



PHARMA DEVILS

QUALITY CONTROL DEPARTMENT

GENERAL TESTING PROCEDURE

Title: Clarity of Solution

SOP No.:		Department :	QC
Effective Date :		Review Date :	
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Supersede SOP No.:	Nil		

1.0 OBJECTIVE:

1.1 To lay down a procedure for Clarity of Solution.

2.0 SCOPE:

2.1 It is applicable for the estimation of Raw material.

3.0 RESPONSIBILITY:

3.1 Analyst / Officer / Executive follow the procedure.

3.2 Head-QC are responsible for effective implementation of this SOP.

4.0 REFERENCE:

4.1 Ph. Eur. method

5.0 DEFINITION:NA

6.0 PROCEDURE:

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

A liquid is considered clear if its clarity is the same as that of water 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 or the solvent used has been performed,

6.1 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



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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, and that reference suspension II can readily be distinguished from reference suspension I (see Table 2.2.1.-1).

6.2 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.
- **Measurement 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



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be used. In turbidimetry or nephelometry with ratio mode, the ratio of the transmission measurement to the 900 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 $90 \pm 2.5^\circ$ 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 900 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: \pm (10 per cent of reading + 0.01 NTU) within the range 0-20 NTU; \pm 7.5 per cent within the range 20-1100 NTU.



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- 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



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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 $25 \pm 3^{\circ}\text{C}$.

- **Hydrazine sulfate solution:** Dissolve 1.0 g of hydrazine sulfate in water 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 in 25.0 mL of water. 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. 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.

	I	II	III	IV
Standard of Opalescence	5 ml	10 ml	30 ml	50 ml
Water	95 ml	90 ml	70 ml	50 ml

Table-2.2.1-1

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).



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

Table 2.2.1-2

7.0 Annexures: NA

8.0 Distribution:

8.1 Display copy 1: Quality Control Lab

9.0 Abbreviation:

GTP : General Test Procedure

QC : Quality Control laboratories

10.0 Revision History:

10.1 Revision history table:

Document Number	CC Number/Date	Brief Description of Change