Stability of Certified Dyes in Tablets III

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A colorimetric method is given for studying the stability of pharmaceutical tablet formulations. The data obtained were employed to calculate color difference units by using the Adams-Nickerson and the MacAdams equations. The fastness of the colorants in the particular formulation studied was rated relative to one another, and these ratings were compared to other pharmaceutical stability references. The methodology of studying color stability is applicable to many types of pharmaceutical preparations.

METHODS OF determining the stability of pharmaceutical colorants have appeared in several reports (1–5). Spectrophotometric measurements have provided a physical evaluation of color; but, in order to obtain a quantitative color measurement which relates to human color perception, colorimetry must be used. With the use of colorimetric instruments, the techniques of measurements and means of analyzing data have been continually expanding. The variation in physical form and composition of pharmaceutical dosage forms present additional problems in both instrumental methods and data analysis.

The pharmaceutical formulator has a need for predicting the fastness or stability of pharmaceutical colorants. Reference tabulations (6, 7) have served as his guide for color selection for many years. Modern instrumentation applied to simple, colored pharmaceuticals aged under standardized conditions provides useful data for the formulator. Similar studies have been carried out in the textile and paint industries (8–10) and furnish a guide for color stability testing of pharmaceuticals.

The purpose of this study is to describe a method of measuring color stability of pharmaceutical tablets. Color difference units are used to describe the chromaticity and lightness changes of the colors before and after aging. Particular colorants will be rated for color stability in the specific pharmaceutical composition studied. The fastness of the colorants will be rated relative to each other, and these ratings will be compared to those listed in other pharmaceutical color stability references (1, 6, 7). Differences in ratings of color stability in several references will be explained and reasons given why the color ranking proposed in this report is regarded as superior. The methodology involved is applicable to color stability testing of many pharmaceutical dosage forms.

EXPERIMENTAL

Colorants.—D&C and FD&C colorants were employed in several concentrations and are listed in Table I.

Formulations.—A basic formula was chosen and used throughout so that the relative inherent color stabilities could be determined. Mannitol was employed as the diluent because of its inertness and its ability to be uniformly colored. Magnesium stearate, 1%, was employed as the lubricant because of its wide usage. Since most formulations contained pigments, a dry blending and milling procedure was used for their preparation. In some instances, soluble colorants were used and these were dissolved in an appropriate solvent, and the resulting solutions were used to dye the mannitol. Tablets weighing 20 Gm. and measuring $2^1/_4$ in. in diameter were compressed on the Carver press at 20,000 lb./sq. in.

Exposure.—After initial measurement, tablets were exposed to cool, white fluorescent light measuring 1000 ft.-candles as determined by a model 614 Weston light meter. The fluorescent light was furnished by a metal cabinet which enclosed a bank of twelve 30-w. and four 20-w. General Electric bulbs (F30T12·CW·RS and F20T12·CW). Heat buildup was prevented by a constantly operating fan.

Measurement.—Colorimetric measurements were made at various time intervals that were appropriate depending on rapidity of color fading. The Color-Eye[1] model D-1 colorimeter was used with the nonspecular insert and white vitriolite standards. The following sets of equations were used to calculate the tristimulus values X_{CIE}, Y_{CIE}, and Z_{CIE}.

$$X_{CIE} = .69664\ X_{CE} + .17416\ Z_{CE} \quad \text{(Eq. 1)}$$

$$Y_{CIE} = .8937\ Y_{CE} \quad \text{(Eq. 2)}$$

$$Z_{CIE} = 1.0574\ Z_{CE} \quad \text{(Eq. 3)}$$

The subscript *CIE* denotes the true tristimulus

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[1] Product of Instrument Development Laboratories, Division of Kollmorgen Corp., Attleboro, Mass.

TABLE I.—LISTING OF RELATIVE LIGHT STABILITY OF CERTIFIED COAL TAR COLORANTS

Colorant	Colorant Concn., %[a]	Exptl. Relative Stability[b]	Δe[c]	Stability Listed by Peacock[d]	Stability Listed by Lachman[e]	Chemical Type, *CI* No.[f]
D&C Red No. 30 Lake	.025–.075	*G*	1.1–2.6	6		Indigoid 73360
FD&C Blue No. 2 Lake	.013–.039	*G*	3.6–2.8	1	10	Indigoid Lake 73015
FD&C Yellow No. 5 Lake	.028–.084	*G*	5.0–5.9	5	9	PyrazoloneLake19140
D&C Yellow No. 10	.30	*G*	1.8	3	3	Quinoline 47005
D&C Yellow No. 11	.30	*G*	3.2	2		Quinoline 47000
D&C Orange No. 17	.162	*G*	3.4	5		Monoazo 12075
D&C Green No. 6	.097	*G*	3.4	4		Anthraquinone 61565
FD&C Red No. 2 Lake	.027–.081	*M*	6.5–14.6	4		Monoazo 16185
D&C Red No. 7 Lake	.031–.093	*M*	12.0–13.5	6		Monoazo 15850
D&C Red No. 36	.09–.27	*M*	8.5–9.8	6		Monoazo 12085
D&C Blue No. 6	.097–.291	*M*	13.4–16.1	6		Indigoid 73000
D&C Orange No. 5	.162	*M*	15.0	2		Fluoran
D&C Green No. 5	.100	*M*	9.8	5	5	Anthraquinone 61570
FD&C Red No. 3 Lake	.019–.057	*P*	30.0	3	1	Xanthene 45430
D&C Red No. 9 Lake	.270	*P*	16.9	6		Monoazo 15585
D&C Red No. 11 Lake	.180	*P*	20.0	5		Monoazo 15630
D&C Red No. 19 Lake	.270	*P*	30.0	3		Xanthene 45170
D&C Red No. 21 Lake	.080	*P*	30.0	2		Fluoran 45380A
D&C Red No. 28	.100	*P*	30.0	3		Xanthene 45410
FD&C Blue No. 1 Lake	.01–.03	*P*	30.0	3	6	Triphenylmethane 42090
FD&C Yellow No. 6 Lake	.06–.10	*P*	24.5–28.0	3		Monoazo 15985
FD&C Violet No. 1 Lake	.042	*P*	30.0	3	2	Triphenylmethane 10316
FD&C Green No. 3	.100	*P*	30.0	3	7	Triphenylmethane 42053

[a] Concentration of pure dye. [b] Based on 10 days exposure to cool white fluorescent light at 1000 ft.-candles. Less than 6 MacAdam units, good (*G*), less than 16 MacAdam units, moderate (*M*), and greater than 16 MacAdam units, poor (*P*). Tablet excipient, mannitol, lubricant magnesium stearate 1%. [c] Δe, MacAdam color difference at 240,000 ft.-candle hr. or 10 days at 1000 ft.-candle hr. exposure. Range of Δe's given when two colorants concentrations were used. [d] From *Reference 6*. [e] From high intensity fluorescent light (1). Best colorant given rating of 10 and next best successive whole number ratings less than 10. [f] From *Reference 7*.

values while the subscript CE denotes Color-Eye readings. The constants in the above equations are furnished for each piece of vitriolite standard.

Computation.—The Univac SS II computer was used for calculation of Adams-Nickerson color difference, ΔE. The Codic (11) was used to calculate MacAdam color differences ΔC and Δe.

DISCUSSION

Colorimetric Measurement.—Colorimetric measurement includes three factors: (*a*) objective measurement of the reflection or transmission characteristics of a material, (*b*) suitable weightings of the measurements dependent on the illuminant properties, and (*c*) inclusion of the sensitivity of the human eye to color. Since illuminant and the eye are included in determining the final data, colorimetry is *subjective*. This is not true of spectrophotometry which involves a purely physical measurement. The Color-Eye has been found to be a very satisfactory instrument for colorimetric measurements of pharmaceutical dosage forms.

Color Differences.—Past work on color stability in pharmaceutical systems has not come to grips with the concept of color difference. Fading has been described as a function of absorbance (1–3) and as a logarithmic function of the Kubelka-Munk equation (4, 5). Since these treatments have not been related to color in the subjective sense, it was suggested that basic colorimetry be applied to the determination of color fading (5). This technique is well developed and documented, and it is reasonable to study color in pharmaceuticals in this way. A number of color difference formulas have been developed (12), but at the present time only two are used extensively and will be described below.

Adams-Nickerson.—Several formulas for the degree of color difference were tested by Nickerson and Stultz (13). One of these was based on the combination of the Adams chromatic-value diagram combined with the Munsell value scale. Color difference, ΔE, is defined as:

$$\Delta E = 40\,\{(0.23\Delta V_y)^2 + [\Delta(V_x - V_y)]^2 + [0.4\,\Delta(V_z - V_y)]^2\}^{1/2} \quad \text{(Eq. 4)}$$

V_x, V_y, and V_z are Munsell value functions given in the following three equations:

$$\frac{X}{98.04} = 1.2219V_x - 0.23111V_x^2 + 0.23951V_x^3 - 0.021009V_x^4 + 0.0008404V_x^5 \quad \text{(Eq. 5)}$$

$$\frac{Y}{100} = 1.2219V_y - 0.23111V^2{}_y + 0.23951V_y^3 - 0.021009V_y^4 + 0.0008404V_y^5 \quad \text{(Eq. 6)}$$

$$\frac{Z}{118.10} = 1.2219V_z - 0.23111V_z^2 + 0.23951V_z^3 - 0.021009_z{}^4 + 0.0008404V_z^5 \quad \text{(Eq. 7)}$$

The terms X, Y, and Z are the tristimulus values obtained by colorimetric measurement. A tabulation of V_x, V_y, and V_z appears in Judd (12) as a function of the tristimulus values. However, the calculations are cumbersome and time consuming;

therefore, the use of a computer for solving the above equations is quite useful.

MacAdam.—The former color difference equations just described assume that color space may be satisfactorily transformed uniformly throughout the *CIE* diagram using one equation. The MacAdam formula (14, 15), however, establishes sets of transformation equations which define a uniform color space within a restricted portion of *CIE* space. The general formulas giving ΔC, the chromaticity difference, and Δe, the over-all color difference in MacAdam units are given below:

$$\Delta C = K(g_{11}\overline{\Delta x^2} + 2g_{12}\Delta x\Delta y + g_{22}\overline{\Delta y^2})^{1/2} \quad \text{(Eq. 8)}$$

$$\Delta e = (\Delta C^2 + g_{33}\Delta Y^2)^{1/2} \quad \text{(Eq. 9)}$$

Substituting Eq. 5 in Eq. 6 gives:

$$\Delta e = K^2(g_x\overline{\Delta x^2} + 2g_{12}\Delta x\Delta y + g_{22}\overline{\Delta y^2}) + g_{33}\overline{\Delta Y^2} \quad \text{(Eq. 10)}$$

In Eqs. 8–10, x and y are chromaticity coordinates, and Y is lightness. The Δ's refer to differences between sample and standard. The constants in the equations K, g_{11}, g_{22}, g_{33} have been determined experimentally and vary depending on the part of color space that includes the color under study.

Since Eq. 10 requires a great deal of time for calculation, simplified means have been devised for its solution. The use of charts for rapid calculation first was described by Davidson and Hanlon (15) and later was formalized by Simon and Goodwin (16). However, when many color differences are to be calculated, the use of a suitable computer is mandatory. A color difference computer, the Codic (11), is marketed by Davidson and Hemmendinger, Easton, Pa. This desk-top-size computer allows one to calculate ΔC (Eq. 5) and ΔE (Eq. 7) in several minutes. The tristimulus values of the sample and standard along with the constants are placed into the computer by means of potentiometer dials, and the chromaticities and color differences are calculated by nulling a meter. The Codic has precision surpassing visual sensitivity; therefore, it is an excellent tool for color matching work as well as fading studies.

Studies of the merits of various color difference formulas have been conducted. Ingle *et al.* (17) compared the loci of unit color differences for the MacAdam, Adams–Nickerson, and NBS color-difference equations. His comparison points out that the three formulas vary in their sensitivities and position throughout the chromaticity diagram. Berger and Brockes (18), in a study of eight color difference formulas, concluded that only the values obtained with the MacAdam equation agreed with the results of visual color comparison. The authors' data, therefore, are interpreted using the MacAdam color difference equation.

RESULTS

Since the Adams-Nickerson color difference also was calculated, a comparison of the numerical values of ΔE and Δe from the MacAdam equation was made. Often it was found that Δe was 2 or 3 times greater than ΔE, however, exceptions were frequent depending on the magnitude of the color differences. Figure 1 gives the various color differences for B6[2] at a concentration of 0.097%. The ratio of Δe to ΔE varies from about 2.28 at the lowest exposure time to about 2.65 at the highest exposure time. This variability illustrates the nonalignment of the two color difference formulas, and this illustration is typical of the results obtained from the other colorants studied. The chromaticity difference, ΔC, gives the color difference without regard to lightness change. It was found in this study that the largest part of the color change was that of chromaticity, and the magnitude of differences in Fig. 1 between ΔC and Δe were the exception rather than the rule. If the Adams-Nickerson equation had been used as a basis of comparison instead of the MacAdam, the over-all ranking of colorant stability that would have resulted would have been nearly the same since gross deviations were not obtained.

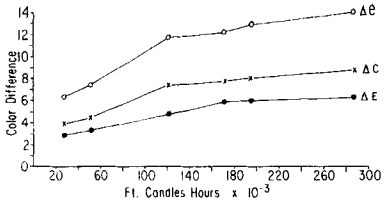

Fig. 1.—Plot of various color differences for D&C Blue No. 6, 0.097%, as a function of ft.-candle hr. exposure at 1000 ft.-candle .

In Fig. 2, MacAdam color differences are illustrated for the blue, green, and violet colorants. A wide range in stabilities can be seen for this group of colors varying from very small changes for B2 and G6 to very high and rapid color differences for B1 and V1.

A nearly consistent dependency of degree of fading on concentration can be seen in Fig. 2. Not all colorants were tested in two concentrations; but of those studied, it is seen that the color of the tablet containing the higher concentration fades to a greater extent over the same time period. The one exception is the colorant B2. This phenomenon readily is apparent visually by comparison of tablets of two colorant concentrations to their respective nonfaded standards. The lighter colored tablet after fading is closer to white, but the color difference is less than that found for the darker tablet. Godlove (19, 20) has written on the perceptibility and acceptability aspects of color acceptance with particular reference to "on tone" and "off tone" fading. He found, for instance, that the consumer is more disturbed by a change in hue than by equally perceptible changes in strength and brightness. Hue change is the exception rather than the rule in this work, but a similar question of acceptance is apparent when the same colorants in two concentrations are compared. While the tablet with the lighter color fades to a smaller degree, it may be less acceptable because of the tendency of its color to change toward white and hence diminution of color.

[2] Abbreviations for colorants will be used; the first letter of the colorant name is used for its designation and D&C and FD&C are omitted.

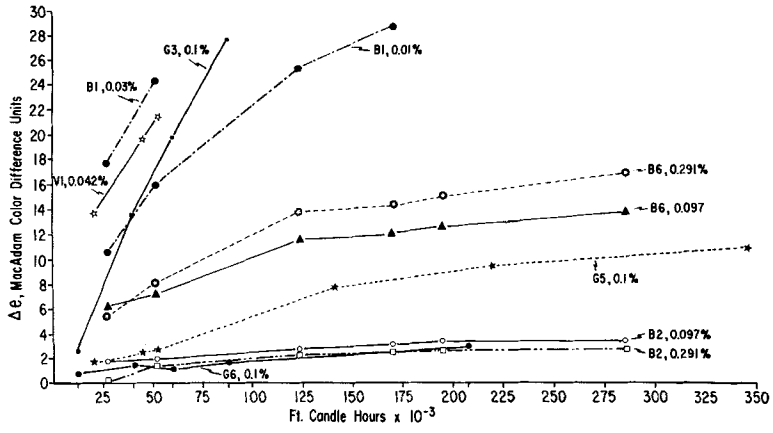

Fig. 2.—Plot of MacAdam color differences for blue, green, and violet colorants as function of ft.-candle hr. exposure at 1000 ft.-candles.

An arbitrary ranking of colorant stability in tablets is given in Table I. Also listed are the concentration ranges employed, the chemical type and CI number, along with stabilities listed by two other authors. The ranking is arbitrary and was deduced from the following considerations. A color showing fading of 6 MacAdam units after 240,000 ft.-candle hr. was given a good ranking (*G*), a color fading 16 MacAdam units after 240,000 ft.-candle hr. was given a moderate ranking (*M*), and color fading of more than 16 MacAdam units over the same period was listed as poor (*P*).

A maximum fading of 6 MacAdam units for a good rating is stringent since 2 to 3 MacAdam units are needed to give a visual color difference. Therefore those colorants in Table I having a good rating only show slightly perceptible color differences after 10 days (240,000 ft.-candle hr.). Testing for longer periods in many cases showed a leveling off of fading, and apparently the colors listed as good would not undergo further change under continued exposure. Some of the colorants given a moderate rating are satisfactory for product usage depending upon the use to which the colorant is put and its concentration. This would be especially true for R2 and R36. That group of colorants having poor stability are probably not satisfactory under any conditions of usage where the product is exposed to any light whatsoever. Among these that are highly unstable are R3, R19, R21, R28, B1, V1, and G3.

At the present time, guides to colorant stability have limited usefulness; and probably the best references are personal ones based on experience. Among the stability references are those found in Peacock's "The Application Properties of the Certified 'Coal Tar' Colorants" (6). The booklet lists the relative fastness of dyes to light as well as to oxidizing agents, reducing agents, and other chemicals. The substrate used in arriving at the rating is unknown but no doubt it had a definite effect, and this could explain the discrepancies. The same relative stabilities are listed in the *Encyclopedia of Chemical Technology* (7). Another listing of relative colorant stabilities has been given by Lachman *et al.* (1). This relative listing is based on reduction of absorbance and not on colorimetric data.

For comparison, Table I also lists colorant stabilities given by Peacock (6) and Lachman (1). It readily is seen that no correlation exists between the authors' ranking and those given by the above authors. The fact that this laboratory's data are based on colorimetry, not spectrophotometry, would explain the lack of correlation. The use of colorimetry affords a means of calculating subsequent color differences and relates directly to the actual psychophysical effect of color on the average human observer. Another point that must be kept in mind is that the substrate and additives may contribute a significant effect toward color stability. It is thought that the authors' colorimetric data should, however, be useful in predicting the stability of colorants in tablets.

Table II.—Color Differences for Duplicate Tablets Containing FD&C Red No. 3 Lake

hr.	ΔE		ΔC		Δe	
	1	*2*	*1*	*2*	*1*	*2*
			Concn., 0.019%[a]			
7	5.80	5.70	13.1	10.9	13.9	11.9
25	10.1	9.34	22.0	18.6	23.4	20.1
48	12.7	...	27.4	...	29.2	...
73	14.6	13.4	30	27.4	30	29.8
144	16.7	15.9		30		30
			Concn., 0.057%[b]			
7	8.7	9.04	12.9	17.6	13.8	18.8
25	14.0	14.9	28.3	30	30	30
48	17.8	18.1	30.0			

[a] Y = 73.2 for tablet *1* and 74.4 for tablet *2*. [b] Y = 61.7 for tablet *1* and 60.7 for tablet *2*.

No general relationship between the chemistry of colorants and stability has been found. However, the indigoid, pyrazolone, and quinoline types rank high in stability. Xanthene, fluoran, and triphenylmethane dyes rank low. Monoazo and anthraquinone colorants generally are intermediate. Functional groups and their positions no doubt are important factors in colorant stability.

Tablets of R3 were run in duplicate in order to obtain an estimate of reproduciblity. The data for the various color differences and two concentrations of the colorant are summarized in Table II. Color differences for each concentration are of different magnitudes as was noted previously. However, an inverse correlation between color difference and lightness, Y, is seen. For the lower concentration, tablet *1* has a lightness of 73.2 *versus* 74.4 for tablet *2*. Tablet *2* shows a smaller degree of fading in all instances, an effect related to the change in concentration. Even though the tablets contained the same amount of colorant, the compression of the powder gave tablets of variable lightness, a factor that alters stability to some extent. The same correlation can be seen for the higher concentration where tablet *1* is lighter than tablet *2*.

SUMMARY AND CONCLUSIONS

A method of testing color stability using a colorimetric procedure has been described. The same methodology is satisfactory for testing a variety of pharmaceutical products. A ranking of colorant stabilities in tablets is given which is intended to provide a guide for selecting a stable color for related formulations.

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Tissue Culture of *Digitalis mertonensis* I

Effect of Certain Steroids on the Callus Growth and Formation of Baljet Positive Substances in *D. mertonensis*

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Static culture conditions for the growth of *Digitalis mertonensis* callus tissue are described. Effects of different steroidal "precursors" on the growth of this callus tissue and the production of total constituents, positive to the Baljet reagent, in the callus and media are estimated.

THE FIRST work on tissue culture of the genus *Digitalis* was reported by Gautheret (1). Staba and co-workers (2–5) also described the growth and metabolism of static and suspension cultures of *Digitalis purpurea* L., *Digitalis lanata* Ehrh., and *D. purpurea* var. *gloxinaeflora* Hort., including a biotransformation study of digitoxigenin using cultures of *Digitalis mertonensis* Buxton and Darlington.

Steroids have been known to stimulate or inhibit growth in microorganisms and plant tissues (6). Tsao (7) found that 0.25% of sodium glycocholate stimulated and 5% concentrations inhibited glycoside production in *D. purpurea*. On the other hand, Chan and Tsao (8) found that sodium cholate inhibited glycoside

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