Modernization of Physical Appearance and Solution Color Tests Using Quantitative Tristimulus Colorimetry: Advantages, Harmonization, and Validation Strategies

BRIAN W. PACK, LAURA L. MONTGOMERY, EVAN M. HETRICK

Small Molecule Design and Development, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

Received 12 May 2015; accepted 12 June 2015

Published online 14 July 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24565

ABSTRACT: Color measurements, including physical appearance, are important yet often misunderstood and underappreciated aspects of a control strategy for drug substances and drug products. From a patient safety perspective, color can be an important control point for detecting contamination, impurities, and degradation products, with human visual acuity often more sensitive for colored impurities than instrumental techniques such as HPLC. Physical appearance tests and solution color tests can also serve an important role in ensuring that appropriate steps are taken such that clinical trials do not become unblinded when the active material is compared with another product or a placebo. Despite the importance of color tests, compendial visual tests are not harmonized across the major pharmacopoeias, which results in ambiguous specifications of little value, difficult communication of true sample color, and significant extra work required for global registration. Some pharmacopoeias have not yet recognized or adopted technical advances in the instrumental measurement of color and appearance, whereas others begin to acknowledge the advantage of instrumental colorimetry, yet leave implementation of the technology ambiguous. This commentary will highlight the above-mentioned inconsistencies, provide an avenue toward harmonization and modernization, and outline a scientifically sound approach for implementing quantitative technologies for improved measurement, communication, and control of color and appearance for both solutions and solids. Importantly, this manuscript, for the first time, outlines a color method validation approach that is consistent with the International Conference on Harmonization's guidance on the topic of method validation. J Pharm Sci 104:3299–3313, 2015
Keywords: analytical chemistry; physical characterization; analysis; formulation; solid dosage form; injectables; colorimetry; physical appearance; description; validation

INTRODUCTION AND BACKGROUND

Color measurement is an important aspect of the control strategy for pharmaceutical development that impacts both small- and large-molecule therapeutics including drug substances and drug products. In addition to being an indicator of quality, control of color is required to maintain consistency across batches, and to ensure that actives and/or placebos do not become unblinded during clinical trials. Color becomes important at several points in the control strategy for a given product, including physical appearance of both the drug substance and drug product, and compendial color tests for drugs administered as solutions. To date, these tests have typically been conducted by visual examination as that is the compendial requirement.[1–4] However, several reports describe the application of instrumental colorimetry to pharmaceutical development and describe the advantages that are obtained from quantitative, objective measures of color and appearance versus subjective visual tests.[5–7]

Instrumental Colorimetry: Background

Tristimulus colorimetry is an objective instrumental approach for color measurements whereby the critical parameters of color perception (i.e., illuminant, sample, and observer) are standardized and well-controlled.[8,9] An in-depth review of the pharmaceutical applications of colorimetry is provided by Subert and Cizmarik[10] in addition to several recent manuscripts.[6,7] As such, only a brief primer on instrumental colorimetry will be provided here. Instrumental colorimetry employs controlled broad-spectrum light sources that enable standardized illuminants designed to mimic common viewing situations including natural daylight (illuminant D65), incandescent light (illuminant A), and fluorescent light (illuminant F). Equally as important as standardized illuminants in generating objective measurements is the application of a standard observer function. The standard observer function serves to represent average human visual sensitivity across the visible spectrum and to eliminate subjectivity because of visual perception differences across observers. It is essential to understand both the illuminant and standard observer function employed when making or comparing color measurements across multiple instruments. For example, a colorimetry measurement at D65/2° or A/10° (illuminant/standard observer angle) would most likely provide different numerical results on the same sample as the illuminant and the standard observer angle are different. Depending upon the sample, this difference could be substantial and will be discussed with the topic of metamerism below. Finally, color measurement instruments ensure that the sample is positioned reproducibly with respect to the illuminant and detector. The entire visible spectrum (reflectance or transmittance), typically between 380 and 700 nm,[11] is recorded by the instrument and adjusted for the spectral sensitivity of the

Correspondence to: Brian W. Pack (Telephone: +317-651-2838; Fax: +317-277-2403; E-mail: packbw@lilly.com)

Journal of Pharmaceutical Sciences, Vol. 104, 3299–3313 (2015)

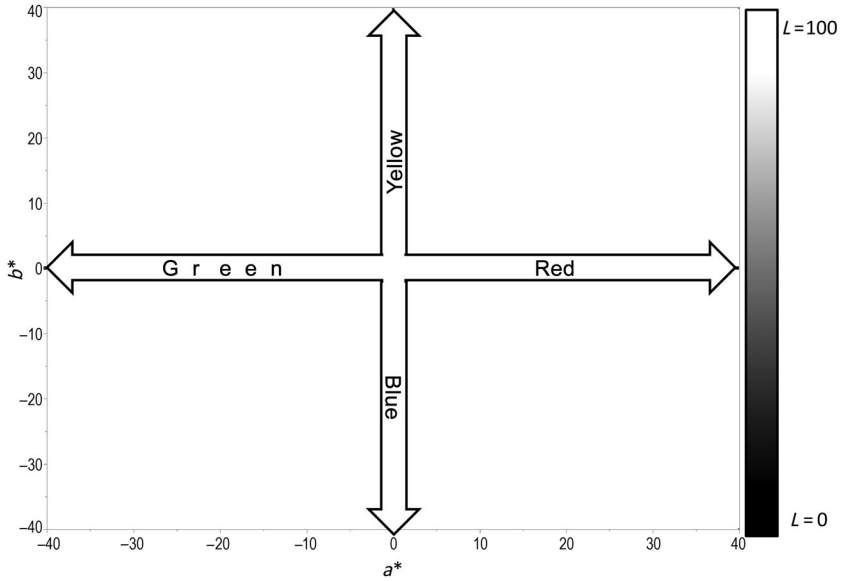

Figure 1. Representation of the CIE L^* a^* b^* three-dimensional color space where a^* represents the red-green color axis, b^* represents the yellow-blue color axis, and L^* represents the lightness axis.

standard observer and converted to a three-dimensional coordinate (i.e., X, Y, Z) that is unique to the hue, saturation, and lightness of that color (i.e., a tristimulus value). Mathematical manipulation of the tristimulus values convert them into the more intuitive CIE $L^*a^*b^*$ (Commission Internationale de l'Eclairage) three-dimensional color space defined by L^*, a^*, and b^* values (see Fig. 1) that have been idealized to correspond most closely with human perception. It is this manipulation via the standard observer function based on human spectral sensitivities that are not constant across the wavelength range that makes the instrumental color measurement different from UV/Vis absorption or transmission measurements.

In the CIE $L^*a^*b^*$ coordinate system, L^* represents the degree of lightness of a color on a scale of 0–100, with 0 being the darkest and 100 the lightest, a^* represents the redness or greenness of a color (positive values of a^* represent red, whereas negative values of a^* represent green), and b^* represents the yellowness or blueness of a sample, with positive values of b^* representing yellow and negative values of b^* representing blue. Color difference from a standard, or from an initial sample in a stability evaluation, can be represented by a change in the individual color components ΔL^*, Δa^*, and Δb^*. The composite change, or difference in color, can be calculated as a simple Euclidian distance in space using the formula:

$$dE^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Solution Color Test: Background and Context

The degree of coloration of a liquid formulation, a reconstituted parenteral drug product, or drug substance solution can be informative as a quality indicating parameter and thus is an essential aspect of a control strategy. In addition, color is a parameter that is immediately apparent to the patient or physician and may impact compliance with a dosing regimen, or impact the blinding of a clinical trial. Therefore, understanding color control during manufacture and stability is of significant importance in order to deliver a product of consistent quality and to avoid end-user (e.g., patient, physician, pharmacist) complaints. As such, each of the major pharmacopoeias has monographs devoted to the visual determination of solution color. Each test involves preparing standard (reference) solutions to which samples are compared visually to determine which standard or reference solution best matches the sample. The similarities and differences between four major pharmacopoeial visual color tests are summarized in Table 1.

In the United States Pharmacopeia, USP <631> *Color and Achromicity* defines 20 "Matching Fluids" that serve as the standards to which samples are compared visually.[1] These solutions contain various ratios of cobaltous chloride (imparting a red color), ferric chloride (yellow color), and cupric sulfate (blue color). Likewise, in the Japanese Pharmacopeia (JP), 9.23 *Matching Fluids for Color*[3] defines the same 20 "Matching Fluids" and in that respect, is harmonized with the USP from a visual measurement standpoint. In contrast, the European Pharmacopeia (EP) is not harmonized with the USP and JP. EP 2.2.2. *Degree of Coloration of Liquids*[2] outlines the preparation of 37 separate "Reference Solutions" that belong to the following five color families: greenish-yellow (GY), yellow (Y), brownish-yellow (BY), brown (B), and red (R). These 37 reference solutions are prepared from the same metal salts as those used for the USP and JP preparation. Finally, the visual color method in the Chinese Pharmacopeia (Ch.P.) differs from the USP, JP, and EP. Appendix IX of the Ch.P. *Colour of Solution*[4] describes five color families, similar to the EP, but unlike the EP, the color families are yellowish green, yellow, orange yellow, orange red, and brownish red, and there are 50 individual reference solutions instead of 37. Moreover, the Ch.P. uses potassium dichromate for preparation of the reference solutions instead of ferric chloride.

In contrast to the USP and JP, which do not specify viewing orientations for the visual comparison, the EP prescribes two different methods for making the comparison. Method I