

1 Q-07 INSTRUMENTAL MEASUREMENT OF COLORATION OF LIQUIDS

2

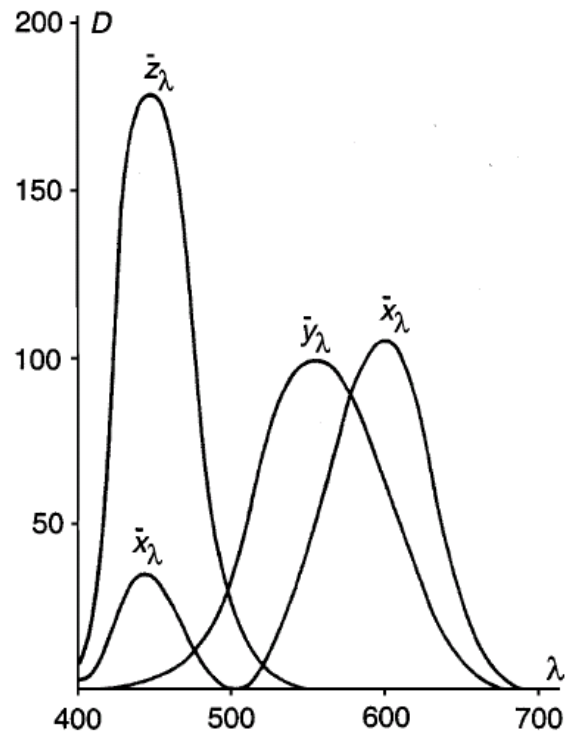
3 PRINCIPLE

4 The observed colour of an object depends primarily on its light absorbing characteristics.
5 However a variety of conditions such as light-source differences, spectral energy of the
6 illuminant, visual sensitivity of the observer, size differences, background differences and
7 directional differences affect the perception of colour. Hue, lightness or brightness and
8 saturation are three attributes of the colour. Instrumental measurement under defined
9 conditions allows numerical expression of a colour. The base of any instrumental
10 measurement of colour is that the human eye has been shown to detect colour via three types
11 of receptors.

12 Instrumental methods for measurement of colour provide more objective data than the
13 subjective viewing of colours by a small number of individuals. With adequate maintenance
14 and calibration, instrumental methods can provide accurate, precise and consistent
15 measurements of colour that do not drift with time. Through extensive colour matching
16 experiments with human subjects having normal colour vision, distribution coefficients
17 (weighting factors) have been measured for each wavelength within the wavelength range of
18 the visible spectrum, giving the relative amount of stimulation of each receptor type caused
19 by the light of that wavelength.

20 The International Commission on Illumination (CIE) has developed models taking into
21 account the light source and the angle at which the observer is looking at the target (field of
22 view). In a visual test for colour of solution, there are requirements that lead to the use of a 2°
23 angle and diffuse daylight. The mean sensitivity of the human eye is represented by the
24 distribution coefficients \bar{x}_λ , \bar{y}_λ and \bar{z}_λ (Fig. 1).

25



26

27 Fig 1. Mean sensitivity of the human eye represented by distribution coefficients, CIE 2°
 28 standard observer. (D = distribution coefficient; λ = wavelength in nm)

29 For any colour, the amount of stimulation of each receptor type is defined by the set of
 30 tristimulus values (XYZ).

31 The relationship between the distribution coefficients and the tristimulus values (X, Y and Z)
 32 is given by the following equations, expressed in terms of integrals:

$$33 \quad X = k \int_0^{\infty} f_{\lambda} \bar{x}_{\lambda} S_{\lambda} d\lambda$$

$$34 \quad Y = k \int_0^{\infty} f_{\lambda} \bar{y}_{\lambda} S_{\lambda} d\lambda$$

$$35 \quad Z = k \int_0^{\infty} f_{\lambda} \bar{z}_{\lambda} S_{\lambda} d\lambda$$

$$36 \quad k = 100 / \int_0^{\infty} \bar{y}_{\lambda} S_{\lambda} d\lambda$$

37 k = normalising constant characterising the stimulation of one receptor type and the used
 38 illumination;

39 S_{λ} = relative spectral power distribution of the illuminant;

40 \bar{x}_{λ} , \bar{y}_{λ} and \bar{z}_{λ} = colour matching distribution coefficients for CIE 2° Standard Observer;

41 f_{λ} = spectral transmittance T_{λ} of the material;

42 λ = wavelength in nanometres.

43 In practical calculations of tristimulus values, the integration is approximated by a summation,
44 as follows:

$$45 \quad X = k \sum_{\lambda} T_{\lambda} \bar{x}_{\lambda} S_{\lambda} \Delta\lambda$$

$$46 \quad Y = k \sum_{\lambda} T_{\lambda} \bar{y}_{\lambda} S_{\lambda} \Delta\lambda$$

$$47 \quad Z = k \sum_{\lambda} T_{\lambda} \bar{z}_{\lambda} S_{\lambda} \Delta\lambda$$

$$48 \quad k = \frac{100}{\sum_{\lambda} S_{\lambda} \bar{y}_{\lambda} \Delta\lambda}$$

49 The tristimulus values can be used to calculate the CIE *Lab* colour space coordinates:
50 L^* (lightness or brightness), a^* (red-green) and b^* (yellow-blue); these are defined by:

$$51 \quad L^* = 116 f\left(\frac{Y}{Y_n}\right) - 16$$

$$52 \quad a^* = 500 \left[f\left(\frac{X}{X_n}\right) - f\left(\frac{Y}{Y_n}\right) \right]$$

$$53 \quad b^* = 200 \left[f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right) \right]$$

54 Where

$$55 \quad f\left(\frac{X}{X_n}\right) = \left(\frac{X}{X_n}\right)^{1/3} \text{ if } \frac{X}{X_n} > (6/29)^3, \text{ otherwise } f\left(\frac{X}{X_n}\right) = \frac{841}{108} \left(\frac{X}{X_n}\right) + \frac{4}{29};$$

$$56 \quad f\left(\frac{Y}{Y_n}\right) = \left(\frac{Y}{Y_n}\right)^{1/3} \text{ if } \frac{Y}{Y_n} > (6/29)^3, \text{ otherwise, } f\left(\frac{Y}{Y_n}\right) = \frac{841}{108} \left(\frac{Y}{Y_n}\right) + \frac{4}{29};$$

$$57 \quad f\left(\frac{Z}{Z_n}\right) = \left(\frac{Z}{Z_n}\right)^{1/3} \text{ if } \frac{Z}{Z_n} > (6/29)^3, \text{ otherwise, } f\left(\frac{Z}{Z_n}\right) = \frac{841}{108} \left(\frac{Z}{Z_n}\right) + \frac{4}{29}.$$

58 X_n , Y_n and Z_n , are the tristimulus values of *purified water*.

59

60 In the spectrophotometric method, transmittance values are obtained at discrete wavelengths
61 throughout the visible spectrum. These values are then used to calculate the tristimulus values
62 through the use of weighting factors \bar{x}_{λ} , \bar{y}_{λ} and \bar{z}_{λ} for a 2° Standard Observer and CIE
63 standard illuminant C (see the International Commission on Illumination publication, CIE).

64 SPECTROPHOTOMETRIC METHOD

65 Operate a suitable spectrophotometer according to the instructions of the manufacturer and
66 determine the transmittance T at least from 400 nm to 700 nm, at intervals of not greater than

67 10 nm. Express the result as a percentage. Calculate the tristimulus values X , Y , and Z and the
68 colour co-ordinates L^* , a^* and b^* .

69

70 DETERMINATION OF COLORATION

71 Calibrate the instrument as per the instrument manufacturer's recommendation. System
72 performance tests are done prior to each measurement or at regular intervals, depending on
73 the use of the apparatus. To this purpose, use certified reference materials¹ within the
74 measurement range.

75 Operate the apparatus according to the manufacturer's instructions and test the sample
76 solution and reference solution(s) under the same conditions (e.g. path length of the cuvette,
77 temperature)

78 For transmittance measurements *purified water* is used as standard and assigned a
79 transmittance of 100.0 per cent at all wavelengths throughout the visible spectrum. Then the
80 tristimulus values for CIE standard illuminant C are: 98.03, 100.00, and 118.11, respectively,
81 corresponding to colour co-ordinates $L^* = 100$, $a^* = 0$ and $b^* = 0$

82 Reference measurements can be made using the colour co-ordinates of *purified water* or
83 freshly prepared pharmacopoeial reference solutions, or using the respective colour co-
84 ordinates stored in the instrument manufacturer's database, provided the latter have been
85 obtained under the same testing conditions.

86 If the test solution is turbid or hazy, it is filtered or centrifuged. If the test solution is not
87 filtered or centrifuged, any haziness or turbidity is reported with the results. Air bubbles are to
88 be avoided and removed.

89 The instrumental method is used to compare two solutions in respect to their colour or colour
90 difference, or a deviation from a defined colour. Calculate the colour difference between the
91 *test solution t* and a *reference r* as ΔE^*_{tr} using the following equation:

$$92 \quad \Delta E^*_{tr} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

93 where ΔL^* , Δa^* and Δb^* are the differences in colour co-ordinates.

94 Instead of the colour coordinates CIE *Lab*, the colour coordinates CIE *LCh* may be used. In
95 this case calculate chroma C^* and compare the colour difference in terms of hue H^* . The hue
96 angle h may also be used.

$$97 \quad C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$98 \quad \Delta H^* = \sqrt{(\Delta E^*_{tr})^2 - (\Delta L^*)^2 - (\Delta C^*)^2}$$

$$99 \quad h_{ab} = \tan^{-1} \left(\frac{b^*}{a^*} \right)$$

¹ Certified filters or certified reference solutions recommended by the instrument's manufacturer.

100 Compliance with a pharmacopoeial requirement

101 For existing monographs which currently include specifications using the visual method, use
102 the following procedure to adapt to instrumental determination. First calculate the value ΔE^*
103 of the test solution versus the reference solutions, as shown in the equation for determination
104 of ΔE^*_{tr} . Determine the nearest neighbour as the reference solution that corresponds to the
105 smallest ΔE^* versus the test solution. The test solution meets the requirements of the
106 monograph if its nearest neighbour is a reference solution whose colour and intensity would
107 pass the monograph requirements.

108 If the nearest neighbour is a reference solution at the colourless end of the reference scale, i.e.
109 in the area between 'colourless' and the faintest reference solution of a scale, determine the
110 ΔE^* of the test solution versus purified water. The test solution meets the monograph
111 requirement if the ΔE^* of the test solution versus purified water is not greater than the ΔE^* of
112 the reference solution versus purified water.

113 Assessment of location within the $L^*a^*b^*$ colour space

114 Instruments may provide information on the actual location of the test solution within the
115 $L^*a^*b^*$ colour space. Using appropriate algorithms, correspondence to pharmacopoeial
116 reference solutions (such as "test solution equals reference solution XY", "test solution close
117 to reference solution XY" or "test solution between reference solutions XY and XZ") can be
118 obtained.