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⟨631⟩ COLOR AND ACHROMICITY

INTRODUCTION

The purpose of this chapter is to provide well-controlled methods for the assessment of color and achromicity of uniform liquid samples. The methods described in this chapter may be applied to the liquid phase of dispersed systems in cases where the dispersed phase can be removed prior to color measurements. Color is an organoleptic characteristic, which means that the value is based on the human response to stimuli. To reliably evaluate color, it is important to control both the illumination and observation parameters. By using a characteristic response for a standard observer, it is possible to perform precise instrumental evaluations of color. This chapter provides guidance for both an organoleptic assessment (*Method I*) and an instrumental assessment (*Method II*) of color and achromicity in liquid samples.

Color may be defined as an observer's perception of or subjective response to a stimulus of radiant energy in the visible spectrum extending over the wavelength range of 400–700 nm. Perceived color is a function of three variables: spectral properties of the object, spectral power distribution of the source of illumination, and visual perception of the observer.

Two objects are said to have a color match for a particular source of illumination when an observer cannot detect a color difference. Where a pair of objects exhibit a color match for one source of illumination and not another, they constitute a metameric pair. Color matches of two objects occur for all sources of illumination if the absorption and reflectance spectra of the two objects are identical.

Achromicity or colorlessness is one extreme of any color scale for transmission of light. It implies the complete absence of color; therefore, the visible spectrum of the sample lacks absorbances. For practical purposes, the observer in this case perceives little if any absorption taking place in the visible spectrum and is unable to discern the difference between an almost colorless sample and a colorless reference.

COLOR ATTRIBUTES AND COLOR SPACE COORDINATES

Because the sensation of color has both a subjective and an objective part, color cannot be described solely in spectrophotometric terms. The common attributes of color therefore cannot be given a one-to-one correspondence with spectral terminology.

Three attributes are commonly used to identify a color: 1) hue (angle), or the color dimension dominant in its purest form, such as red, yellow, blue, green, and intermediate terms; 2) lightness, or the quality that distinguishes a light color from a dark one; and 3) chroma, or the quality that distinguishes an intense color from a weak one, such as the extent to which a color differs from a gray of the same lightness. The three attributes of color may be used to define a three-dimensional color space in which any color is located by its coordinates. The color space chosen is visually uniform if the geometric distance between two colors in the color space is correlated with the perceived difference between the two colors. Cylindrical coordinates are often chosen for convenience. Points along the vertical axis represent the lightness from dark to light (or black to white) and have indeterminate hue (angle) and no chroma. Focusing on a cross-section perpendicular to the lightness axis, hue is determined by the angle about the long axis and chroma is determined by the geometric distance from the vertical axis. Red, yellow, green, blue, and intermediate hues are differentiated by various hue angles. Colors of the same hue angle become more intense (i.e., chromatic) as they move further from the vertical axis (larger chroma). For example, colorless or achromic water has an indeterminate hue angle, high lightness, and little to no chroma. If a colored solute is added, the water takes on a particular hue (angle). As more is added, the color becomes darker, more intense, or deeper; that is, the chroma increases and lightness decreases. However, if the solute is a neutral color, such as gray, the lightness decreases, no increase in chroma occurs, and the hue (angle) remains indeterminate.

Laboratory spectroscopic measurements can be converted to measurements of the color attributes by using the response factors for a standardized observer. Spectroscopic results are weighted by three distribution functions to yield the tristimulus values X, Y, and Z (for additional information, see *Color—Instrumental Measurement* ⟨1061⟩).

The tristimulus values are not coordinates in a visually uniform color space; however, they can be transformed into a more uniform chroma–hue color space known as the CIELAB color space, defined by the International Commission on Illumination (CIE) in 1976. Also known as CIE L*a*b* or sometimes abbreviated as simply "Lab" color space, CIELAB was designed so that the same amount of numerical change in these values corresponds to roughly the same amount of visually perceived change. The resulting L^*, a^*, b^*, C^*_{ab}, and h^*_{ab} values indicate the following:

- L^* is the lightness coordinate. L is always positive and ranges from 0 to 100; 100 is the colorless (white) standard and 0 is the zero (black) standard.
- a^* is the red/green coordinate. $+a^*$ is red, and $-a^*$ is green.
- b^* is the yellow/blue coordinate. $+b^*$ is yellow, and $-b^*$ is blue.
- C^*_{ab} is the chroma in the (a^*, b^*) plane. $C^*_{ab} = [(a^*)^2 + (b^*)^2]^{1/2}$.

- h^*_{ab} is the hue angle in the (a^*, b^*) plane, measured from the a^* axis increasing counterclockwise, and is reported as a value from 0 to 360 degrees. The hue angles for the elementary colors are approximately 25 (red), 92 (yellow), 162 (green), 220 (cyan), 271 (blue), and 337 (magenta) degrees.

Additionally, the difference between two points in the CIELAB color space (ΔE^*) can be calculated as follows:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Slight differences in colors are not perceptible in visual comparisons. Although it is possible for the human eye to differentiate between colors with a ΔE^* of about 1, it is frequently difficult for an observer with normal color vision to reliably differentiate between colors with a ΔE^* of less than 3. Therefore, it is preferable to rely on quantitative assessment of color and color differences when precise, reliable comparisons are required.

COLOR DETERMINATION AND STANDARDS

The perception of color and color matches is dependent on conditions of viewing and illumination. Determinations should be made using diffuse, uniform illumination under conditions that reduce shadows and minimize non-spectral reflectance. Liquids should be compared in matched color-comparison containers that are transparent to the illumination light. Either organoleptic (qualitative) or instrumental (quantitative) assessment of color may be used.

Containers for Liquid Samples and Matching Fluids

For liquid samples, the containers used to hold the samples during the color measurements should be non-absorbing in the 400–700 nm range and must be identical for the sample and matching fluids. The containers may be round or square, and the diameter or depth of the container may be adjusted to provide a shorter path length for darker samples or a longer path length for lighter samples. The use of cuvettes or glass tubes with a path length of about 10 mm is recommended.

Turbid and Opaque Samples

When using transparent color-matching fluids, the color evaluation of the sample should be based on evaluation of the clarified sample. If the sample is turbid or opaque, the dispersed phase should be removed by centrifugation or filtration before evaluating the color. If the sample or matching fluid contains air bubbles or foam, these should be removed by centrifugation before evaluation of the color.

Matching Fluids

The colors of standards should be as close as possible to those of test specimens for quantifying color differences. Any well-defined and well-characterized matching fluid is permitted (*1*). For example, matching fluids may be prepared according to *Table 1* or may be prepared as described in the *European Pharmacopoeia* (*2*) (*2.2.2. Degree of Coloration of Liquids*) or *Chinese Pharmacopoeia* (*3*) (*Appendix IX, Colour of Solution*). To prepare the matching fluids in *Table 1*, pipet the prescribed volumes of the colorimetric test solutions (see *Reagents, Indicators, and Solutions—Colorimetric Solutions*) and water into one of the matching containers and mix the solution in the container.

Table 1. Matching Fluids

Matching Fluid	Parts[1] of Cobaltous Chloride CS	Parts of Ferric Chloride CS	Parts of Cupric Sulfate CS	Parts of Water
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	0.0	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	0.0	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	0.0	0.0

Matching Fluid	Parts[1] of Cobaltous Chloride CS	Parts of Ferric Chloride CS	Parts of Cupric Sulfate CS	Parts of Water
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	0.0	4.9	0.1	0.0
O	0.1	4.8	0.1	0.0
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	0.0	4.7
T	0.5	0.5	0.4	3.6

[1] The composition in Table 1 is described on a v/v basis and indicates the relative volume of each ingredient required to make the reference solution.

If a matching fluid is prepared, it must be freshly prepared on the day of use unless suitable data demonstrating the stability of the color in the matching fluid are available to support the use for longer time periods. For purchased color standards or matching fluids, follow the appropriate use instructions and expiry dates.

METHOD I: ORGANOLEPTIC (QUALITATIVE) ASSESSMENT OF COLOR AND COLOR MATCHES

Illuminant

A standard illuminant is a theoretical source of visible light with a profile (its spectral power distribution) that is published. Standard illuminants are designated by a letter or by a letter-number combination. Illuminants A, B, and C were introduced in 1931, with the intention of representing average incandescent light, direct sunlight, and average daylight, respectively. The illumination of the sample(s) and matching fluid(s) should be solely due to the controlled illuminant source. Any additional illumination from room lighting or windows should be minimized (for example, by use of a light box or light booth). A daylight illuminant capable of providing a correlated color temperature (CCT) of 6000–7000 K should be used. Filtered tungsten halogen lamps are recommended, but daylight fluorescent lamps with comparable spectral and illuminance characteristics also can be used. The spectral power distribution should conform to ISO 23603/CIE S 012 (4) for the D65 illuminant with a grade of B/C or better. The illumination of the sample and matching fluid should be diffuse and not at an angle that will provide specular reflections to the observer. Recommended geometries include 0:45 and 45:0 illuminant viewer, in degrees.

Observer

The observer angle may be normal (vertical) or at 90 degrees (horizontal) if the illumination angle is controlled to avoid specular reflection and provides diffuse illumination relative to the observer angle. The sample and one or more matching fluids should subtend a viewing angle of 2–10 degrees and be in the field of view simultaneously. Using identical tubes of colorless, transparent, and neutral glass of 12-mm external diameter, compare 2.0 mL of the liquid to be examined with 2.0 mL of water or matching fluid. A small, discernible gap should exist between the sample and matching fluid. The surrounding field should be white, black, or neutral gray.

Procedures

Evaluate the sample and at least one other matching fluid simultaneously. Evaluate the brightness, saturation, and hue (angle). Hue (angle) differences between the sample and the reference should be interpreted as a failure of the comparative test unless there are explicit instructions in the monograph to ignore hue (angle) differences.

When analyzing samples that are expected to have color, there are three approaches:

1. Establish a minimum level of color. If the sample matches the reference sample or has more color, it passes the test.
2. Establish a maximum level of color. If the sample matches the reference sample or has less color, it passes.
3. Select a matching (or reference) solution to determine an indiscernible difference between the test sample and the reference. In this case, the sample must match the matching solution. It is preferred, in this case, to establish a standard of the same compound as the analyte of interest to avoid situations of metamerism.

Table 2 illustrates how the results of these color evaluations should be interpreted and reported.

Table 2. Interpretation of Color Comparisons for Qualitative Color Evaluations

Color Evaluation Test	Test Requirement	Sample Has More Color	Reference Has More Color	Sample Matches Reference
Almost Colorless	Compare sample to Purified Water.	Fails the test	Passes the test	Passes the test
Maximum Level of Color	Compare to a matching fluid that should exhibit discernibly more color than sample.	Fails the test	Passes the test	If used for achromicity, fails the test. Otherwise, passes the test
Minimum Level of Color	Identify a matching fluid that represents minimum acceptable color.	Passes the test	Fails the test	Passes the test
Indiscernible from Color Standard	Prepare color standard; compare test sample to color standard.	Fails the test	Fails the test	Passes the test

Achromicity is a special case of an evaluation of the maximum level of color. For achromicity evaluations, one must either perform an *Indiscernible from Color Standard* test using Purified Water as the standard or perform a *Maximum Level of Color* test with a color standard with a low level of color that is discernibly different from Purified Water (i.e., a ΔE^* relative to Purified Water of 3–6). Matching fluids that are almost colorless are generally not useful for qualitative color evaluations and should be avoided. *Table 3* lists the matching fluids that should be avoided and those that are recommended for achromicity evaluations using the *Maximum Level of Color* test.

Table 3. Matching Fluids for Use in Achromicity Evaluations (*5*)

Source of Matching Fluid	Matching Fluids Almost Colorless (not recommended for use)	Matching Fluids Darker and Differentiated from Colorless (recommended for maximum level of color in an achromicity test)
This chapter	None	A, P, Q, S
European Pharmacopoeia, *2.2.2. Degree of Coloration of Liquids*	B7, B8, B9, BY7, Y7, GY7, R7	B6, BY5, Y5, GY5, R6
Chinese Pharmacopoeia, Color Standard	YG1, Y1, OY1, OR1, BR1	YG2, YG3, Y2, Y3, OY3, OY4, OR3, OR4, BR3, BR4, BR5

Change to read:

METHOD II: INSTRUMENTAL (QUANTITATIVE) ASSESSMENT OF COLOR AND COLOR MATCHES

Instrumentation

For evaluating the color of solutions, it is acceptable to use any spectrometer or colorimeter (in transmission mode) that has been qualified for use, according to *Ultraviolet–Visible Spectroscopy ⟨857⟩*, in the operating range of 400–700 nm and has a bandwidth of 10 nm or less.

Calibration

For verification of the calibration in transmission mode, a black tile (or block of the light path) is used to determine the zero end of the lightness scale; Purified Water (or relevant solvent) is used for 100% transmission (for more information, see *Analytical Instrument Qualification ⟨1058⟩*).

Recording of Spectroscopic Data

The spectroscopic data should be recorded from 400–700 nm at 10-nm intervals. If results are acquired at more frequent intervals, only the results from the 10-nm intervals will be used to calculate the instrumental color values. If the samples are turbid, they should be clarified by centrifugation or filtration prior to loading into the cuvette. Samples also should be checked to ensure that the light transmission path is free from air bubbles or foam.

Calculation of CIELAB Values

This instrumental method relies on calculation of the color values in the CIELAB color space using either illuminant C with the 2-degree observer or illuminant D65 with the 10-degree observer. *Table 4* and *Table 5* contain the weighting factors for illuminant C with the 2-degree

observer (C/2) and for illuminant D65 with the 10-degree observer (D65/10), respectively. These weighting factors should be used to convert the spectroscopic data into CIELAB values (for additional information, see ⟨1061⟩). As an example, *Table 6* contains three sets of spectroscopic transmittance data for the ideal Lovibond 10R, 10Y, and 10B glasses (*6*). *Table 7* shows the CIELAB (C/2) and CIELAB (D65/10) values calculated from these data using the weighting factors in *Table 4* and *Table 5*.

Table 4. Weighting Factors for Illuminant C with 1931 (2-degree) Observer (400–700 nm, 10-nm spacing)[a,b]

λ (nm)	*Wx(λ)* = xλPλ	*Wy(λ)* = yλPλ	*Wz(λ)* = zλPλ
400	0.099	0.003	0.463
410	0.325	0.009	1.547
420	1.292	0.038	6.207
430	2.968	0.123	14.496
440	3.959	0.261	19.860
450	3.931	0.443	20.728
460	3.360	0.692	19.286
470	2.283	1.061	15.022
480	1.116	1.612	9.479
490	0.363	2.358	5.286
500	0.048	3.414	2.868
510	0.092	4.842	1.512
520	0.578	6.449	0.720
530	1.519	7.936	0.381
540	2.786	9.145	0.195
550	4.285	9.831	0.086
560	5.877	9.834	0.038
570	7.323	9.148	0.020
580	8.414	7.990	0.015
590	8.985	6.629	0.010
600	8.958	5.321	0.007
610	8.324	4.177	0.003
620	7.055	3.146	0.001
630	5.327	2.196	0.000
640	3.692	1.442	0.000
650	2.352	0.887	0.000
660	1.360	0.503	0.000
670	0.713	0.261	0.000

λ (nm)	Wx(λ) = xλPλ	Wy(λ) = yλPλ	Wz(λ) = zλPλ
680	0.364	0.132	0.000
690	0.172	0.062	0.000
700	0.154	0.055	0.000
Sum	98.074	100.000	118.230
White point[c]	98.074	100.000	118.232

[a] Adapted from ASTM E308 (*Standard Practice for Computing the Colors of Objects by Using the CIE System*).

[b] The weighting factors (*Wx*, *Wy*, *Wz*) are obtained by multiplying the illuminant power at the particular wavelength (*P*) by the response factor at that wavelength (*x*,*y*,*z*).

[c] The white point value is the exact value for the colorless/white standard. A slight difference may exist between the sum and white point values due to rounding in the last decimal place.

Table 5. Weighting Factors for Illuminant D65 with 1964 (10-degree) Observer (400–700 nm, 10-nm spacing)[a,b]

λ (nm)	Wx(λ) = xλPλ	Wy(λ) = yλPλ	Wz(λ) = zλPλ
400	0.145	0.015	0.643
410	0.676	0.069	3.110
420	1.603	0.168	7.627
430	2.451	0.300	12.095
440	3.418	0.554	17.537
450	3.699	0.890	19.888
460	3.064	1.290	17.695
470	1.933	1.838	13.000
480	0.802	2.520	7.699
490	0.156	3.226	3.938
500	0.039	4.320	2.046
510	0.347	5.621	1.049
520	1.070	6.907	0.544
530	2.170	8.059	0.278
540	3.397	8.668	0.122
550	4.732	8.855	0.035
560	6.070	8.581	0.001
570	7.311	7.951	0.000
580	8.291	7.106	0.000
590	8.634	6.004	0.000
600	8.672	5.079	0.000

λ (nm)	Wx(λ) = xλPλ	Wy(λ) = yλPλ	Wz(λ) = zλPλ
610	7.930	4.065	0.000
620	6.446	2.999	0.000
630	4.669	2.042	0.000
640	3.095	1.290	0.000
650	1.859	0.746	0.000
660	1.056	0.417	0.000
670	0.570	0.223	0.000
680	0.274	0.107	0.000
690	0.121	0.047	0.000
700	0.109	0.043	0.000
Sum	▲94.809▲ (ERR 1-Dec-2023)	100.000	107.307
White point	▲94.811▲ (ERR 1-Dec-2023)	100.000	107.304

[a] Adapted from ASTM E308 (*Standard Practice for Computing the Colors of Objects by Using the CIE System*).

[b] The weighting factors (*Wx*, *Wy*, *Wz*) are obtained by multiplying the illuminant power at the particular wavelength (*P*) by the response factor at that wavelength (*x*,*y*,*z*).

Table 6. Ideal Lovibond Transmission Spectra for 10R, 10Y, and 10B plates (6)

λ (nm)	10R	10Y	10B
400	0.3368	0.0000	0.9007
410	0.3429	0.0001	0.8938
420	0.3480	0.0013	0.8815
430	0.3514	0.0073	0.8630
440	0.3538	0.0271	0.8414
450	0.3527	0.0750	0.8154
460	0.3475	0.1704	0.7780
470	0.3370	0.3024	0.7112
480	0.3144	0.4441	0.6119
490	0.2753	0.5671	0.5013
500	0.2164	0.6615	0.4136
510	0.1472	0.7319	0.3172
520	0.0939	0.7801	0.2373
530	0.0709	0.8116	0.1722
540	0.0866	0.8354	0.1525

λ (nm)	10R	10Y	10B
550	0.1483	0.8486	0.1822
560	0.2509	0.8521	0.2231
570	0.3703	0.8530	0.1967
580	0.4763	0.8472	0.1235
590	0.5666	0.8382	0.0674
600	0.6351	0.8264	0.0631
610	0.6861	0.8127	0.0712
620	0.7251	0.7989	0.0750
630	0.7558	0.7860	0.0709
640	0.7788	0.7776	0.0601
650	0.7968	0.7740	0.0645
660	0.8115	0.7734	0.0786
670	0.8230	0.7774	0.1457
680	0.8330	0.7834	0.2734
690	0.8419	0.7869	0.4701
700	0.8485	0.7918	0.6607

Table 7. CIELAB Values Calculated from the Data in *Table 6* and Using the Weighting Factors from *Table 4* and *Table 5*

CIELAB Values	Calculation with Illuminant C and 1931 (2-degree) Observer			Calculation with Illuminant D65 and 1964 (10-degree) Observer		
	10R	10Y	10B	10R	10Y	10B
L^*	64.32	91.20	51.83	63.67	90.02	54.75
a^*	49.79	−21.97	23.36	47.75	−15.48	9.65
b^*	−0.27	71.64	−65.31	−1.77	73.38	−61.29
C^*_{ab}	49.79	74.93	69.37	47.79	75.00	62.05
h^*_{ab}	359.69	107.05	269.68	357.88	101.91	278.95

METHOD IIA: COMPARATIVE TEST OF COLORS USING CIELAB VALUES

The CIELAB values for a sample and a matching solution may be evaluated to determine the acceptability of the sample. *Table 8* lists the different metrics that may be used to compare the color values of a sample to the color values of a matching solution. In evaluating stability, the color values may be tracked over time to assess trends. The variability in the color values may be used to assess process control and variability due to raw materials or processing.

Table 8. Interpretation of Color Comparisons[a,b]

Color Evaluation Test	Test	Requirement
Almost Colorless	Compare the sample to Purified Water.	Measure the sample and Purified Water. Calculate ΔE^* relative to Purified Water.

Color Evaluation Test	Test	Requirement
		Requirement is that $\Delta E^* < 1$.
Maximum Level of Color	Identify a matching fluid of similar hue angle ($\Delta h^*_{ab} < 15$) that is discernibly more colored than the sample.	Measure the matching fluid and calculate the $\Delta E0^*$ relative to Purified Water. Measure the sample and calculate the ΔE^* relative to Purified Water. Requirement is $\Delta E^* < \Delta E0^*$.
Minimum Level of Color	Identify a matching fluid of similar hue angle ($\Delta h^*_{ab} < 15$) that is discernibly less colored than the sample.	Measure the matching fluid and calculate the $\Delta E0^*$ relative to Purified Water. Measure the sample and calculate the ΔE^* relative to Purified Water. Requirement is $\Delta E^* > \Delta E0^*$.
Indiscernible from Color Standard	Prepare color standard; compare the test sample to the color standard.	Calculate ΔE^* relative to selected color standard. Requirement is that $\Delta E^* < 3$.

[a] Comparisons rely on the numerical values of *L**, *a**, and *b**, and the comparison of these values to those of the matching solution or standard.

[b] *CIELAB values must be calculated with the same illuminant and observer for evaluating differences between the sample and matching fluid.*

METHOD IIB: INSTRUMENTAL COLOR ASSESSMENT

The CIELAB values (calculated as described in *Method IIa: Comparative Testing of Colors Using CIELAB Values*) may be used directly as a quantitative measure of the color attributes of a sample. Acceptable specifications for the color attributes can be established in the CIELAB color space and applied to color values obtained for a sample.

For new monographs under development, which do not already have a visual color determination method, the following approach harnesses the advantages of instrumental colorimetry to its fullest and removes drawbacks associated with limiting the control space to what is encompassed by existing visual standards. Also, for new monographs under development, acceptable numerical limits within color space should be determined based on process capability, stability, and analytical variability. A specification would then take the form of one of the following:

- A solution with *L** NLT XX, NMT XX; *a** NMT XX, NLT XX; and *b** NMT XX, NLT XX (XX being a numerical value).
- ΔE^* versus a single point in 3-dimensional space. Ideally this point would represent the average based on multiple batches or based on representative material, such as a well-characterized reference standard. Other points may be justified.

Limits may be set with individual color parameters (e.g., *L**, *a**, and *b** or another suitably validated color space) or as a ΔE^* versus a single point in color space when considering batch release or stability studies.

Reporting of Results

The calculated CIELAB color values are dependent on the illuminant and observer used and, therefore, should be reported. The CIELAB values for the coordinates *L**, *a**, and *b** should be reported. The values for chroma (C^*_{ab}) and hue angle (h^*_{ab}) are derived from *a** and *b** and do not need to be reported. A recommended method for reporting the CIELAB results is to report the color values as shown in the following examples:

CIELAB ([illuminant]/[observer]) = (*L**, *a**, *b**)

CIELAB (C/2) = (50.70, −11.30, −33.30)

CIELAB (D65/10) = (52.69, −19.43, 29.61)

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