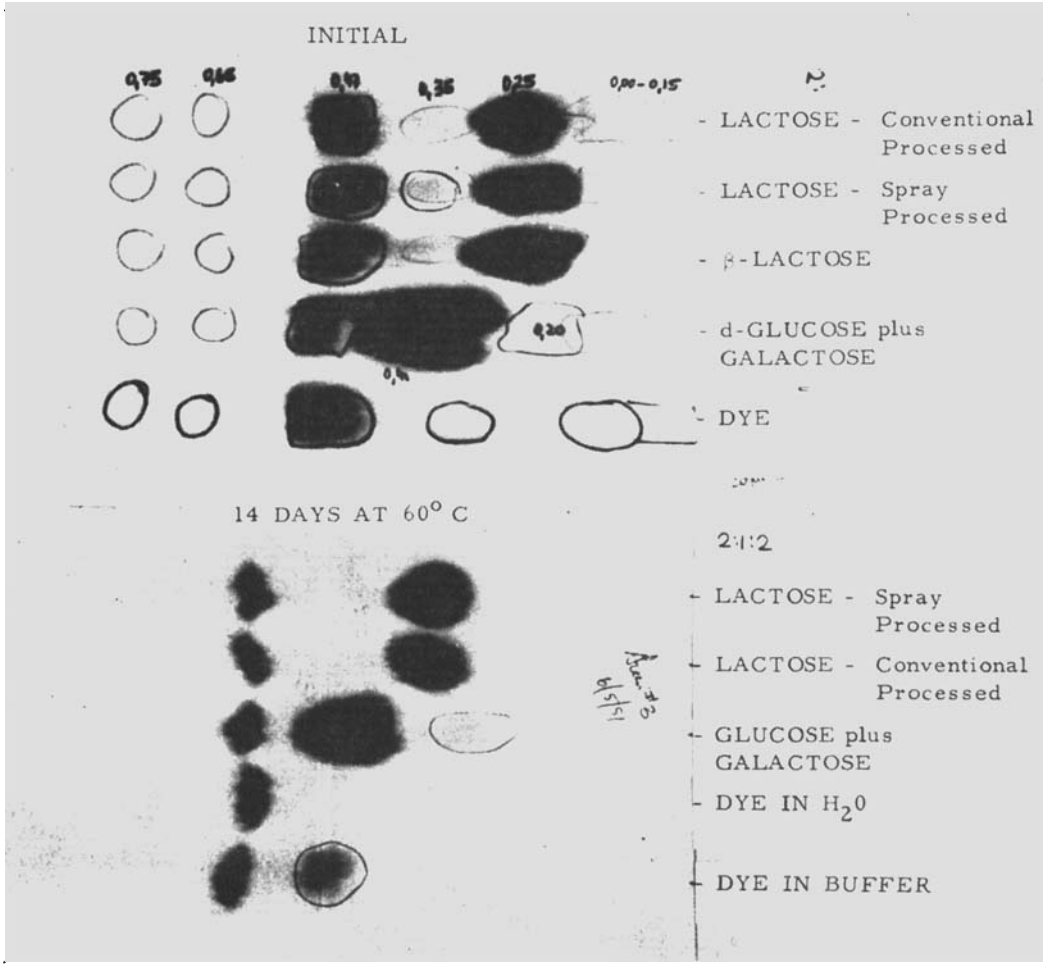

Fig. 2.—Paper chromatograms of FD&C Green No. 3 and FD&C Green No. 3 plus sugar solutions indicating change in R_f value after 14 days at 60°.

TABLE VII.—THE EFFECT OF LACTOSE, D-GLUCOSE, AND D-GALACTOSE ON THE ABSORBANCE OF FD&C GREEN NO. 3 AT 628 mμ

FD&C Green No. 3–0.15% in	Zero Time	150 Days RT	150 Days 60°	92 Days E.L.[a]
Distilled water	0.420	0.399	0.406	0.418
0.15 *M* Phosphate buffer solution (pH 6.6)	0.420	0.395	0.405	0.425
5% Lactose U.S.P. (regular) solution	0.410	0.400	0.418	0.422
5% Lactose U.S.P. (spray-processed) solution	0.420	0.403	0.418	0.436
5% D-Glucose solution	0.420	0.403	0.418	0.423
5% D-Galactose solution	0.420	0.403	0.418	0.423

[a] Exaggerated lighting.

TABLE VIII.—THE EFFECT OF LACTOSE, D-GLUCOSE, AND D-GALACTOSE ON THE ABSORBANCE OF FD&C GREEN NO. 3 AT 302 mμ

FD&C Green No. 3–0.15% in	Zero Time	150 Days RT	150 Days 60°	92 Days E.L.[a]
Distilled water	0.264	0.253	0.260	0.258
0.15 *M* Phosphate buffer solution (pH 6.6)	0.260	0.259	0.261	0.264
5% Lactose U.S.P. (regular) solution	0.255	0.260	0.280	0.263
5% Lactose U.S.P. (spray-processed) solution	0.260	0.259	0.279	0.267
5% D-Glucose solution	0.262	0.259	0.287	0.270
5% D-Galactose solution	0.262	0.259	0.282	0.270

[a] Exaggerated lighting.

oxidized colors. This postulation is in keeping with the spectrophotometric and partition chromatographic findings of Jones, *et al.* (15). Their analysis of various samples of commercial triphenylmethane colors accounted for an average of 94% of the material as the sum of pure dye, leuco compound, volatile material, and inorganic salts. These investigators concluded that the remaining material was chiefly overoxidized color.

FD&C Blue No. 1.—Aqueous solutions of FD&C Blue No. 1 with the various sugars were stored similarly to the FD&C Green No. 3 and no change in absorbance value at 625 mμ was noticed to occur with this dye in the presence of the sugars stored at room temperature and 60° as presented in Table IX.

TABLE IX.—THE EFFECT OF LACTOSE, D-GLUCOSE, AND D-GALACTOSE ON THE ABSORBANCE OF FD&C BLUE NO. 1 AT 625 mμ

FD&C Blue No. 1–0.15% in	Zero Time	90 Days RT	90 Days 60°	90 Days E.L.[a]
Distilled water	0.870	0.870	0.860	0.860
0.15 *M* Phosphate buffer solution (pH 6.6)	0.870	0.870	0.860	0.850
5% Lactose U.S.P. (regular) solution	0.875	0.870	0.870	0.870
5% Lactose U.S.P. (spray-processed) solution	0.870	0.870	0.870	0.830
5% D-Glucose solution	0.875	0.870	0.870	0.810
5% D-Galactose solution	0.870	0.870	0.870	0.710

[a] Exaggerated lighting.

However, at 625 mμ, the spectrophotometric data indicated a decrease in absorbance for the FD&C Blue No. 1 in solution with spray-processed lactose, D-glucose, and D-galactose exposed to exaggerated lighting for 90 days. The data indicated that the effect on the absorbance was greatest with the D-galactose.

No absorbance at 307 mμ was observed for this dye in the presence of the sugars at 60° as illustrated in Table X. The absorption maximum was found to

TABLE X.—THE EFFECT OF LACTOSE, D-GLUCOSE, AND D-GALACTOSE ON THE ABSORBANCE OF FD&C BLUE NO. 1 AT 307 mμ

FD&C Blue No. 1–0.15% in	Zero Time	90 Days RT	90 Days 60°	90 Days E.L.[a]
Distilled water	0.115	0.118	0.113	0.107
0.15 *M* Phosphate buffer solution (pH 6.6)	0.114	0.113	0.113	0.107
5% Lactose U.S.P. (regular) solution	0.117	0.113	...	0.113
5% Lactose U.S.P. (spray-processed) solution	0.115	0.110	...	0.110
5% D-Glucose solution	0.115	0.113	...	0.110
5% D-Galactose solution	0.117	0.118	...	0.105

[a] Exaggerated lighting.

shift from 307 to 280 mμ as shown in Fig. 3. This effect can possibly be explained by the sugar molecule being thermally degraded producing products which absorb at 280 mμ as previously shown in Fig. 1.

No changes were detected in the FD&C Blue No. 1 solutions stored at room temperature and 60° by paper partition chromatographic procedures. There was some indication of dye loss in the samples kept for 90 days in the light cabinet.

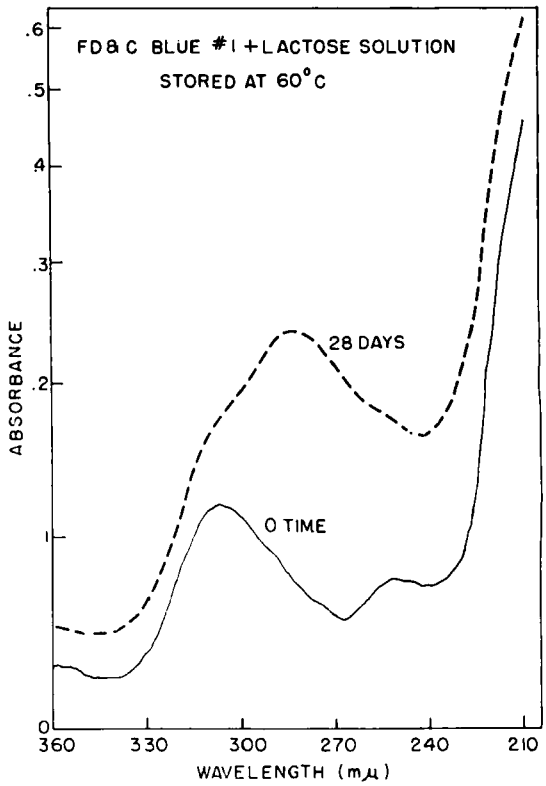

Fig. 3.—Plot showing the influence of lactose on the ultraviolet absorption maximum of FD&C Blue No. 1 after 28 days' storage at 60°.

FD&C Blue No. 2.—Although studies with the dye FD&C Blue No. 2 are still in progress, it is felt worth while to mention certain observations obtained thus far. The preliminary data indicated that lactose, D-glucose, and D-galactose were very deleterious to the stability of this colorant and that the monosaccharides were more destructive than the unhydrolyzed disaccharide. The absorption data obtained from these conditions of testing were very erratic and did not permit kinetic interpretation. Figure 4 shows the spectra changes for FD&C Blue No. 2 in the presence of D-glucose after 3, 6, and 10 days exposure to light. For the 10-day sample, two different spectra (A and B) were obtained for the same sample taken within minutes of one another. Paper partition chromatographic examination of the aqueous buffered solutions of FD&C Blue No. 1 and FD&C Blue No. 2 plus the sugars further substantiated the findings of instability for this colorant. The initial R_f values for FD&C Blue No. 2 at 0.50, and 0.60 for trace impurities, diminished rapidly until no dye was visible after 14 days of heat and light storage (Fig. 5). After 12 weeks, no dye was detected in the room temperature sample. It was observed that two new R_f values appeared at 0.16 and 0.35, which probably indicated the decomposition products of the dye.

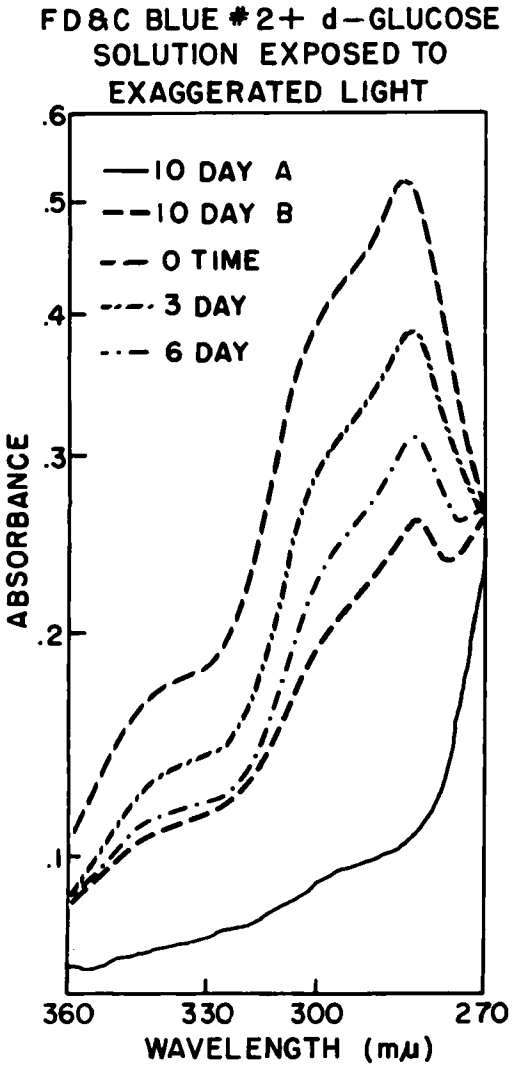

Fig. 4.—A plot of the ultraviolet absorption spectra of FD&C Blue No. 2 indicating changes in the presence of D-glucose after varying periods of storage at 60°.

The decomposition of this indigo disulfonate dye has been studied by several investigators. Scott, *et al.* (8), reporting on the accelerated color loss of certified dyes in the presence of nonionic surfactants, showed that the rate of fading of FD&C Blue No. 2 in the presence or absence of Pluronic F68 follows pseudo first-order kinetics. Kuramoto, *et al.* (7), evaluated the influence of several pharmaceutical materials on the rate of fading of FD&C Blue No. 2 in aqueous solution at pH 6.64 and found that sugars, such as dextrose, lactose, and sucrose, increased the rate of fading of this dye. These investigators postulated that the decomposition of FD&C Blue No. 2 took place by reduction *via* a semiquinone formation to a colorless leuco compound.

Preisler, *et al.* (16), investigated the oxidation–reduction potentials of the indigo sulfonates and their reduced forms and their absorption coefficients in alkaline buffers. These investigators observed that "when indigo in alkaline alcoholic solution or indigo disulfonate in aqueous buffers of about pH 11.0 to 12.5 are reduced by the gradual addition of reducing agent, a red intermediate color appears between the initial blue (or green) of the fully oxidized and the yellow of the reduced compound; on reoxidation the red color appears in the reverse color sequence. Outside this pH zone, the red color is not observed; in less alkaline solutions of the sulfonates, the color changes from blue, through the green of the mixture, to yellow; in more strongly alkaline solutions, the initial color is a yellow, which on reduction changes to a lighter yellow." The data collected by Preisler established conclusively that the red intermediate color was due to a semiquinone formation.

In the present study, a similar reduction pattern was observed. A red color developed after 6 days at 60° in the FD&C Blue No. 2—D-glucose solution between the initial blue and the final yellow color of the faded solution. This red color lasted for only a short period of time. The appearance of this red intermediate at the lower pH's of this study indicated that the semiquinone formation was possible under less alkaline conditions than those observed by Preisler and his co-workers. These data tended to support the postulation of Kuramoto, *et al.*, that reduction to a semiquinone formation occurred in the decomposition of this certified color.

Preliminary results in this investigation indicated that when the sealed ampuls of FD&C Blue No. 2 plus the sugar solutions were exposed to atmospheric conditions, the green-yellow color of the apparently reduced compound changed to a pale blue. On further exposure, the blue color of the solution faded to a pale yellow. The solution at this time was observed to be strongly fluorescent when viewed under ultraviolet light. These changes appeared to indicate that the decomposition of FD&C Blue No. 2 was not solely one of complete reduction to the leuco compounds, but that the mechanism of fading of this indigo disulfonate dye could possibly be reduction followed by oxidation. Inskeep and Kretlow (17)

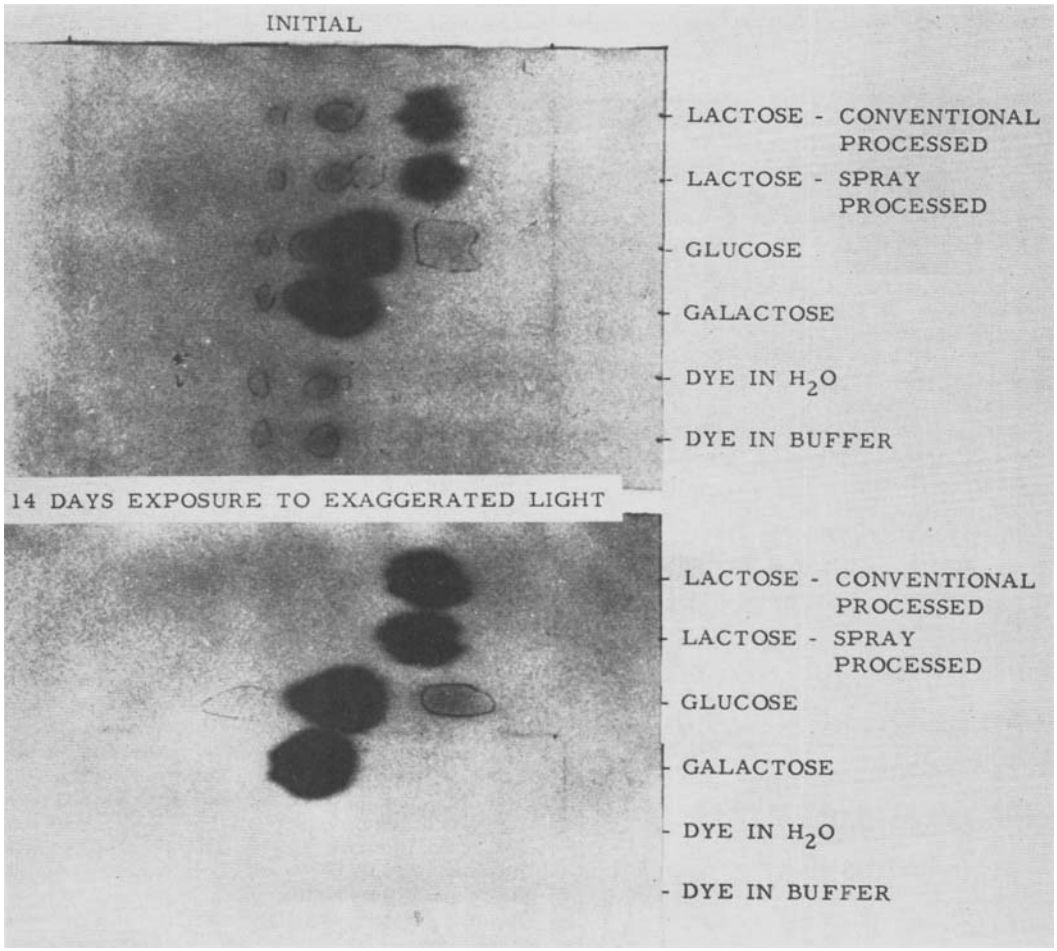

Fig. 5.—Chromatograms showing the R_f values for FD&C Blue No. 2 initially and their absence after 14 days' exposure to exaggerated lighting.

observed that although a common property of dyestuffs 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.

Jones, *et al.* (18), studied the photodecomposition of dilute solutions of FD&C Blue No. 2 exposed to ordinary and diffuse laboratory light. Spectrophotometric examination showed that the chief component was isatinsulfonic acid. When viewed under ultraviolet light, the faded solutions exhibited a bluish fluorescence characteristic of the sulfonated anthranilic acids. These experiments showed that the fading of solutions of FD&C Blue No. 2 was due almost entirely to the oxidation of the colorant to isatinsulfonic acid and finally to sulfonated anthranilic acid. In alkaline solution, these investigators observed that the indigo disulfonate is converted to disulfonated crysanilic acid which is oxidized by potassium persulfate in acid solution to approximately equal amounts of isatinsulfonic acid and sulfonated anthranilic acid.

That oxidative decomposition was more destructive to this colorant than reduction is stated in the review made by Desai and Giles (19). Hibbert (20) was able to isolate a small quantity of isatin from samples of indigo-dyed cotton cloth which had been faded by exposure to sunlight or to the light from a carbon arc. Zuckerman (21) also reported that FD&C Blue No. 2 had the poorest light resistance of all the certified colors and because of its structure exhibited the best resistance to reducing agents.

SUMMARY AND CONCLUSIONS

In this study the influence of lactose, D-glucose, and D-galactose on the stability of five certified dyes has been investigated. From the results obtained the following conclusions can be drawn.

1. In aqueous solutions buffered to pH 6.6 to 6.8, FD&C Red No. 4, FD&C Yellow No. 5, FD&C Green No. 3, and FD&C Blue No. 1 are relatively stable upon exposure to exaggerated lighting and temperature in the presence of spray-dried and conventional-processed lactose, D-glucose, and D-galactose.

2. FD&C Blue No. 2 is very unstable, particularly when exposed to light, and the sugars significantly accelerate the decomposition of this

colorant. The decomposition of FD&C Blue No. 2 appears to proceed by reduction to a semiquinone followed by oxidation.

3. There appears to be some evidence to indicate molecular changes in the lactose upon storage at high temperature. These changes seem to interfere with the spectra analysis in the ultraviolet range for colorants which absorb between 228 and 284 mμ.

As a result of this investigation, further studies are underway to thoroughly evaluate the thermal stability of lactose and lactose-and-colorants, both in aqueous solutions and in solid dosage forms. In addition studies are continuing to elucidate the degradation reaction for FD&C Blue No. 2.

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Drug Standards

Assay of Progesterone in Oil Injectables

By JACOB WOLFF

A method is presented for the quantitative determination of progesterone in oil solutions. The ketosteroid is isolated by partition chromatography using nitromethane on purified siliceous earth as the immobile phase and *n*-heptane as the eluent. The assay is based on the ultraviolet absorbance in ethanol solution at the maximum near 241 mμ. The identity of the separated progesterone is confirmed by determining the infrared spectrum in carbon disulfide solution.

THE U.S.P. XVI (1) assay procedure for progesterone injection is a welcome simplification of the method in the previous edition (2). However, the assay is still based on the reaction with 2,4-dinitrophenylhydrazine, a general reagent for aldehydes and ketones. The melting point of the resulting hydrazone is rather high, 267–275°, and no other purity criteria are specified. Monty (3) reports that some chromatographically inhomogeneous 2,4-dinitrophenylhydrazones exhibit melting points no lower than the accepted values for the pure compounds. Umberger (4) used the color reaction with isonicotinic acid hydrazide for the determination of progesterone and testosterone. This reaction is more specific since it depends on the presence of a conjugated carbonyl linkage as in Δ^4-3-ketosteroids. Touchstone and Murawec (5) used spectrofluorometric techniques for the determination of progesterone in biological fluids. It appeared that a procedure based on the physical separation of progesterone from oil solutions, with its subsequent assay and identification, would be a more desirable approach than any of these.

Column chromatographic techniques have been successfully applied to similar problems.

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