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COLOR STABILITY: AN EVALUATION OF THREE NATURAL-SOURCE COLORANTS AS COMPONENTS IN COMPRESSED TABLETS

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ABSTRACT

A modified colorimetric procedure was developed in order to measure changes in color in multiple-tablet beds rather than in individual tablets or other dosage forms. Fading, induced by exposure of the test product to an exaggerated illumination source, was assumed to be the nechanism for color change during the short-term studies. The color changes encountered during the long-term studies were attributed to the effects of chromatic distortion produced by storage conditions (time, temperature, humidity, and/or package).

This study encompassed an evaluation of three natural-source colorants included in the composition of compressed tablets. These colorants (carmine. cranberry, and raspberry) were selected as model components in the tablet formulas for the long-term storage studies because of their favorable photostability encountered during the short-term screening trials.

INTRODUCTION

Many pharmaceutical dosage forms use color, siize and shape for easy identification and for psycogological reasons. The proper selection of a colorant can have decided repercussions for the desired pharmacologoc effect when considering the reaction of the target population [1].

The pharmaceutical formulator may encounter many problems when attempting to provide product identification through the use of a particular

colorant in his product. Among these problems are color fastness and chromatic changes. Parameters for consideration include the photostability of the colorant, per se, or its reactivity with either the active or with other excipients in the formula.

There are two classifications for coloring agents used in the pharmaceutical industry, namely, certified colors and those exempt from certification. Certified colors are produced synthetically. Each production batch must meet certain stringent requirements, when tested by the Food and Drug Administration, before it can be certified. They are identified by specific designation, e. g., FD&C Yeloow No. 6,, FD&C Blue No 1, etc. Further designation may include lake (dye adsorbed on a substrate, usually alumina) or water soluble dye.

Colorants whcih are exempt from certification are derived from animal, miineral, or vegetable sources or can be synthetic duplicates of the naturally-occurring substance. Most natural-source colorants have limited pharmaceutical usefulness because (a) they commonly have weak tinctorial strength, (b) they often impart a taste and/or an odor to the product in which they are incorporated, and (c) most natural products have inherent variability among batches. Interest in this class of colorants, as alternate sources for product imagery, has increased , in recent years, because of the decertification of so many of the extensively used certified colors.

Color fastness may be defined as the resistance of the colorant to fading when the dosage form, in whch it is included, is exposed to illumination. The subject of fading, as it relates to the use of certified colorants in pharmaceutical dosage forms, has been investigated extensively [2-13]. Conversely, comparable studies concerned with the fading of products containing natural-source colorants has been investigated, almost exclusively, by the food industry [14-16].

Various forms of exaggerated light, e. g. fluorescent [7], incandescent [8], or ultraviolet [12] have been used to provide quantifiable illumination intensity whereby the photostability of a new formulation may be evaluated. The effects of the foregoing light sources on the samples being studied are obtained, spectrophotometrically by reflectance, usually from a single dosage form. The present study addresses the measurements reflected from a multiple-tablet bed.

The spectrophotometric scan will provide a very precise quantification of the color being measured. Color change can be calculated from the data obtained from a sample after exposure to one of the light sources but it is complicated. Tristimulus data, on the other hand, provides a simpler means of

comparing colors and, hence, color change [17, 18]. The calculation of color change uses the following equation:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \qquad \text{Eq. 1}$$

where L is the luminosity factor and a and b are the chromaticity factors.

The objectives of this present study were achieved by modifying the method of obtaining colorimetric readings in order to measure the relative color stability of multiple-tablet samples. This method was used, initially, for screening from among the many candidates of those colorants which would provide the models for the long-term study. It was modified , further, for the evaluation of those model colorants subjected to the long-term study.

No attempt has been made, in this study, to elucidate the specific mechanism for the incidence of fading or chromatic distortion of any of the specific colorants being studied.

EXPERIMENTAL

Material

The colorants and other excipients used in this study include the following:

Carmine 40 Calcium/Aluminum Lake - Biocon
Cranberry Juice Concentrate - Ocean Spray
Raspberry Juice Concentrate - Ocean Spray
FD&C Yellow No 6 alumina lake - Warner Jenkins
Microcrystalline Cellulose (Avicel PH 101) - FMC
Pregelatinized Starch 1500 - Colorcon
Hydrous Lactose - Foremost
Magnesium Stearate - WCD

Equipment

Colorlab Colorimeter and Sensor - Gardner
Fluorescent Light Cabinet - Forma Scientific
Refrigerator (5° C) - Norlake Scientific
Stability Cabinets - Hot Pak - maintained at
30 ° C/ Ambient
30 ° C/ 75% Relative Humidity

40 ° C/ Ambient
40 ° C/ 75% Relative Humidity
50 ° C/ Ambient
Petri Dishes (5 cm and 9 cm)

Preparation of the Samples

The following general placebo formula was used for the preparation of all test and control tablets:

Colorant	0.1 to 5.0 mg.*
Microcrystalline Cellulose	25.0 mg
Pregelatinized Starch 1500	25.0 mg
Hydrous Lactose	50.0 mg
Granulating Solution	---
Magnesium Stearate	0.5 mg

* Sufficient to produce comparable color intensity (L value)

The test colorant was dispersed in the hydroalcoholic granulating solution and used to granulate the mixed powders. After drying and milling, the granules were lubricated with magnesium stearate and compressed (100 mg tablets using 0.25 inch , round, standard-concave tooling).

Colorimetric readings were obtained, using 10 g of sample (equivalent to 100 tablets), initially and at each observation period. When the samples would be cascaded into the 5 cm petri dish, the tablets would not lie in the same plane. Light reflected from such a surface would be irregular. It was theorized that if the reflectance would be read at two different angles, the average of the two readings would "neutralize" the lack of a plane surface.

During the short-term trials, the same orientation of the tablet bed was assured with the aid of a mark on the side of the smaller (5 cm) dish and in the base of the larger (9 cm) dish. Two marks on the outside edge of the larger dish (the second at 90 ° to the first) ensured the same positioning of a given sample for each observation when these marks would be alligned with a mark on the laboratory jack. The 9 cm dish was larger than the view port of the sensor unit. When it would be brought into juxtaposition with the unit, the sample dish which had been placed into the larger dish would be at a constant distance from the sensor bulbs., for any given sample, at each observation time.

The same sample dish was used for any given sample throughout the entire test period, during the short-term trials. It was easy to maintain the orientation for any given sample for these screening trials. On the other hand, tablets were transferred to a particular container (plastic or glass) after initial readings and then cascaded from the container at the specific observation period into a sample dish (not the same one with which readings were obtained initially). This situation required a further modification of the method for obtaining readings. The test sample (100 tablets) was cascaded into a 5 cm dish and readings obtained as previously described. This was repeated ten times for both initial values and for those determined at any of the designated observation times (1. 3. 6. or 12 months) for samples containg each colorant.

Samples for the long-term study were packaged in high density polyethylene and in glass bottles and placed at stability stations for storage. Readings (L, a, and b values) were obtained on selected samples at the designated time for the observation.

RESULTS AND DISCUSSION

Surface color evaluation is very subjective, visually, and dependent upon the ability of the individual to determine color differences reproducibly [9]. The human eye is not sufficiently sensitive to light reflected from all regions of the visible spectrum, particularly at the extremes (violets and reds) [19]. Instrumental colorimetry provides a precise description of a colored sample and aids in defining, quantitatively, differences between a colored sample and its established standard [20].

Color change, encountered during the short-term screening trials, was attributed to fading caused by the exposure of the test product to exaggerated light The angle of reflectance was maintained constant for any given sample by reason of the same orientation for that sample each time readings were obtained. The data contained in Table 1 represents ΔE values generated during the short-term screening trials when samples were exposed to the exaggerated light directly (open) or with the protection of an amber-acetate sheet (covered). When Δ E approaches a value of 6, color differences between a test product and its standard can be discerned, visually, in a side-by-side comparison [21].

The data in Table 1 can be evaluated more readily graphically (Figures 1, 2, and 3). Each figure shows the comparison of Δ E values (opened and covered) of a specific colorant with those for the experimental control, FD&C

TABLE 1.
Relative Color Stability (ΔE) Short-term Screening

Colorant	Conc.	Storage	Day 2	4	7	14
Carmine Lake	0.1%	Open	3.61	7.40	8.81	10.9
		Covered	2.56	3.83	4.68	6.03
Cranberry Conc.	5.0%	Open	3.80	6.72	6.73	8.44
		Covered	1.46	1.56	2.05	3.82
Raspberry Conc.	5.0%	Open	1.07	2.63	3.87	4.99
		Covered	0.36	1.18	2.22	3.14
Yellow No 6	0.2%	Open	4.78	5.81	8.17	10.7
		Covered	2.77	2.94	3.17	4.64

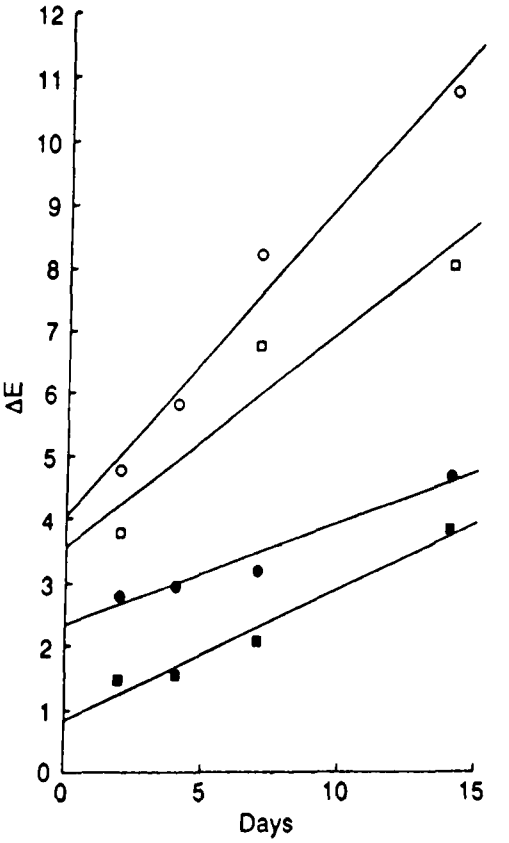

Figure 1: Relative Color Stability (Δ E) of tablets containing Carmine 40 calcium aliminum lake (□ open; ■ covered) or the experimental control FD&C Yellow No 6 alumina lake (○ open; ● covered).

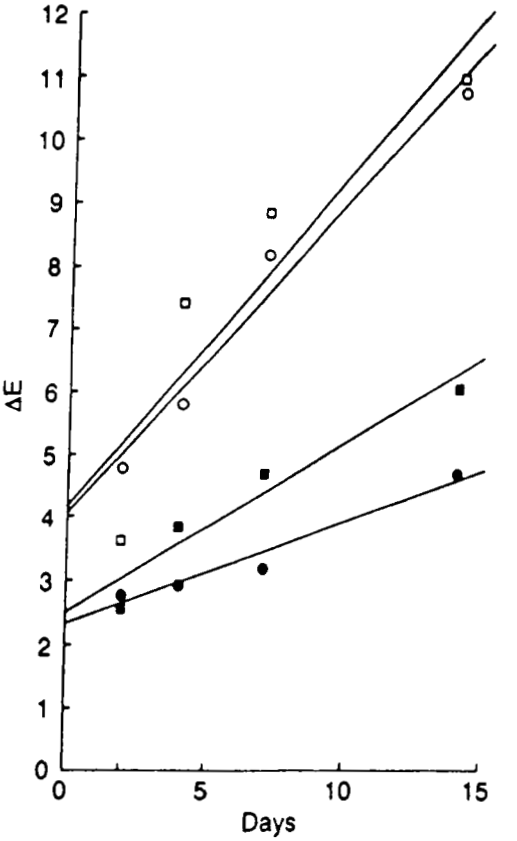

Figure 2: Relative Color Stability (Δ E) of tablets containing Cranberry Juice Concentrate (□ open; ■ covered) or the experimental control FD&C Yellow No 6 alumina lake (○ open; ● covered).

Yellow No 6 alumina lake. The photostability for Carmine is shown in Figure 1. That of Cranberry is shown in Figure 2 ; Raspberry in Figure 3.

The family of lines in each of the figures represent conputer-generated, least-squares-regression lines. The validity of the fit of that line for the data points shown becomes greater as the regression coefficient (R^2) for that line approaches unity. The slope of each line is indicative of the rate of fading of the test sample as induced by exposure to the exaggerated light.

A comparison is shown in Table 2 of the regression coefficients and slopes for the regression lines for each of the test colorants and the control and for the

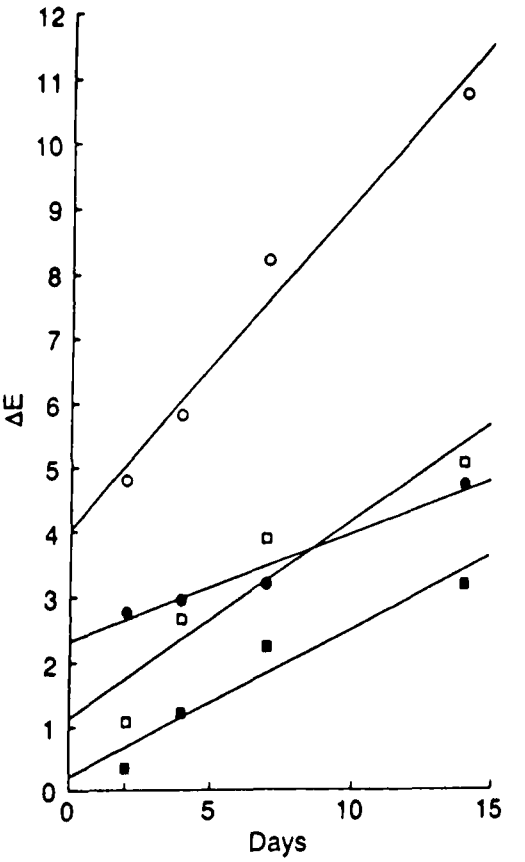

Figure 3: Relative Color Stability (Δ E) of tablets containing Raspberry Juice Concentrate (□ open; ■ covered) or the experimental control FD&C Yellow No 6 alumina lake (○ open; ● covered).

TABLE 2.
Relative Color Stability (ΔE) Short-term Screening

Colorant	Conc.	Storage	Regression Coefficient	Regression Line Slope
Carmine Lake	0.1%	Open	0.817	0.52846
		Covered	0.933	0.26822
Cranberry Conc.	5.0%	Open	0.901	0.33917
		Covered	0.916	0.20622
Raspberry Conc.	5.0%	Open	0.876	0.30006
		Covered	0.928	0.22193
Yellow No 6	0.2%	Open	0.970	0.49468
		Covered	0.957	0.19540

TABLE 3.
Relative Color Stability (ΔE) Long-term Storage Study: Carmine

Time (Mo.)	Package	Relative Humidity	Temperature 50°C	40°C	30°C	5°C
1	Plastic	Amb	1.24			
		75 %		0.9		
	Glass	Amb	1.17			
		75 %		1.49		
3	Plastic	Amb	2.13			
		75 %		2.83		
	Glass	Amb	0.53			
		75%		1.01		
6	Plastic	Amb		0.61		
		75%			2.20	
	Glass	Amb		1.74		
		75%			0.61	
12	Plastic	Amb		1.42	1.36	0.92
		75%			3.00	
	Glass	Amb		1.80	1.09	0.47
		75%			1.36	

conditions of exposure to the light (open or covered). The ranking for the effects of fading , using the slopes of the regression lines, indicates that the raspberry was the best followed by cranberry, Yellow No 6 and finally carmine.

The angle of reflectance would be different, for any given sample, each time the tablets would be cascaded from the bottle into the sample dish at a particular observation time, during the long-term studies. These differences were "neutralized" by using the averages of twenty readings resulting from multiple transfers from the bottle into a sample dish. The effects of storage conditions (time, temperature, humidity, and / or package) on color stability

TABLE 4.
Relative Color Stability (ΔE) Long-term Storage Study: Cranberry

Time (Mo.)	Package	Relative Humidity	Temperature 50°C	40°C	30°C	5°C
1	Plastic	Amb	4.00			
		75 %		1.99		
	Glass	Amb	4.22			
		75 %		3.42		
3	Plastic	Amb	5.07			
		75 %		5.98		
	Glass	Amb	5.42			
		75%		4.12		
6	Plastic	Amb		1.75	1.36	
		75%			8.88	
	Glass	Amb			0.69	
		75%			6.71	
12	Plastic	Amb		2.70		
		75%			9.05	
	Glass	Amb		2.45	1.31	1.21
		75%				8.80

during the long-term studies are shown as Δ E values for tablets containing Carmine (Table 3), Cranberry (Table 4), Raspberry (Table 5), and the experimental control Yellow No 6 alumina lake (Table 6). Color changes, here, were attributed to chromatic distortion produced by conditions of storage. The ranking from this part of the study indicates that the carmine was the best, followed by cranberry, Yellow no 6 and finally the raspberry.

Generally, color stability seemed to be affected less severely for test products packaged in glass bottles. It would appear that humidity has a more marked adverse effect on color stability than temperature or time.

TABLE 5
Relative Color Stability (ΔE) Long-term Storage Study: Raspberry

Time (Mo.)	Package	Relative Humidity	Temperature 50°C	40°C	30°C	5°C
1	Plastic	Amb	2.83			
		75 %		4.66		
	Glass	Amb	2.58			
		75 %		6.90		
3	Plastic	Amb	5.55			
		75 %		15.2		
	Glass	Amb	9.74			
		75%		8.74		
6	Plastic	Amb		17.1	3.08	
		75%				
	Glass	Amb		14.4	3.33	
		75%				
12	Plastic	Amb		2.01	1.94	1.07
		75%			1.52	
	Glass	Amb		19.2	0.44	0.93
		75%			11.6	

CONCLUSIONS

The results from the first part of this study have demonstrated an ability to measure the effects of color change as it relates to a more realistic evaluation of a multiple-tablet bed. This method allows comparisons to be made for the selection of one colorant in preference to another. Further, it can be used to establish relative rates of degradation.

It was the close approximation of the data for the three colorants (Carmine, Cranberry, and Raspberry) to that of the experimental control (Yellow No 6), encountered during the screening trials, which led to their selection for the long-term, storage studies.

TABLE 6
Relative Color Stability (ΔE) Long-term Storage Study: Yellow No. 6

Time (Mo.)	Package	Relative Humidity	Temperature 50°C	40°C	30°C	5°C
1	Plastic	Amb	1.59			
		75 %		2,05		
	Glass	Amb	1.48			
		75 %		2.62		
3	Plastic	Amb	1.85			
		75 %		2,89		
	Glass	Amb	1,86			
		75%		3.69		
6	Plastic	Amb		2.20	1.81	1.90
		75%				
	Glass	Amb		2.83	1.31	1.78
		75%				
12	Plastic	Amb		2.66	2.02	2.23
		75%			1.09	
	Glass	Amb		2.96	2.16	1.88
		75%			2.34	

The results of the second part of this study suggest the potential for using carmine or cranberry juice concentrate as colorants for compressed tablets. Their specific use, of course, would be dependent upon the outcome of compatability tests with a desired active or with other excipients.

REFERENCES

1. A. M. Raff. J. Pharm Sci. 52 (3): 291 - 295 (1963)

2. T. Urbanyi, C. J. Schwartz, and L. Lachman, J. Am. Pharm. Assoc. (Sci. Ed.) 49 (3) : 163-164 (1960

3. L. Lachman, C. J. Schwartz, T. Urbanyi, and J. Cooper, *ibid)* 49 (3): 165-169 (1960)

4. L. Lachman, S. Weinstein, C. J. Schwartz, T. Urbanyi, and J. Cooper, J. Pharm. Sci. 50 (2) : 141-144 (1961)

5. C. J. Schwartz, L. Lachman, T. Urbanyi, and J. Cooper, *ibid* 50 (2) : 145 - 148 (1961)

6. L. Lachman, T. Urbanyi, S. Weinstein, J. Cooper, and C. J. Schwartz, *ibid* 51 (4) : 321--326 (1962)

7. C. J. Schwartz, L. Lachman, T. Urbanyi, S. Weinstein and J. Cooper, *ibid* 51 (4) : 326-328 (1962)

8, M. E. Everhard and F. W. Goodhart, *ibid* 52 (3) : 281 - 283 (1963)

9. M. E. Everhard, D. A. Dichcaus, and F. W. Goodhart, *ibid* 53 (2) : 173 - 178 (1964)

10 F.. W. Goodhart, M. E. Everhard, and D. A. Dickcaus, *ibid* 53 (3) : 338 - 340 (1964)

11. F. W. Goodhart, H. A. Lieberman, D. S. Mody, and F. C. Ninger, *ibid* 56 (1) : 63 - 67 (1967)

12. P. Turi, D. Brusco, H. V. Maulding, R. A. Tausenfreund, and A. F. Michaelis, *ibid* 61 (1 1) : 1811 - 1814 (1972)

13. P. Turi, R. A. Tausenfreund, A. F. Michaelis, and C. Steren *ibid* 63 (8) : 1309 - 1311 (1974)

14. A. J. Finberg, Food Product Development, 5 (8) : 146 - 147 (1971)

15. Anonymous, The Flavor Industry, 2 (4) :217 - 219 (1971)

16. J. H. von Elbe and I. Y. Maing, General Science Today 18 (9) : 263 - 264 (1973)

17. L. Lachman, C. J. Schwartz, and J. Cooper, J. Am Pharm Assoc. (Sci. Ed.) 49 (4) : 213 - 218 (1960)

18. R. S. Hunter, J. Ophth Soc. Amer. 38 : 161 (1948)

19. F. M. Bogdansky, J. Pharm. Sci., 64 (2) : 322 - 328 (1 9 75)

20. R. W. Jurgens, jJ. P. Lacz, and A. D. Weiss, *ibid* 67 (10):1485 - 1486 (1978)

21. B. Judd, " Color in Business, Science, and Industry ", John Wiley and Sons, New York, N. Y. (1952)