CONTROL OF INSTRUMENT PERFORMANCE

– *Calibration*: performed with at least 4 reference suspensions of formazin covering the measuring range of interest. Reference suspensions described in this chapter or suitable reference standards calibrated against the primary reference suspensions may be used.
– *Stray light*: < 0.15 NTU within the range 0-10 NTU; < 0.5 NTU within the range 10-1100 NTU. Stray light is defined as that light that reaches the nephelometric detector without being a result of scatter from the sample. Stray light is always a positive interference and is a significant source of error in low-range turbidity measurements. Sources of stray light include: imperfections in and scratches on sample cells, internal reflections of the optical system, contamination of the optics or sample cell chamber with dust, and electronic noise. Instrument design can also affect stray light. The influence of stray light becomes negligible in ratio mode measurements.

The test methodology for the specific substance/product to be analysed must also be verified to demonstrate its analytical capability. The instrument and methodology shall be consistent with the attributes of the substance to be examined.

Measurements of standards and samples should be carried out under the same temperature conditions, preferably between 20 °C and 25 °C.

REFERENCE SUSPENSIONS

Formazin has several desirable characteristics that make it an excellent turbidity standard. It can be reproducibly prepared from assayed raw materials. The physical characteristics make it a desirable light-scatter calibration standard. The formazin polymer consists of chains of different lengths, which fold into random configurations. This results in a wide variety of particle shapes and sizes, which allows the analysis of different particle sizes and shapes that are found in real samples. Stabilised formazin suspensions that can be used to prepare stable, diluted turbidity standards are commercially available and may be used after comparison with the standards prepared as described.

All steps of the preparation of reference suspensions as described below are carried out at 25 ± 3 °C.

Hydrazine sulfate solution. Dissolve 1.0 g of *hydrazine sulfate R* in *water R* and dilute to 100.0 mL with the same solvent. Allow to stand for 4-6 h.

Primary opalescent suspension (formazin suspension). In a 100 mL ground-glass-stoppered flask, dissolve 2.5 g of *hexamethylenetetramine R* in 25.0 mL of *water R*. Add 25.0 mL of the hydrazine sulfate solution. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be mixed thoroughly before use.

Standard of opalescence. Dilute 15.0 mL of the primary opalescent suspension to 1000.0 mL with *water R*. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspensions. Prepare the reference suspensions according to Table 2.2.1.-1. Mix and shake before use.

Table 2.2.1.-1

	I	II	III	IV
Standard of opalescence	5.0 mL	10.0 mL	30.0 mL	50.0 mL
Water R	95.0 mL	90.0 mL	70.0 mL	50.0 mL

Measurements of reference suspensions I-IV in ratio mode show a linear relationship between the concentrations and measured NTU values (see Table 2.2.1.-2).

Table 2.2.1.-2

Formazin suspensions	Opalescent values (NTU)
Reference suspension I	3
Reference suspension II	6
Reference suspension III	18
Reference suspension IV	30
Standard of opalescence	60
Primary opalescent suspension	4000

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corrected 10.5

2.2.2. DEGREE OF COLORATION OF LIQUIDS(1)

◊A solution is *colourless* if it has the appearance of *water R* or the solvent used for the preparation of the solution to be examined, or is not more intensely coloured than reference solution B_9.

Report the results together with the method used (method I, method II or method III).

VISUAL METHODS

The examination of the degree of coloration of liquids in the range brown-yellow-red is carried out using one of the 2 methods below, as prescribed in the monograph.

METHOD I

Using identical tubes of colourless, transparent, neutral glass with an external diameter of 12 mm, compare 2.0 mL of the liquid to be examined with 2.0 mL of *water R*, of the solvent used for the preparation of the solution to be examined, or of the reference solution (see Tables of reference solutions) prescribed in the monograph. Compare the colours in diffuse daylight, viewing horizontally against a white background.

METHOD II

Using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15-25 mm, compare the liquid to be examined with *water R*, with the solvent used for the preparation of the solution to be examined, or with the reference solution (see Tables of reference solutions) prescribed in the monograph, the depth of the layer being 40 mm. Compare the colours in diffuse daylight, viewing vertically against a white background.

PREPARATION OF REFERENCE SOLUTIONS

Primary solutions

Yellow solution. Dissolve 46 g of *ferric chloride R* in about 900 mL of a mixture of 25 mL of *hydrochloric acid R* and 975 mL of *water R* and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 45.0 mg of $FeCl_3,6H_2O$ per millilitre by adding the same acidic mixture. Protect the solution from light.

Titration. In a 250 mL conical flask fitted with a ground-glass stopper, introduce 10.0 mL of the solution, 15 mL of *water R*, 5 mL of *hydrochloric acid R* and 4 g of *potassium iodide R*, close the flask, allow to stand in the dark for 15 min and add 100 mL of *water R*. Titrate the iodine released with *0.1 M sodium thiosulfate*, using 0.5 mL of *starch solution R*, added towards the end of the titration, as indicator.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 27.03 mg of $FeCl_3,6H_2O$.

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

Red solution. Dissolve 60 g of *cobalt chloride R* in about 900 mL of a mixture of 25 mL of *hydrochloric acid R* and 975 mL of *water R* and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 59.5 mg of $CoCl_2,6H_2O$ per millilitre by adding the same acidic mixture.

Titration. In a 250 mL conical flask fitted with a ground-glass stopper, introduce 5.0 mL of the solution, 5 mL of *dilute hydrogen peroxide solution R* and 10 mL of a 300 g/L solution of *sodium hydroxide R*. Boil gently for 10 min, allow to cool and add 60 mL of *dilute sulfuric acid R* and 2 g of *potassium iodide R*. Close the flask and dissolve the precipitate by shaking gently. Titrate the iodine released with *0.1 M sodium thiosulfate*, using 0.5 mL of *starch solution R*, added towards the end of the titration, as indicator. The end-point is reached when the solution turns pink.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 23.79 mg of $CoCl_2,6H_2O$.

Blue primary solution. Dissolve 63 g of *copper sulfate pentahydrate R* in about 900 mL of a mixture of 25 mL of *hydrochloric acid R* and 975 mL of *water R* and dilute to 1000.0 mL with the same mixture. Titrate and adjust the solution to contain 62.4 mg of $CuSO_4,5H_2O$ per millilitre by adding the same acidic mixture.

Titration. Into a 250 mL conical flask fitted with a ground-glass stopper, introduce 10.0 mL of the solution, 50 mL of *water R*, 12 mL of *dilute acetic acid R* and 3 g of *potassium iodide R*. Titrate the iodine released with *0.1 M sodium thiosulfate*, using 0.5 mL of *starch solution R*, added towards the end of the titration, as indicator. The end-point is reached when the solution shows a slight pale brown colour.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 24.97 mg of $CuSO_4,5H_2O$.

Standard solutions

Using the 3 primary solutions, prepare the 5 standard solutions as follows.

Table 2.2.2.-1.

Standard solution	Volumes in millilitres			
	Yellow solution	Red solution	Blue solution	Hydrochloric acid (10 g/L HCl)
B (brown)	3.0	3.0	2.4	1.6
BY (brownish-yellow)	2.4	1.0	0.4	6.2
Y (yellow)	2.4	0.6	0.0	7.0
GY (greenish-yellow)	9.6	0.2	0.2	0.0
R (red)	1.0	2.0	0.0	7.0

Reference solutions for Methods I and II

Using the 5 standard solutions, prepare the following reference solutions.

Table 2.2.2.-2. – *Reference solutions B*

Reference solution	Volumes in millilitres	
	Standard solution B	Hydrochloric acid (10 g/L HCl)
B_1	75.0	25.0
B_2	50.0	50.0
B_3	37.5	62.5
B_4	25.0	75.0
B_5	12.5	87.5
B_6	5.0	95.0
B_7	2.5	97.5
B_8	1.5	98.5
B_9	1.0	99.0

Table 2.2.2.-3. – *Reference solutions BY*

Reference solution	Volumes in millilitres	
	Standard solution BY	Hydrochloric acid (10 g/L HCl)
BY_1	100.0	0.0
BY_2	75.0	25.0
BY_3	50.0	50.0
BY_4	25.0	75.0
BY_5	12.5	87.5
BY_6	5.0	95.0
BY_7	2.5	97.5

Table 2.2.2.-4. – *Reference solutions Y*

Reference solution	Volumes in millilitres	
	Standard solution Y	Hydrochloric acid (10 g/L HCl)
Y_1	100.0	0.0
Y_2	75.0	25.0
Y_3	50.0	50.0
Y_4	25.0	75.0
Y_5	12.5	87.5
Y_6	5.0	95.0
Y_7	2.5	97.5

Table 2.2.2.-5. – *Reference solutions GY*

Reference solution	Volumes in millilitres	
	Standard solution GY	Hydrochloric acid (10 g/L HCl)
GY_1	25.0	75.0
GY_2	15.0	85.0
GY_3	8.5	91.5
GY_4	5.0	95.0
GY_5	3.0	97.0
GY_6	1.5	98.5
GY_7	0.75	99.25

Table 2.2.2.-6. – *Reference solutions R*

Reference solution	Volumes in millilitres	
	Standard solution R	Hydrochloric acid (10 g/L HCl)
R_1	100.0	0.0
R_2	75.0	25.0
R_3	50.0	50.0
R_4	37.5	62.5
R_5	25.0	75.0
R_6	12.5	87.5
R_7	5.0	95.0

Storage

For Method I, the reference solutions may be stored in sealed tubes of colourless, transparent, neutral glass of 12 mm external diameter, protected from light.

For Method II, prepare the reference solutions immediately before use from the standard solutions.◊

INSTRUMENTAL METHOD – METHOD III

PRINCIPLE

The observed colour of an object depends primarily on its light-absorbing characteristics. However, a variety of conditions such as light-source differences, spectral energy of the illuminant, visual sensitivity of the observer, size differences, background differences and directional differences affect the perception of colour. Hue, lightness (or brightness) and saturation are 3 attributes of the colour. Instrumental measurement under defined conditions allows numerical expression of a colour. The base of any instrumental measurement of colour is that the human eye has been shown to detect colour via 3 types of receptors.

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

The International Commission on Illumination (CIE) has developed models taking into account the light source and the angle at which the observer is looking at the target (field of view). In a visual test for coloration of solutions, there are requirements that lead to the use of a 2° angle and diffuse daylight (illuminant C). The mean sensitivity of the human eye is represented by the distribution coefficients $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ (Figure 2.2.2.-1).

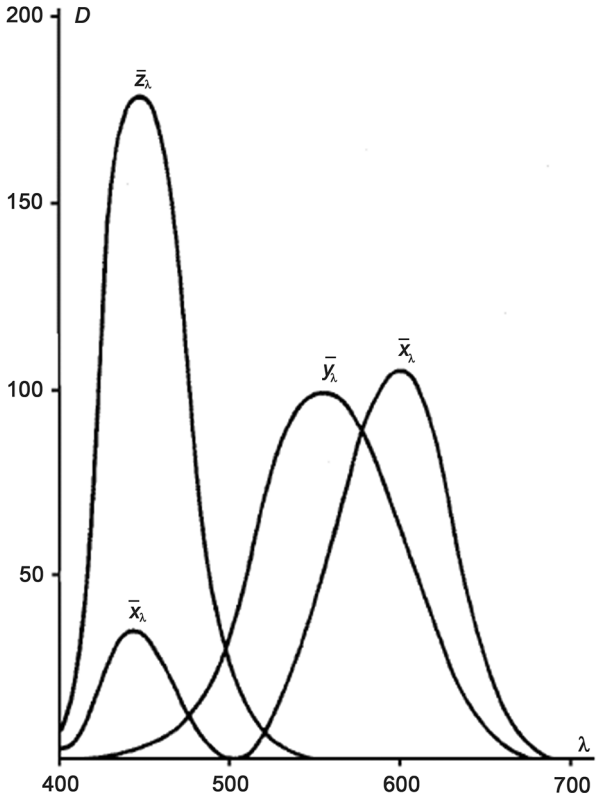

Figure 2.2.2.-1. – *Mean sensitivity of the human eye represented by distribution coefficients, CIE 2° Standard Observer (D = distribution coefficient; λ = wavelength in nanometres)*

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

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

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

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

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

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

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

S_λ = relative spectral power distribution of the illuminant;

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

f_λ = spectral transmittance T_λ of the material;

λ = wavelength, in nanometres.

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

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

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

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

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

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

$$L^* = 116 f(Y/Y_n) - 16$$

$$a^* = 500\,[f(X/X_n) - f(Y/Y_n)]$$

$$b^* = 200\,[f(Y/Y_n) - f(Z/Z_n)]$$

where X_n, Y_n and Z_n are the tristimulus values of *water R* and

$f(X/X_n) = (X/X_n)^{1/3}$ if $X/X_n > (6/29)^3$,

otherwise $f(X/X_n) = 841/108\,(X/X_n) + 4/29$;

$f(Y/Y_n) = (Y/Y_n)^{1/3}$ if $(Y/Y_n) > (6/29)^3$,

otherwise $f(Y/Y_n) = 841/108\,(Y/Y_n) + 4/29$;

$f(Z/Z_n) = (Z/Z_n)^{1/3}$ if $(Z/Z_n) > (6/29)^3$,

otherwise $f(Z/Z_n) = 841/108\,(Z/Z_n) + 4/29$.

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

SPECTROPHOTOMETRIC METHOD

Using a suitable spectrophotometer according to the manufacturer's instructions, determine the transmittance (*T*) at least over the range 400-700 nm, at intervals of not greater than 10 nm. Express the result as a percentage. Calculate the tristimulus values *X*, *Y*, and *Z* and the colour co-ordinates L^*, a^* and b^*.

DETERMINATION OF COLORATION

Calibrate the instrument according to the manufacturer's recommendations. Carry out system performance tests prior to each measurement or at regular intervals, depending on the use of the apparatus. For this purpose, use certified reference materials within the measurement range.

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

For transmittance measurements, use *water R* as the standard, assigning it a transmittance of 100.0 per cent at all wavelengths in the visible spectrum. Then the weighting factors $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$ for CIE standard illuminant C are used to calculate the tristimulus values corresponding to colour co-ordinates $L^* = 100$, $a^* = 0$ and $b^* = 0$.

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

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

The instrumental method is used to compare 2 solutions in terms of their colour or colour difference, or a deviation from a defined colour. Calculate the colour difference (ΔE^*_{tr}) between the test solution (*t*) and a reference solution (*r*) using the following equation:

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

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

The CIE *LCh* colour co-ordinates may be used instead of the CIE *Lab* colour co-ordinates.

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

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2.2.3. POTENTIOMETRIC DETERMINATION OF pH

The pH of an aqueous solution is defined as the negative logarithm of the activity of its hydrogen ions, expressed conventionally as the hydrogen ion concentration of the solution. For practical purposes, its definition is an experimental one. The pH of a solution to be examined is related to that of a reference solution (pH_s) by the following equation:

$$pH = pH_s - \frac{E - E_s}{k}$$

in which *E* is the potential, expressed in volts, of the cell containing the solution to be examined and E_s is the potential, expressed in volts, of the cell containing the solution of known pH (pH_s), *k* is the change in potential per unit change in pH, expressed in volts and calculated from the Nernst equation.

Table 2.2.3.-1. – *Values of k at different temperatures*

Temperature (°C)	*k* (V)
15	0.0572
20	0.0582
25	0.0592
30	0.0601
35	0.0611

The potentiometric determination of pH is made by measuring the potential difference between 2 appropriate electrodes immersed in the solution to be examined; 1 of these electrodes is sensitive to hydrogen ions (usually a glass electrode) and the other is the reference electrode (e.g. a silver-silver chloride electrode). They are often combined as 1 compact electrode, together with a temperature probe.

Apparatus. The measuring apparatus is usually a voltmeter with an input resistance at least 100 times that of the electrodes used. It is normally graduated in pH units and has a sensitivity such that discrimination of at least 0.05 pH unit or at least 0.003 V may be achieved.

Recent pH meters are microprocessor-controlled and are operated using the manufacturer's firmware or software, according to given instructions.

Management of electrodes. The electrodes are stored appropriately and according to the manufacturer's recommendations (e.g. in an electrolyte solution or a suitable storage solution). Before measurement, the electrodes are visually checked. Refillable electrodes are checked for the absence of air bubbles in the glass bulb and to ensure that the inner electrolyte solution level is satisfactory. The filling orifice has to remain open during the measurement. It is also recommended that the diaphragm of the reference electrode is checked. Before first use, or if the electrode has been stored out of electrolyte solution, it is usually necessary to condition it, according to the recommendations of the manufacturer. If pH stabilisation is too slow (i.e. a long response time), or a zero point shift, reduced slope or any other difficulties in calibration are observed, the electrode will probably need to be cleaned or replaced. The cleaning is performed depending on the type of sample and as prescribed in the manufacturer's manual. Regular cleaning is recommended.

Calibration and measurement conditions. Unless otherwise prescribed in the monograph, all measurements are carried out at the same temperature as that used for calibration (± 2.5 °C), usually between 20 °C and 25 °C. Table 2.2.3.-2 shows the variation of pH with respect to temperature of a number of reference buffer solutions used for calibration. Follow the manufacturer's instructions for temperature correction.

The calibration consists of the determination of the slope (e.g. 95-105 per cent) and the offset of the measuring system. Most commercial pH meters offer a "self test" or "start-up test" where, for example, the slope and asymmetry potential are tested and compared to the manufacturer's specifications. The apparatus is calibrated using at least 2 buffer solutions chosen so that the expected pH value of the solution to be examined lies between the pH values of the buffer solutions. The range must be at least 2 pH units. The pH of another buffer solution of intermediate pH, read from the scale, must not differ by more than 0.05 pH units from the value corresponding to that solution.

Reference buffer solutions are preferably commercially available certified reference materials. Alternatively, buffer solutions can be prepared in-house according to Table 2.2.3.-2.