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Short communication

Color measurement of a solid active pharmaceutical ingredient as an aid to identifying key process parameters

Paul D. Oram [a,*], Jeffrey Strine [b]

[a] *Department of Analytical Research, Merck & Co. Inc., P.O. Box 2000, Rahway, NJ 07065, USA*
[b] *Department of Chemical Engineering Research and Development, Merck & Co. Inc., P.O. Box 2000, Rahway, NJ 07065, USA*

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Abstract

The color of a powdered active pharmaceutical ingredient (API) in an investigational drug was found to be the result of a number of process parameters. Color measurements of the solid material were derived from reflectance data obtained from a sphere spectrophotometer. These data provided a convenient and non-destructive way to track the effects of variations to the process parameters on the resulting material. Visual evaluation could not provide an objective, quantitative assessment of the material. Color of solution (COS) measurements did not provide adequate sensitivity to distinguish some of the samples from each other, even when visual differences were apparent.

Keywords: Powder; Color measurement; Sphere spectrophotometer; Process control

1. Introduction

The color of the active ingredient in pharmaceutical products (API) is typically evaluated as part of the suite of tests performed on the bulk powder before it is released for formulation. Deviation from the expected color may be an indication of a process abnormality which results in a change in the physical properties of the material or an indication that the level of some impurity has increased. Intensely colored impurities may be present at concentrations too low to be detected by typical assay methods such as HPLC or titration. Even if the cause of the dis-coloration is not a concern with regard to purity or physical properties, it may still be unacceptable from an aesthetic standpoint when the compound is formulated into final product.

Two tests are commonly used in the pharmaceutical industry to assess the color of powdered ingredients. The first is visual evaluation. An experienced analyst can instantly tell if there is a significant problem with a batch by looking at it. But this test poses some difficulties, especially when performed in two or more different laboratories. A typical specification for color might be 'white to off-white'. The term 'off-white' is often used to describe any light shade of color. A typical 'off-white' batch might be light yellow, while light pink material would indicate an abnormality. Unless the analyst has been trained to recognize typical material, both batches could be described as off-white and pass the test. The specification could be changed to 'white to light yellow' to avoid this situation, but the analyst still has to learn whether light tan or light brown equals light yellow. A more significant difficulty with visual evaluation is how to establish a threshold of failure. For example, at what point is a sample so dark that it is no longer light yellow, but tan or brown? A second test is commonly used to help answer this question.

In the color of solution (COS) test, the material is dissolved in a suitable solvent and the absorbance is measured at a selected wavelength. Typically, 440 nm is used because this is the wavelength where yellowing associated with degradation of white materials should be detected. However, the characteristic absorption bands of the impurities or degradates in a material may or may not have maximum sensitivity around 440 nm. In this case, a complete scan may reveal a more appropriate wavelength for analysis. It is also possible that two or more different impurities contribute to the color, and the individual levels of these may vary from batch to batch. In this case, measurements

* Corresponding author. Tel.: +1 732 594 1228; fax: +1 732 594 3887.
E-mail address: paul_oram@merck.com (P.D. Oram).

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at multiple wavelengths may be necessary in order to obtain an accurate measure of what is observed visually. Even after a suitable wavelength is identified, the signal to noise ratio of the measurements may still be too low to give numbers that accurately reflect subtle differences in the color of the solid that are easily detected by the human eye. An additional complication in COS measurements is when the absorbance of the compound in solution is enhanced or diminished due to solvent effects. This is most obvious for compounds whose color is affected by pH.

The problems associated with visual evaluation and color of solution measurements may be avoided if the color of the solid API is measured directly. This is done using a commercially available spectrophotometer that measures the reflectance of the material across the visible wavelength region. Mathematical treatment of the reflectance data yields tristimulus values, which can be converted into a variety of numeric results that provide quantitative assignments of the observed color. This is a well developed technique that is widely used for quality control in industries where color is a critical attribute of the product (this includes almost all consumer products, with the paint and textile industries being notable examples). It has also been used in a variety of research applications where color is an indicator of physical properties of the material being measured, and a number of applications in the pharmaceutical industry have been published [1–7]. Additionally, the technique is listed in a U.S. Pharmacopeia monograph [8].

2. Materials and methods

An X-rite 8400 bench top sphere spectrophotometer was used. The instrument has a d/8° geometry with a 6 in. sphere and a pulsed xenon lamp. Reflectance data were collected over the full wavelength range of the instrument (360–740 nm, 10 nm interval) with the specular component included, through a 19-mm viewing aperture. The samples were contained in 35 mm × 10 mm polystyrene Petri dishes. The active ingredient used in the study is an investigational drug, produced by the Department of Chemical Engineering Research and Development at Merck & Co. Inc., during pilot plant runs and laboratory process development experiments.

Tristimulus values were calculated by X-rite Color Master QA II software v6.0. The theoretical basis of these calculations is described in a number of reference works [9–11]. Several standard illuminants are available for use in the calculations, along with either the 2° or 10° standard observer, as defined by the Commission Internationale de l'Eclairage (CIE, 1931, 1964). The software is capable of converting the tristimulus data into a variety of standard chromaticity scales, tolerance systems, and other indices. In this study, samples were compared using two derived values, delta *E* and the American Society for Testing and Materials (ASTM) 313 whiteness index. Delta *E* is an indication of the geometric distance in color space of the sample being measured with regard to a reference sample. In the present case, a batch of recrystallized material was used as a reference. The delta *E* values are based on CIELAB color space and derived using illuminant F2, along with the CIE 1931 2° standard observer. Illuminant F2 was selected as the best approximation of the cool white fluorescent laboratory lighting under which visual observations of the samples were made. For visual evaluation, all samples were compared simultaneously on white paper. The whiteness index is a one-dimensional scale derived from the tristimulus values that gives an indication of the difference of the sample from a perfect reflecting diffuser. The whiteness index is derived using a defined illuminant and standard observer, which in this case is illuminant C with the 2° observer. Although this does not match the conditions under which the visual observations were made, this number was found experimentally to be useful for sorting the samples under investigation.

Color of solution values were obtained by measuring the blank corrected absorbance of a 1% (w/v) solution at the specified wavelength on a Cary 300 spectrophotometer using a 1.0 cm quartz cell. The COS values are the absorptivity of the solution calculated using g/mL as the concentration units, multiplied by 1000.

3. Results and discussion

Production of the API originally included a recrystallization as the final step to reduce the levels of potential impurities, and which consequently reduced residual color. After a change in the chemical synthesis, it was observed that impurity levels (reported from HPLC analysis) were no longer affected by the recrystallization step, so a proposal was made to remove it from the process. The color of seven pilot plant batches made prior to recrystallization was acceptable by visual evaluation. However, when they were compared side by side, three of them appeared to be different, and all of these batches were somewhat darker than the recrystallized material. This raised the concern that removing the recrystallization step might produce material with unacceptably high color. So it was necessary to identify the extent to which various process parameters affect the color, and an accurate measurement of the color of the solid material produced at this step was needed to track the impact of those parameters.

At that time, COS at 440 nm data were used as a means of quantifying the color appearance of the solid API. As shown in Table 1, not only does this number not distinguish the batches from each other, but also it fails to distinguish them from the white recrystallized material. Measurement of the COS at 390 nm gives better signal to noise ratio, so the difference between the recrystallized and non-recrystallized batches could be measured, but data taken at this wavelength still did not distinguish the three batches that appear to be most different by visual examination. Subsequently, the sphere spectrophotometer was implemented as a measurement tool. The CIELAB delta *E* and the ASTM E313 whiteness index both gave good correlation to the visual appearance of the samples. The data in Table 1 are sorted by ascending whiteness index; the delta *E* values follow the same trend, in inverse order. The recrystallized batch (B8) was used as a reference sample, and this was also measured against itself to provide an indication of the method precision.

In order to identify which process parameters contributed to the variations in the appearance of the batches, a series of delib-

Table 1
Color data for seven batches of powder API prior to recrystallization, and one recrystallized batch used as reference

COS 440	COS 390	Batch sample	Appearance	Delta *E*	Whiteness (E313)
4	9	B1	Yellow tinge	6.7	41
5	12	B2	Yellow tinge	4.4	52
5	10	B3	Pink tinge	3.4	58
4	10	B4	Off-white–gray	2.8	62
4	7	B5	Off-white–gray	2.6	62
4	10	B6	Off-white–gray	2.4	63
3	9	B7	Off-white–gray	2.1	65
4	4	B8(recrystallized)[a]	White	NA	73
		B8 (recrystallized)		0.3	75

Specular component was included in reflectance measurement. COS is absorptivity at the indicated wavelength (×1000) based on a 1% (w/v) solution.
[a] Reference.

Table 2
Results for stress experiments on penultimate step of API synthesis, and of a front run that used a different lot of synthetic intermediate carried out at target reaction conditions

Experiment	Equiv SOCI2	Reaction temp (°C)	Time to quench (h)	Appearance	COS 390	Delta *E*	Whiteness (E313)
A	1.20	20	NA	Light tan/brown	82	14.8	2
B	1.05	20	NA	Light yellow	58	14.2	5
C	1.20	20	4	Light yellow	65	12.9	10
D	1.20	−15	24	Off-white	23	4.4	53
E	1.10	0	NA	Off-white	12	1.9	71
Reference	NA	NA	NA	White	4	NA	73
F	1.01	−15	4	White	7	0.8	76
G	1.01	20	24	White	5	0.8	76
Front run	1.05	−10	NA	Off-white	15	5.2	52

erate stress experiments were carried out on the penultimate synthetic step. This step involves the coupling of two intermediate compounds using thionyl chloride in dimethylacetamide, along with pyridine as a reagent. It was known from previous work with these reagents that trace levels of highly colored impurities may be created depending on the reaction conditions, but usually the concentration of these impurities is too low to be detected by HPLC analysis. Three process conditions were suspected of causing off colored product: the number of equivalents of thionyl chloride used, the temperature of the batch during the thionyl chloride charge, and the elapsed time before the reaction was quenched. The results of these experiments (Table 2, experiments A–G) demonstrated that the amount of thionyl chloride charged has the biggest impact on the resulting color. The temperature during the charge also has an effect, but the extent of the effect depends on the amount of thionyl chloride charged. The time elapsed before quenching the batch did not have an impact. These results showed that the process parameters could have a fairly wide range without adversely affecting the color.

The batches shown in Table 1 were all produced close to the process target conditions of 1.05 equivalents of thionyl chloride and at −10 °C, so what was the cause of the apparent difference of the first three batches? This question was answered during a front run of a new batch of the synthesis intermediate. This particular batch of intermediate was light tan, not the off-white typically seen for this material. The source of the color could not be attributed to any previously known impurity. However, the impurity was isolated by extracting a large portion of the batch to confirm the source of the color. As shown in Table 2, the color data for the API produced from the front run of this intermediate falls in the region of the first three pilot plant batches, showing that the source of the color causing trace impurities does not necessarily arise from the coupling conditions, but may be carried in from the intermediates. The history of the pilot plant batches shown in Table 1 supports this conclusion. Batch 1 was made from an intermediate obtained from an older synthetic route, and hence contained different impurities from subsequent batches. Batch 2 was made from a mixture of the intermediate from the old synthetic process and the first batch of material obtained from a new process. The first batch of intermediate from the new process (B3) had an elevated level of an impurity compared to subsequent batches, which may account for its slightly different appearance. Thus, the color data obtained from the solid material by the sphere spectrophotometer provided an indication of process variations between the batches.

4. Conclusions

Direct measurement of the color of solid bulk API provides a convenient and accurate supplement to visual evaluation. While visual evaluation is a quick and sensitive test, it requires an

experienced analyst to determine what constitutes typical material, and subtle differences between batches may not be noticed unless they are compared side by side. Measurement of the color of a solution of the material may not provide meaningful information when working near the sensitivity limit of the method. By measuring the color of the solid directly, the sample is not consumed in the analysis. This is an important advantage in development work, since the material from a small scale lab run can be measured and retained for other experiments. The reflectance spectrum also provides qualitative information about the absorbance of the sample. The delta *E* values and whiteness index values provided a sensitive quantitative and objective measure of how different the samples were from a reference batch and from each other. The ability to sort the samples using the color data from the sphere spectrophotometer helped to identify and define the key process parameters that need to be controlled in the final step in order to produce material of acceptable color without recrystallization.

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