made lie in the range of 1750-2000 NTU. Linearity must be demonstrated by constructing a calibration curve using at least 4 concentrations.

TURBIDIMETRY

The optical property expressed as turbidity is the interaction between light and suspended particles in liquid. This is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in a straight line through the sample. The quantity of solid material in suspension can be determined by the measurement of the transmitted light. A linear relationship between turbidity and concentration is obtained when the particle sizes are uniform and homogeneous in the suspension. This is true only in very dilute suspensions containing small particles. Linearity between turbidity and concentration must be established by constructing a calibration curve using at least 4 concentrations.

RATIO TURBIDIMETRY

In ratio turbidimetry the relationship of the transmission measurement to the 90° scattered light measurement is determined. This procedure compensates for the light that is diminished by the colour of the sample. The influence of the colour of the sample may also be eliminated by using an infrared light-emitting diode (IR LED) at 860 nm as the light source of the instrument. The instrument's photodiode detectors receive and measure scattered light at a 90° angle from the sample as well as measuring the forward scatter (light reflected) in front of the sample along with the measurement of light transmitted directly through the sample. The measuring results are given in NTU(ratio) and are obtained by calculating the ratio of the 90° angle scattered light measured to the sum of the components of forward scattered and transmitted light values. In ratio turbidimetry the influence of stray light becomes negligible. Nephelometers are used for measurements of the degree of opalescence of colourless liquids.

Measurements of reference suspensions I-IV with a ratio turbidimeter show a linear relationship between the concentrations and measured NTU values. Reference suspensions I-IV (Ph. Eur.) may be used as calibrators for the instrument.

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

INSTRUMENTAL DETERMINATION OF OPALESCENCE

Requirements in monographs are expressed in terms of the visual examination method with the defined reference suspensions. Instrumental methods may also be used for determining compliance with monograph requirements once the suitability of the instrument as described below has been established and calibration with reference suspensions I-IV and with *water R* or the solvent used has been performed.

Apparatus. Ratio turbidimeters or nephelometers with selectable ratio application use as light source a tungsten lamp with spectral sensitivity at about 550 nm operating at a filament colour temperature of 2700 K, or IR LED having an emission maximum at 860 nm with a 60 nm spectral bandwidth. Other suitable light sources may also be used. Silicon photodiodes and photomultipliers are commonly used as detectors and record changes in light scattered or transmitted by the sample. The light scattered at 90 ± 2.5° is detected by the primary detector. Other detectors are those to detect back and forward scatter as well as transmitted light. The instruments used are calibrated against standards of known turbidity and are capable of automatic determination of turbidity. The test results expressed in NTU units are obtained directly from the instrument and compared to the specifications in the individual monographs.

Instruments complying with the following specifications are suitable.

- *Measuring units*: NTU. NTU is based on the turbidity of a primary reference standard of formazin. FTU (Formazin Turbidity Units) or FNU (Formazin Nephelometry Units) are also used, and are equivalent to NTU in low regions (up to 40 NTU). These units are used in all 3 instrumental methods (nephelometry, turbidimetry and ratio turbidimetry).
- *Measuring range*: 0.01-1100 NTU.
- *Resolution*: 0.01 NTU within the range of 0-10 NTU, 0.1 NTU within the range of 10-100 NTU, and 1 NTU for the range > 100 NTU. The instrument is calibrated and controlled with reference standards of formazin.
- *Accuracy*: 0-10 NTU: ± (2 per cent of reading + 0.01) NTU. 10-1000 NTU: ± 5 per cent.
- *Repeatability*: 0-10 NTU: ± 0.01 NTU. 10-1000 NTU: ± 2 per cent of the measured value.
- *Calibration*: with 4 reference suspensions of formazin in the range of interest. Reference suspensions described in this chapter or suitable reference standards calibrated against the primary reference suspensions may be used.
- *Stray light*: this is a significant source of error in low level turbidimetric measurement; stray light reaches the detector of an optical system, but does not come from the sample; < 0.15 NTU for the range 0-10 NTU, < 0.5 NTU for the range 10-1000 NTU.

Instruments complying with the above characteristics and verified using the reference suspensions described under Visual method may be used instead of visual examination for determination of compliance with monograph requirements.

Instruments with range or resolution, accuracy and repeatability capabilities other than those mentioned above may be used provided they are sufficiently validated and are capable for the intended use. The test methodology for the specific substance/product to be analysed must also be validated to demonstrate its analytical capability. The instrument and methodology should be consistent with the attributes of the product to be tested.

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2.2.2. DEGREE OF COLORATION OF LIQUIDS

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

A solution is *colourless* if it has the appearance of *water R* or the solvent or is not more intensely coloured than reference solution B_9.

METHOD I

Using identical tubes of colourless, transparent, neutral glass of 12 mm external diameter, compare 2.0 ml of the liquid to be examined with 2.0 ml of *water R* or of the solvent or of the reference solution (see Tables of reference

solutions) prescribed in the monograph. Compare the colours in diffused 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 mm to 25 mm, compare the liquid to be examined with *water R* or the solvent or the reference solution (see Tables of reference solutions) prescribed in the monograph, the depth of the layer being 40 mm. Compare the colours in diffused daylight, viewing vertically against a white background.

REAGENTS

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. Place in a 250 ml conical flask fitted with a ground-glass stopper, 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 liberated iodine with *0.1 M sodium thiosulphate*, using 0.5 ml of *starch solution R*, added towards the end of the titration, as indicator.

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

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. Place in a 250 ml conical flask fitted with a ground-glass stopper, 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 sulphuric acid R* and 2 g of *potassium iodide R*. Close the flask and dissolve the precipitate by shaking gently. Titrate the liberated iodine with *0.1 M sodium thiosulphate*, 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 thiosulphate* is equivalent to 23.79 mg of $CoCl_2,6H_2O$.

Blue primary solution. Dissolve 63 g of *copper sulphate 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. Place in a 250 ml conical flask fitted with a ground-glass stopper, 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 liberated iodine with *0.1 M sodium thiosulphate*, 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 thiosulphate* 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	Volume 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.

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2.2.3. POTENTIOMETRIC DETERMINATION OF pH

The pH is a number which represents conventionally the hydrogen ion concentration of an aqueous 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:

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

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 (for example, a saturated calomel electrode).

Apparatus. The measuring apparatus is 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.

Method. Unless otherwise prescribed in the monograph, all measurements are made at the same temperature (20-25 °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. For the temperature correction, when necessary, follow the manufacturer's instructions. The apparatus is calibrated with the buffer solution of potassium hydrogen phthalate (primary standard) and 1 other buffer solution of different pH (preferably one shown in Table 2.2.3.-2). The pH of a third buffer solution of intermediate pH read off on the scale must not differ by more than 0.05 pH unit from the value corresponding to this solution. Immerse the electrodes in the solution to be examined and take the reading in the same conditions as for the buffer solutions.

When the apparatus is in frequent use, checks must be carried out regularly. If not, such checks should be carried out before each measurement.

All solutions to be examined and the reference buffer solutions must be prepared using *carbon dioxide-free water R*.

PREPARATION OF REFERENCE BUFFER SOLUTIONS

Potassium tetraoxalate 0.05 M. Dissolve 12.61 g of $C_4H_3KO_8,2H_2O$ in *carbon dioxide-free water R* and dilute to 1000.0 ml with the same solvent.

Potassium hydrogen tartrate, saturated at 25 °C. Shake an excess of $C_4H_5KO_6$ vigorously with *carbon dioxide-free water R* at 25 °C. Filter or decant. Prepare immediately before use.

Potassium dihydrogen citrate 0.05 M. Dissolve 11.41 g of $C_6H_7KO_7$ in *carbon dioxide-free water R* and dilute to 1000.0 ml with the same solvent. Prepare immediately before use.

Potassium hydrogen phthalate 0.05 M. Dissolve 10.13 g of $C_8H_5KO_4$, previously dried for 1 h at 110 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 ml with the same solvent.

Potassium dihydrogen phosphate 0.025 M + disodium hydrogen phosphate 0.025 M. Dissolve 3.39 g of KH_2PO_4 and 3.53 g of Na_2HPO_4, both previously dried for 2 h at 120 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 ml with the same solvent.

Potassium dihydrogen phosphate 0.0087 M + disodium hydrogen phosphate 0.0303 M. Dissolve 1.18 g of KH_2PO_4 and 4.30 g of Na_2HPO_4, both previously dried for 2 h at 120 ± 2 °C, in *carbon dioxide-free water R* and dilute to 1000.0 ml with the same solvent.

Disodium tetraborate 0.01 M. Dissolve 3.80 g of $Na_2B_4O_7,10H_2O$ in *carbon dioxide-free water R* and dilute to 1000.0 ml with the same solvent. Store protected from atmospheric carbon dioxide.