

Tristimulus Color Measurements in Fading Studies and Other Color Changes of Pharmaceutical Dosage Forms

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Synopsis—The concept of color as a psychological-physical phenomenon is reviewed, and examples are given illustrating this viewpoint. The need for and subsequent development of tristimulus color measurements are briefly described. A procedure for following the degradation of colors is discussed, and then methods of measuring the degree of color change are evaluated. These methods of measuring color change include R (reflectance), K/S (Kubelka-Munk function), and ΔE (change in color).

INTRODUCTION

Everyone, including those who may have some form of color blindness, will see some colors when viewing a “colored” object. Not everyone will see the same colors, but all will see colors.

In Fig. 1 there is no color—just a curved line. It does not convey as much information as the object which generated the curve. Even with a number of transformations it can, at best, only start to convey the information that is desired, namely, the color that the viewer will see when looking at the object which generated this curve.

It should be noted at the onset that the subject of this paper is color, not dye or colorant concentration in a dosage form.

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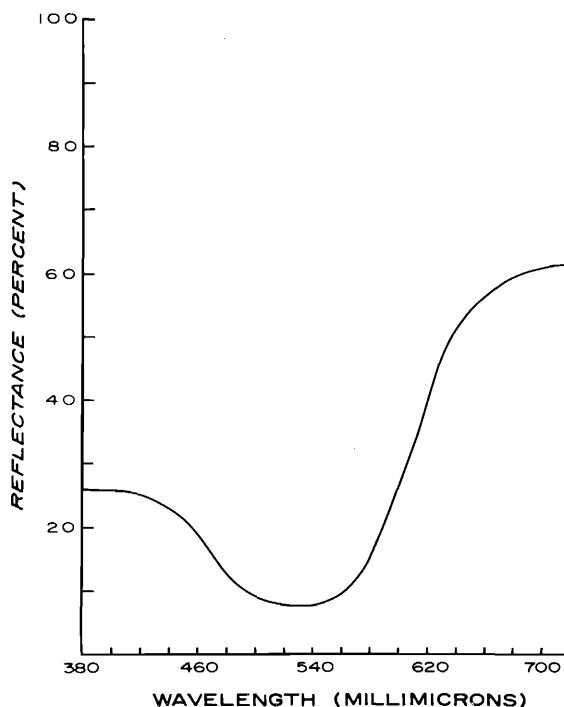


Figure 1. Reflectance measurement of a colored object

Nickerson (1) said: "It is true that a spectral distribution curve may serve to define unambiguously the *stimulus* for the color of a light source, but the spectral-reflectance or spectral-transmittance curve of an object is not sufficient to define even the stimulus for its color. Its spectral properties must be combined with those of the light source under which it is to be viewed before even the stimulus for the object's color is defined. To define its *color* the concept of an observer must also be introduced." Essentially what Nickerson is saying is that to see an object as colored there are three requirements: the object, the observer, and an illuminant. If any one of these three is absent then there is no colored object in the true meaning of the word. This is more than a semantic nicety.

USE OF SPECTROPHOTOMETRIC RESULTS

A spectrophotometric curve alone will not serve to define a color. In order to describe color with any degree of adequacy it is therefore necessary to have some form of color space defined. Color space de-

velopment can be said to have started with Newton, and refinements are still being made in the accepted standards today. Without going into any great detail the following is a description of what is known as the CIE (Commission Internationale de l'Eclairage) color space.

In the late 1920's and early 30's Wright and Guild, in England, independently of each other had a number of observers match the color of the spectrum at each wavelength with three primary lights, red, green, and blue. The values obtained by these two experimenters were re-

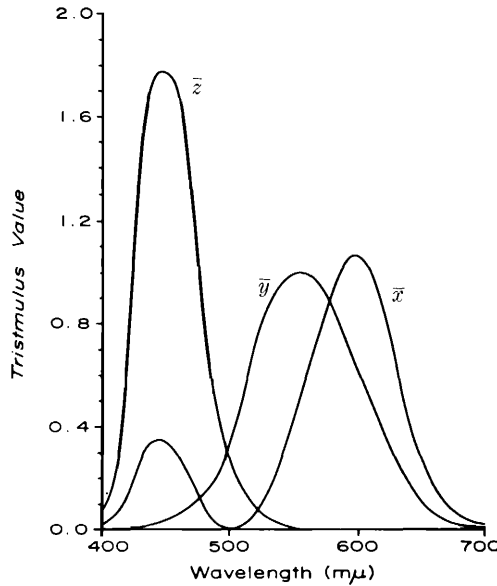


Figure 2. CIE tristimulus values, \bar{x} , \bar{y} , and \bar{z} for the spectrum colors

markably close to each other even though they used different observers, primary lights, and instruments. Because of this close agreement on the values obtained the CIE adopted their average values as a standard.

A number of mathematical transformations were performed on the values of the real primaries used in the studies of Wright and Guild in order to make subsequent calculations relatively simple and rapid. The results are the unreal or imaginary primaries shown in Fig. 2 as the tristimulus values \bar{x} , \bar{y} , and \bar{z} .

At any wavelength the values X , Y , and Z correspond to the magnitude of the CIE primaries \bar{x} , \bar{y} , and \bar{z} needed by the standard observer to match the color of the particular wavelength. Chromaticity coordinates

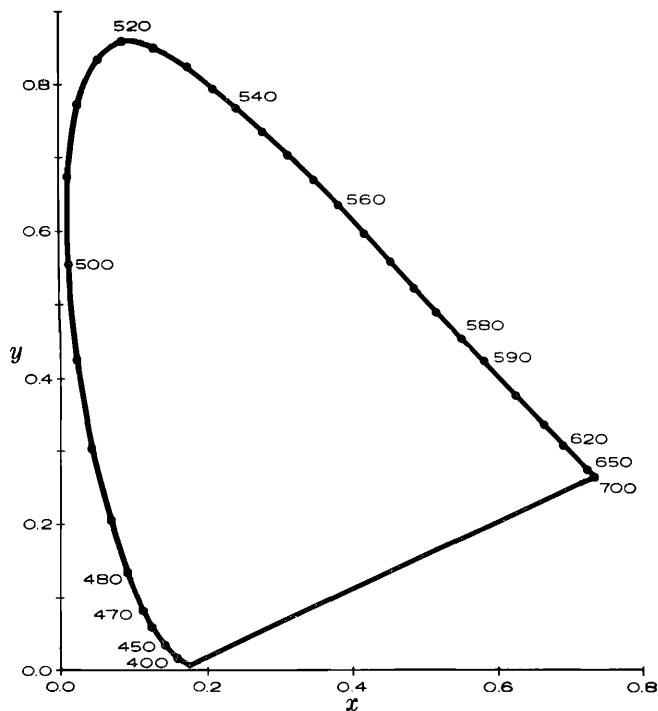


Figure 3. CIE chromaticity diagram

TABLE I
Sample Calculation (Weighted Ordinate Method)

Wavelength (m μ)	E_c	\bar{x}	$E_c\bar{x}$	R	$RE_c\bar{x}$
400	63.3	0.0144	0.91	0.09	0.082
700	76.3	0.0114	0.87	0.45	0.391
					420.0
			$\Sigma RE_c\bar{x} = 420 = X$	$\frac{X}{X + Y + Z} = \frac{420}{1603} = 0.2620 = x$	
			$\Sigma RE_c\bar{y} = 758 = Y$	$\frac{Y}{X + Y + Z} = \frac{758}{1603} = 0.4729 = y$	
			$\Sigma RE_c\bar{z} = 425 = Z$	$Y = \frac{Y \text{ actual}}{Y \text{ hypothetical}} = \frac{758}{1079} = 0.7025 = 70.25\%$	

are the tristimulus values expressed as fractions of their total. Since they must total one, it is necessary to calculate only two values to define all three.

$$x = \frac{X}{X + Y + Z} \quad y = \frac{Y}{X + Y + Z} \quad z = \frac{Z}{X + Y + Z}$$

If, in turn, the chromaticity coordinates of the tristimulus values are calculated and plotted on an x,y diagram the spectrum locus is generated (Fig. 3). The wavelengths are indicated in nanometers ($m\mu$).

Assuming that one has a spectrophotometric curve generated by some object it is necessary to convert this information into some form that will allow it to be plotted in color space. Table I contains a sample calculation used to determine the tristimulus values and the lightness value of a sample using the weighted ordinate method. This is just one of several methods that may be used to determine these values.

At each wavelength between 400 and 700 $m\mu$ (usually in 10 $m\mu$ increments) the relative energy of the light source E_c is multiplied by the tristimulus value \bar{x} and the reflectance value of the sample R . These values are then summed up over their total range to yield X , Y , and Z , the tristimulus values for the sample. The chromaticity coordinates are obtained by expressing the tristimulus values as fractions of their total values. The lightness value is a relative measure of the actual *versus* the theoretical Y (or lightness) value.

It might appear that, if one wished to measure color differences between several sets of colored objects, it would only be necessary to follow the above procedure carefully and finally plot the points obtained on the chromaticity diagram and measure the distances between the various sets of points. Ideally two points twice as far apart as another set of two points could then be said to have a greater difference in their colors. Unfortunately, this will not prove to be correct in all instances because color space is not linear. In other words, equal distances do not represent the same degree of color change throughout the color space.

This lack of uniformity of color space is well illustrated by a study published by MacAdam (2). In this study he had one observer make a series of 25,000 color matches around 25 color centers distributed throughout the color space. The data were plotted on the CIE chromaticity diagram, and Fig. 4 shows the figures that were obtained when a curve was drawn around the points representing a color match for each color center. These ellipses have been enlarged for the purposes of illustration. The diagram shows the nonlinearity of color space.

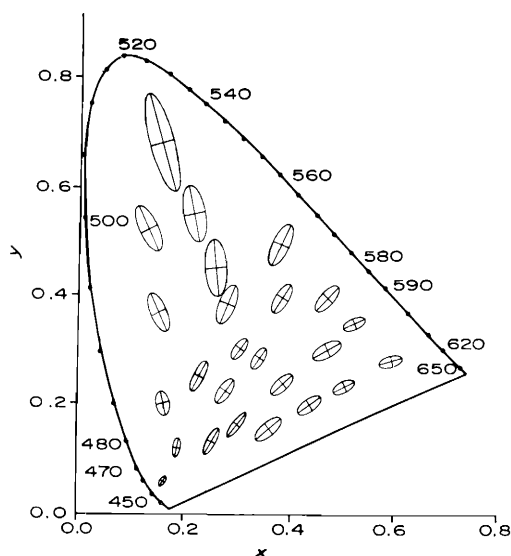


Figure 4. Graphical representation of data by D. L. MacAdam showing the visible effect of chromaticity changes throughout the CIE diagram. The axis of each ellipse have been multiplied by 10 for illustrative purposes

According to MacAdam no linear projection of the CIE diagram will result in a uniformly spaced chromaticity diagram; nor will any plane uniform chromaticity diagram plotted by any method conform to the chromaticity spacing judged by one observer. Rather than pursue such a course, MacAdam attempted to construct a uniform color space within small areas of the CIE diagram, each small area being treated separately. This may be considered analogous to considering the earth as a plane surface if one intends to measure only comparatively short distances. However, as longer distances are measured, the earth's curvature becomes most significant.

The MacAdam system of determining color differences is considered as one of the better means of establishing color differences in a field that is still being developed and is still considered to be the most difficult area in the field of color measurement.

PRACTICAL PROCEDURE

To demonstrate a technique for making color measurements and to do fading studies tablets were chosen. However, essentially everything described here will be applicable to any other dosage form, with few exceptions.

Throughout this discussion the major concern will be with "color," not the dye or colorant content in the dosage form. This point must be emphasized because it is the crux of the entire technique. "Color" and colorant content of a dosage form are not necessarily the same.

Consider a new product which is to be colored green. There is a choice of two green colorants and a blue and yellow combination that will result in green. That is the total colorant gamut for this color. It is also desired to color this product with a colorant or colorants such that it will appear unchanged to the consumer for the longest possible period of time. It should be noted that it is important that the product need only appear to remain unchanged—not that its dye or pigment content remain the highest or that its chemical degradation rate be the slowest. The objective is a measure of the total effect that the consumer sees when he looks at the product. This includes the background in the product caused by any and all additives. In other words, the colorant is of interest not as an isolated chemical entity but as an integral part of a specific, well-defined formulation. It is essential to achieve a situation where a consumer cannot tell any difference in color between the last of one purchased product and a fresh container of the same item.

For this study a green insoluble colorant (non-FDA approved), an aluminum lake, and a combination of a blue and a yellow FD&C dye were used to achieve three blue-green colored compacts.* A white control was also made. The insoluble colorants were first mixed with lactose and passed through a Mikro-Samplmill fitted with a number 0.010 HB screen. They were then granulated using an acacia solution as the binding agent, dried for sixteen hours at 54 °C, and subsequently screened through a fine screen on a comminution mill. The two soluble dyes were added to the lactose as a solution. This was then air-dried, finally screened, and subsequently similarly treated as the other two granulations. The granulations were compressed on a Carver Press using 1¼ in. (32 mm) bevel-edge, flat faced punches, each compact being compressed at the same pressure.

To effect the color change the compacts were mounted on a white board and placed in windows having a southern exposure. They were exposed to sunlight from the hours of 11 a.m. to 4 p.m. each day. The amount of sunlight that the tablets received each day was measured by the use of a Sunlight Integrator manufactured by the Self-Organizing Systems, Inc.† The instrument is designed to measure the solar energy

* The percentage of colorant in each formulation is deliberately omitted because products are colored up to a certain depth, within certain legal limits, and the interest here is to follow the change in the color.

† 6612 Denton Drive, Dallas, Tex.

at the sensor over a period of time. A cadmium sulfide light-dependent resistor is used as the sensor and a solion tetrode as the integrator. The instrument is calibrated against an Eppley Pyrheliometer to give one count for each 2 cal/cm^2 or one count per two Langley units. The Langley unit is used by the U. S. Meteorological Service so that the number of units to which the tablets have been exposed may be transposed into the equivalent number of days or weeks of sunshine in any one of several major areas in the United States.

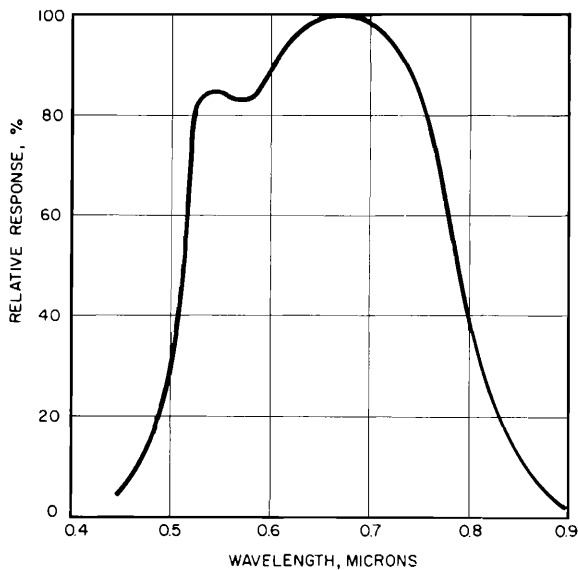
Figure 5 is a photograph of the Sunlight integrator showing the sensing unit. The spectral response of the light-detecting unit is shown in Fig. 6. Its maximum sensitivity lies in the visible range.

The compacts were exposed to the sunlight for varying periods of time. As soon as a difference in color was detected in each formulation,



Figure 5. Sunlight integrator and sensing unit

a compact was removed from the exposure area and placed in a case to await its subsequent evaluation by reflectance spectrophotometry. When a series of compacts had reached a point of nonacceptability as determined by laboratory personnel making a visual evaluation, the particular test series was discontinued. When all of the colored tablets, along with the uncolored control, had been exposed to the sunlight for the desired periods of time, the samples of an entire series were measured to determine their changes in reflectance. The tablets were measured using a G. E. recording spectrophotometer equipped with a D&H integra-



SPECTRAL RESPONSE OF LIGHT DEPENDENT RESISTOR (LDR - C1)

Figure 6. Spectral response of light dependent resistor

tor. Barium sulfate was used as the white standard, the illuminant was Source C, and the specular component was excluded.

In the next several figures are the spectrophotometric curves made of the compacts prior to exposure and then after the indicated number of Langley units of direct sunlight exposure.

Figure 7 shows the curves of the white controls. The curves exhibit the very slightest change at the low end, perhaps ultimately fading toward yellowness if the test had been carried out for a much longer period of time.

Figure 8 contains the curves of the compacts colored with a green insoluble colorant. These compacts showed very little change during the period in which they were evaluated. The curves attest to the same fact. They show very little change, regardless of what criterion is used.

Figure 9 shows the curves of compacts colored with a combination of water-soluble blue and yellow FD&C dyes. The compacts exhibited a continuous fading as well as a loss in saturation as they were exposed to the direct sunlight. The curves show the same things qualitatively. The compacts are getting lighter, as indicated by the greater reflectance; there is a slight shift in hue; and the tendency of the slopes to become less steep shows a loss in saturation.

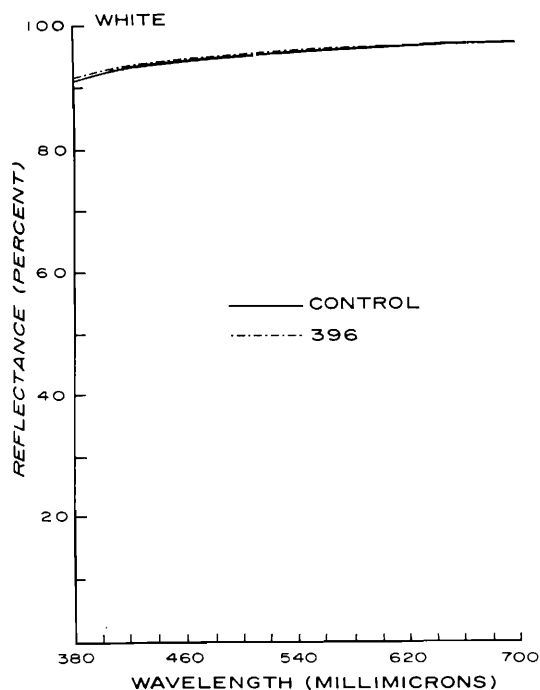


Figure 7. Reflectance measurements of white controls. The numerals refer to the number of Langley units of direct sunlight to which the specimen has been exposed

The curves of compacts colored with an aluminum lake are shown in Fig. 10. These compacts appeared to have suffered the greatest degree of color change of this series. The curves indicate essentially the same characteristics as those of the FD&C dye combination.

In this particular study the use of color difference formulas obviously is not necessary; the pigment is the most stable colorant. However, to illustrate the point, the different methods for following the fading of colorants are used to see what results.

Table II contains a summary of the data for this first series of compacts. The first column identifies the compacts and the next column the number of Langley units to which each was exposed. Next are the chromaticity coordinates and the Y , or lightness value. Following these are the Δe , c , and L values which indicate change in color, chromaticity, and lightness, respectively. These were computed using a color difference computer programmed on the basis of the previously mentioned MacAdam color difference formula.

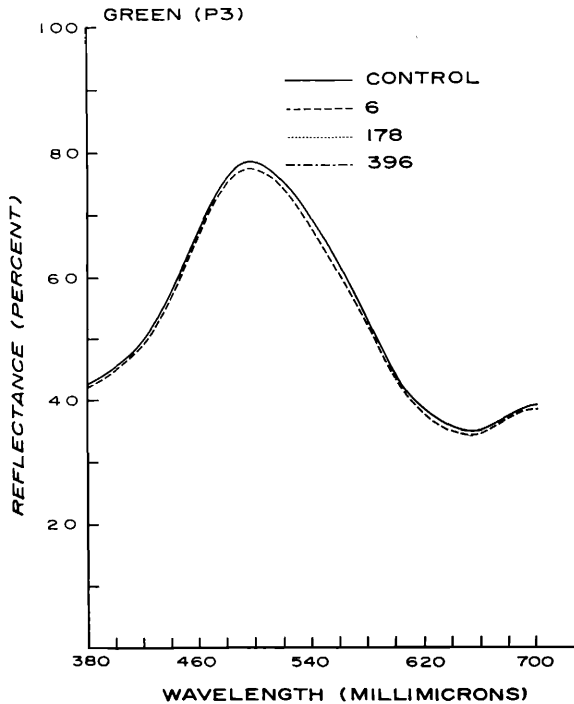


Figure 8. Reflectance measurements of compacts colored with green pigment. The numerals refer to the number of Langley units of direct sunlight exposure for each compact. (In this case the curves for the three exposed samples are superimposable)

The next column is the value of the **minimum reflectance** (R_{min}) of the unexposed control. The wavelength of the minimum reflectance was then used for the balance of the test for that compact, even though the minimum may have shifted. The last column is the Kubelka-Munk value for this particular reflectance value. Its function will be examined later.

USE OF EXPERIMENTAL RESULTS

These data could be used in one of several ways to answer the question of which colorant combination yields the most light-stable formulation. It is very important here to point out that the methods can be chosen only by hindsight, i.e., after all the data are available, the curves, the dosage forms that have been exposed to the lights, and all the numbers that have been generated by this laborious procedure are analyzed.

Table II
Summary of Data for First Series of Compacts

Tablet	Exposure (Langley Units)	<i>x</i>	<i>y</i>	<i>Y</i>	(MacAdam Units)				
					Δe	Δc	ΔL	R_{min}	K/S_{min}
White	Control	0.3120	0.3186	96.27	91.5	0.0039
White	396	0.3120	0.3185	96.39	0.0	0.0	0.0	92.0	0.0035
Green P3	Control	0.2698	0.3254	60.39	34.5	0.6218
Green P3	6	0.2692	0.3255	59.78	1.1	0.8	0.3	34.0	0.6406
Green P3	178	0.2695	0.3251	59.70	1.0	0.3	0.7	34.0	0.6406
Green P3	396	0.2698	0.3250	59.81	0.8	0.3	0.5	34.5	0.6218
Green 3D4	Control	0.2739	0.3366	58.38	32.5	0.7010
Green 3D4	6	0.2751	0.3360	58.40	1.4	1.4	0.0	33.0	0.6802
Green 3D4	178	0.2814	0.3306	59.01	10.5	10.4	0.1	38.5	0.4912
Green 3D4	396	0.2862	0.3305	60.75	16.0	15.5	0.5	42.0	0.3890
Green 5D5	Control	0.2474	0.3166	44.14	23.5	1.245
Green 5D5	6	0.2487	0.3162	44.17	1.4	1.2	0.2	24.0	1.203
Green 5D5	50	0.2560	0.3131	46.08	8.4	8.1	0.3	28.0	0.9257
Green 5D5	142	0.2662	0.3082	49.44	21.1	19.8	1.3	34.5	0.6036

Table III
Changes in Color for First and Second Series of Compacts

Tablet	$\Delta e/L$	$K/S^*/L$	$\Delta A/L \dagger$
P3	0.002	0.000	0.0
Yellow	0.011	0.039	9.6
Red	0.024	0.049	1.3
3D4	0.040	0.112	27.0
5D5	0.148	0.362	117.0

* Calculated from $100 \times [(K/S)_o - (K/S)_L] / [L(K/S)_o]$.

† $\times 10^5$.

Table IV
Summary of Data for Second Series of Compacts

Tablet	Exposure (Langley units)	<i>x</i>	<i>y</i>	<i>Y</i>	(MacAdam Units)				
					Δe	Δc	ΔL	R_{min}	K/S_{min}
Red	Control	0.3392	0.2862	33.63	27.0	0.9868
Red	28	0.3400	0.2864	33.69	0.8	0.7	0.1	27.0	0.0968
Red	122	0.3384	0.2888	34.67	3.5	2.8	0.7	28.0	0.9257
Red	452	0.3358	0.2928	37.17	11.0	8.5	2.5	31.0	0.7679
Yellow	Control	0.3896	0.4050	83.10	30.7	0.7798
Yellow	28	0.3904	0.4062	83.20	0.8	0.7	0.1	30.5	0.7918
Yellow	122	0.3876	0.4026	83.13	1.6	1.6	0.0	32.0	0.7225
Yellow	452	0.3837	0.3980	83.11	4.8	4.7	0.1	34.0	0.6406

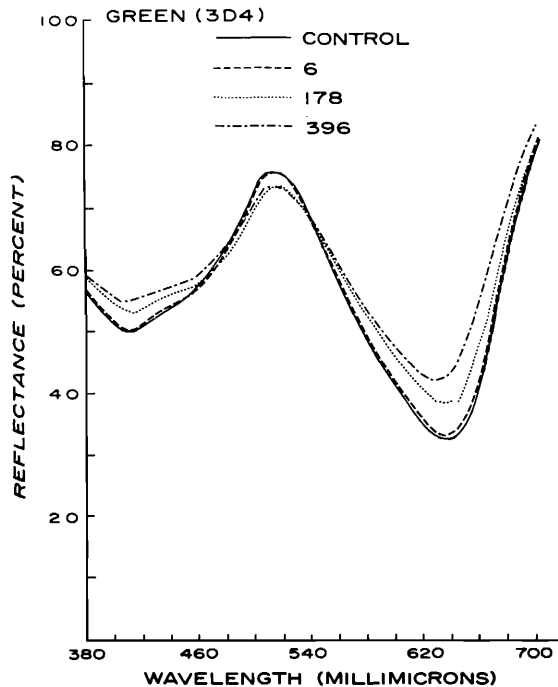


Figure 9. Reflectance measurements of compacts colored with a combination of water-soluble blue and yellow dyes. The numerals refer to the number of Langley units of direct sunlight exposure for each compact

It may be of value to plot the chromaticity coordinates to see if the larger change in color can be accounted for by a shift in the chromaticity or if the main change is a change of lightness of the color.

These considerations are of importance in many instances. There are cases in which an on-hue fading can be tolerated within relatively wide limits while an off-hue fading will be a cause for rejection. In other cases the chromaticity limits may be asymmetrical around the object's color. Mackinney and Little (3) point out that butter is a case in which the acceptability limit is unlike that of the perceptibility ellipsoid. Fairly wide variations from yellow to orange will be tolerable, but the slightest shift from yellow to a greenish tinge would be a cause for rejection. These instances, and there are many of them, tend to complicate a rigorous study of colorant fading, but they are of practical importance. It is axiomatic that one tries to use the simplest measurement as long as it correlates with visual observations.

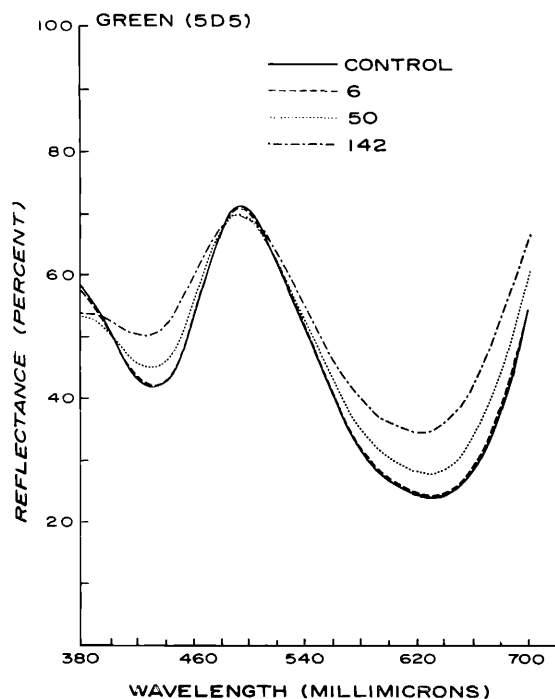


Figure 10. Reflectance measurements of compacts colored with a green aluminum lake. The numerals refer to the number of Langley units of direct sunlight exposure for each compact

One way to follow the progress of the color changes is to calculate the "absorbance" value at the point of minimum reflectance. To use the negative log of R to follow the progress of the color change is to assume that R is a linear function of the colorant concentration. This has been shown not to be the case by a number of workers in the field of color measurement. The relationship of colorant concentration to reflectance was developed from theoretical and empirical considerations by Kubelka and Munk among others. Reflectance is shown to be related to colorant concentration by the use of their formula:

$$K/S = (1 - R_{\infty})^2 / 2R_{\infty}$$

where K is the absorption coefficient and S is the scattering coefficient. A second way of following the color change would be to use the K/S values of the Kubelka-Munk equations. This technique assumes, on an

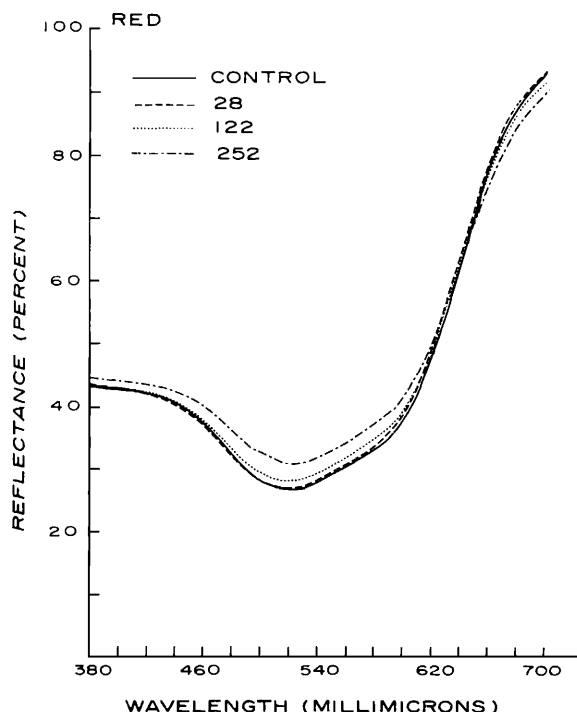


Figure 11. Reflectance measurements of compacts colored with a red aluminum lake. The numerals refer to the number of Langley units of direct sunlight exposure for each compact

arbitrary basis, that an apparent 50% fade involves the destruction of 50% of the colorant. It makes the further assumption that the fading is on-hue when the course of the color change is followed at only a single wavelength. Neither of these assumptions may prove to be valid in some studies. Furthermore, even when the assumptions are valid the equation may be linear only over a small range.

The third technique for following the change in color utilizes one of the color difference formulas. Of these the MacAdam color difference units appears to be one of the better ones. Mudd (4) stated that colorant stability can be evaluated either by determining how long it takes to alter a color to a predetermined point or by subjecting samples to definite periods of exposure and measuring the color changes.

Using these concepts it is possible to calculate the change in color, Δe /Langley unit, as shown by the figures in the second column next to

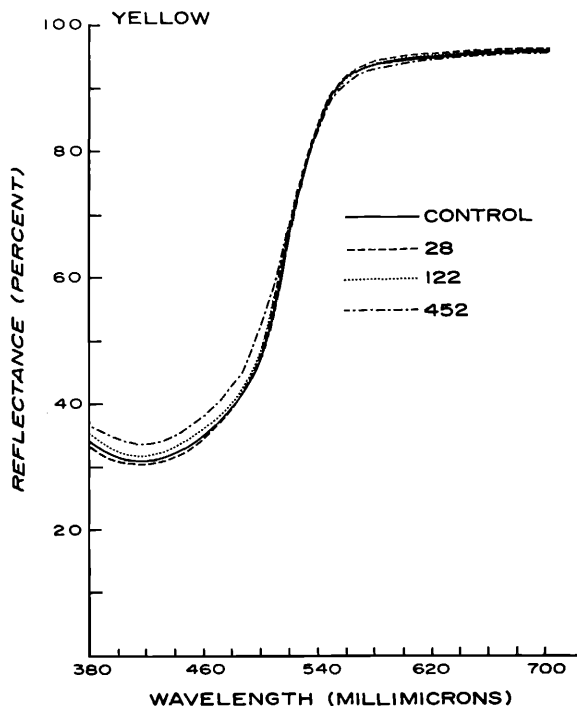


Figure 12. Reflectance measurements of compacts colored with a yellow aluminum lake. The numerals refer to the number of Langley units of direct sunlight exposure for each compact

the colorant designation in Table III. In the third column are the changes in the K/S values/Langley units. The figures in the last column represent the changes in absorbancy units/Langley units.

These figures illustrate that qualitatively all three methods rank the colorant stabilities in the same order. This is due to the fact that all of them were, essentially, on hue and also because the stability of these colorants was so dissimilar that a method would have to be grossly in error to cause a rank inversion. It should be noted that the Δe and K/S show 3D4 and 5D5 in about the same ratio to each other, while the absorbancy value yields a figure double that.

Frequently it may be necessary to find the most stable colorant from a series in which the colors are not the same. The ranking of colorant stabilities has been reported as such in the past. Figure 11 shows the curves of red-colored tablets, prepared with an aluminum lake (in a manner similar to those of the green tablets). Figure 12 illustrates a

series made using a yellow aluminum lake. These compacts have had a finite change in their chromaticity although there is essentially no change in their lightness values.

Table IV shows the same type of data as was previously shown in Table II for the three sets of green tablets. It may be seen that there is very little indication of a change in lightness which would be expected in a case of "fading."

Table III includes the calculated values for the red and yellow tablets. It can be seen that when more than one color is being evaluated there is a rank inversion using absorbance units, and the K/S values fail to indicate the magnitude of the color change and its type, that is, chromaticity or lightness.

Another way of illustrating this point of considering color as more than colorants is shown in Table V. It lists the calculated number of Langley units that the tablets would have been exposed to when the K/S value had dropped to 75% of its original value. The ranking has not been changed, which indicates that qualitatively this is still a satisfactory procedure. However if the Δe value, the total color change, is calculated for this period of exposure it can be seen that a 25% reduction in the Kubelka-Munk value does not result in the same degree of color change in every case.

CONCLUSIONS

It is suggested that fading studies be conducted as follows. The particular formulations under consideration, not the colorants as isolated entities, should be exposed to the desired lighting conditions. These can be either normal or exaggerated conditions. Samples should be withdrawn at periodic intervals, with the time of exposure being noted. Samples should not be allowed to fade to a washed-out color or even close to this. All small color difference formulas are invalid if the color differences are too great.

Table V
Relative Light Stability of the Colorants

Tablet	Langley Units at $K/S_{75\%}$	Δe at $K/S_{75\%}$
P3	∞	0.0
Yellow	620	6.2
Red	150	3.9
3D4	111	8.2
5D5	64	9.7

Spectrophotometric curves can then be run on the samples, as has been discussed here, and the chromaticity coordinates and lightness values obtained. A look at the samples, their curves, and the numbers obtained should then indicate which technique should be used to follow the course of color changes and to select the most stable colorant. It may be of value to plot color change, as Δe , versus Langley units or time, and then determine how long an exposure it takes for the sample to reach a point of five color-change units.

In any case it cannot be overstressed that there must be a visual correlation between the ranking given by an observer and whatever system of measurements is chosen. Any criterion which disregards this is unsatisfactory.

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