2.2. PHYSICAL AND PHYSICOCHEMICAL METHODS

2.2.1. CLARITY AND DEGREE OF OPALESCENCE OF LIQUIDS

Using identical test 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 a reference suspension freshly prepared as described below, the depth of the layer being 40 mm. Compare the solutions in diffused daylight 5 min after preparation of the reference suspension, viewing vertically against a black background. 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.

A liquid is considered *clear* if its clarity is the same as that of *water R* or of the solvent used when examined under the conditions described above, or if its opalescence is not more pronounced than that of reference suspension I.

REAGENTS

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

Hexamethylenetetramine solution. Dissolve 2.5 g of *hexamethylenetetramine R* in 25.0 ml of *water R* in a 100 ml glass-stoppered flask.

Primary opalescent suspension. To the solution of hexamethylenetetramine in the flask add 25.0 ml of hydrazine sulphate 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 well mixed 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 at most 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

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.

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).

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

Temperature °C	*k*
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: one