2.2. PHYSICAL AND PHYSICO-CHEMICAL METHODS

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2.2.1. CLARITY AND DEGREE OF OPALESCENCE OF LIQUIDS

Opalescence is the effect of light being absorbed or scattered by submicroscopic particles or optical density inhomogeneities. The absence of any particles or inhomogeneities in a solution results in a clear solution.

A liquid is considered *clear* if its clarity is the same as that of *water R* or of the solvent used, or if its opalescence is not more pronounced than that of reference suspension I (see Table 2.2.1.-1), when examined under the conditions described below.

Requirements in monographs are expressed in terms of the visual method by comparing with the defined reference suspensions (see Table 2.2.1.-1). However, instrumental methods may also be used for determining compliance with monograph requirements once the suitability of the instrument has been established as described below and calibration with reference suspensions I-IV and with *water R* or the solvent used has been performed.

VISUAL METHOD

Using identical test-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 a reference suspension freshly prepared as described below. Ensure that the depths of the layers in the 2 test-tubes are the same (about 40 mm).

Compare the liquids in diffused daylight 5 min after preparation of the reference suspension, viewing vertically against a black background.

System suitability. The diffusion of light must be such that reference suspension I can readily be distinguished from *water R*, and that reference suspension II can readily be distinguished from reference suspension I (see Table 2.2.1.-1).

INSTRUMENTAL METHOD

The instrumental assessment of clarity and opalescence provides a more discriminatory test that does not depend on the visual acuity of the analyst. Numerical results are more useful for process control and quality monitoring, especially in stability studies. For example, previous numerical data on stability can be extrapolated to determine whether a given batch of a preparation will exceed shelf-life limits prior to the expiry date.

TURBIDIMETRY AND NEPHELOMETRY

When a suspension is viewed at right angles to the direction of the incident light, the system appears opalescent due to the scattering of light by the particles of the suspension (Tyndall effect). A certain portion of the light beam entering a turbid liquid is transmitted, another portion is absorbed and the remaining portion is scattered by the suspended particles. The light-scattering effect of suspended particles can be measured either indirectly by observation of the transmitted light (turbidimetry) or directly by measuring the scattered light (nephelometry). Turbidimetry and nephelometry are more reliable in low turbidity ranges, where there is a linear relationship between turbidity values and detector signals.

As the degree of turbidity increases, not all the particles are exposed to the incident light and the scattered or the transmitted radiation of other particles is hindered on its way to the detector.

For quantitative measurements, the construction of calibration curves is essential. Linearity must be based on at least 4 levels of concentrations. Reference suspensions must show a sufficiently stable degree of turbidity and must be produced under well-defined conditions.

MEASUREMENTS IN RATIO MODE

The determination of opalescence of coloured liquids is done using instruments with ratio mode, since colour provides a negative interference, attenuating both incident and scattered light and lowering the turbidity value. The effect is so great, even for moderately coloured samples, that conventional nephelometers cannot be used.

In turbidimetry or nephelometry with ratio mode, the ratio 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. Instruments with ratio mode use as light source a tungsten lamp with spectral sensitivity at about 550 nm operating at a filament colour temperature of 2700 K. 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 measured by the primary detector. Other detectors measure back and forward scatter (reflected light) as well as transmitted light. The results are obtained by calculating the ratio of the 90° scattered light measured to the sum of the components of forward scattered and transmitted light values.

The instruments used are calibrated against standards of known turbidity and are capable of automatic measurement of turbidity. The test results are obtained directly from the instrument and compared to the specifications in the individual monograph.

Alternatively, the influence of the colour of the sample may also be eliminated by using an infrared light-emitting diode (IR LED) having an emission maximum at 860 nm with a 60 nm spectral bandwidth as the light source of the instrument.

INSTRUMENT REQUIREMENTS

Instruments complying with the following characteristics and verified using reference suspensions as described below may be used instead of visual examination for determination of compliance with monograph requirements.

- *Measuring unit*: NTU (nephelometric turbidity units). NTU is based on the turbidity of a primary standard of formazin. FTU (formazin turbidity units) or FNU (formazin nephelometric units) are also used, and are equivalent to NTU in regions of low turbidity (up to 40 NTU). These units are used in all 3 instrumental methods (nephelometry, turbidimetry and in ratio mode).
- *Measuring range*: 0.01-1100 NTU.
- *Resolution*: 0.01 NTU within the range 0-9.99 NTU; 0.1 NTU within the range 10.0-99.9 NTU; and 1 NTU for the range > 100 NTU.
- *Accuracy*: ± (10 per cent of reading + 0.01 NTU) within the range 0-20 NTU; ± 7.5 per cent within the range 20-1100 NTU.
- *Repeatability*: ± 0.05 NTU within the range 0-20 NTU; ± 2 per cent of the reading within the range 20-1100 NTU.

Instruments with measuring 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.

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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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*.