acid R, and dissolve it in 15 ml of *chloroform R* unless otherwise prescribed. Add very slowly 25.0 ml of *iodine bromide solution R*. Close the flask and keep it in the dark for 30 min unless otherwise prescribed, shaking frequently. Add 10 ml of a 100 g/l solution of *potassium iodide R* and 100 ml of *water R*. Titrate with *0.1 M sodium thiosulphate*, shaking vigorously until the yellow colour is almost discharged. Add 5 ml of *starch solution R* and continue the titration adding the *0.1 M sodium thiosulphate* dropwise until the colour is discharged (n_1 ml of *0.1 M sodium thiosulphate*). Carry out a blank test under the same conditions (n_2 ml of *0.1 M sodium thiosulphate*).

$$I_{\mathrm{I}} = \frac{1.269\ (n_2 - n_1)}{m}$$

METHOD B

Unless otherwise prescribed, use the following quantities (Table 2.5.4.-2) for the determination.

Table 2.5.4.-2

Presumed value I_{I}	Mass (g) (corresponding to an excess of 150 per cent ICl)	Mass (g) (corresponding to an excess of 100 per cent ICl)	Iodine chloride solution (ml)
<3	10	10	25
3	8.4613	10.5760	25
5	5.0770	6.3460	25
10	2.5384	3.1730	20
20	0.8461	1.5865	20
40	0.6346	0.7935	20
60	0.4321	0.5288	20
80	0.3173	0.3966	20
100	0.2538	0.3173	20
120	0.2115	0.2644	20
140	0.1813	0.2266	20
160	0.1587	0.1983	20
180	0.1410	0.1762	20
200	0.1269	0.1586	20

The mass of the sample is such that there will be an excess of *iodine chloride solution R* of 50 per cent to 60 per cent of the amount added, i.e. 100 per cent to 150 per cent of the amount absorbed.

Introduce the prescribed quantity of the substance to be examined (*m* g) into a 250 ml flask fitted with a ground-glass stopper and previously rinsed with *glacial acetic acid R* or dried, and dissolve it in 15 ml of a mixture of equal volumes of *cyclohexane R* and *glacial acetic acid R*, unless otherwise prescribed. If necessary, melt the substance before dissolution (melting point greater than 50 °C). Add very slowly the volume of *iodine chloride solution R* stated in Table 2.5.4.-2. Close the flask and keep it in the dark for 30 min, unless otherwise prescribed, shaking frequently. Add 10 ml of a 100 g/l solution of *potassium iodide R* and 100 ml of *water R*. Titrate with *0.1 M sodium thiosulphate*, shaking vigorously until the yellow colour is almost discharged. Add 5 ml of *starch solution R* and continue the titration adding the *0.1 M sodium thiosulphate* dropwise until the colour is discharged (n_1 ml of *0.1 M sodium thiosulphate*). Carry out a blank test under the same conditions (n_2 ml of *0.1 M sodium thiosulphate*).

$$I_{\mathrm{I}} = \frac{1.269\ (n_2 - n_1)}{m}$$

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2.5.5. PEROXIDE VALUE

The peroxide value I_{P} is the number that expresses in milliequivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance, as determined by the methods described below.

When the monograph does not specify the method to be used, method A is applied. Any change from method A to method B is validated.

METHOD A

Place 5.00 g of the substance to be examined (*m* g) in a 250 ml conical flask fitted with a ground-glass stopper. Add 30 ml of a mixture of 2 volumes of *chloroform R* and 3 volumes of *glacial acetic acid R*. Shake to dissolve the substance and add 0.5 ml of *saturated potassium iodide solution R*. Shake for exactly 1 min then add 30 ml of *water R*. Titrate with *0.01 M sodium thiosulphate*, adding the titrant slowly with continuous vigorous shaking, until the yellow colour is almost discharged. Add 5 ml of *starch solution R* and continue the titration, shaking vigorously, until the colour is discharged (n_1 ml of *0.01 M sodium thiosulphate*). Carry out a blank test under the same conditions (n_2 ml of *0.01 M sodium thiosulphate*). The volume of *0.01 M sodium thiosulphate* used in the blank titration must not exceed 0.1 ml.

$$I_{\mathrm{p}} = \frac{10\,(n_1 - n_2)}{m}$$

METHOD B

Carry out the operations avoiding exposure to actinic light.

Place 50 ml of a mixture of 2 volumes of *trimethylpentane R* and 3 volumes of *glacial acetic acid R* in a conical flask and replace the stopper. Swirl the flask until the substance to be examined (*m* g; see Table 2.5.5.-1) has dissolved. Using a suitable volumetric pipette, add 0.5 ml of *saturated potassium iodide solution R* and replace the stopper. Allow the solution to stand for 60 ± 1 s, thoroughly shaking the solution continuously, then add 30 ml of *water R*.

Table 2.5.5.-1

Expected peroxide value I_{p}	Mass of substance to be examined (g)
0 to 12	2.00 to 5.00
12 to 20	1.20 to 2.00
20 to 30	0.80 to 1.20
30 to 50	0.500 to 0.800
50 to 90	0.300 to 0.500

Titrate the solution with *0.01 M sodium thiosulphate* (V_1 ml), adding it gradually and with constant, vigorous shaking, until the yellow iodine colour has almost disappeared. Add about 0.5 ml of *starch solution R1* and continue the titration, with constant shaking especially near the end-point, to liberate all of the iodine from the solvent layer. Add the sodium thiosulphate solution dropwise until the blue colour just disappears.

Depending on the volume of *0.01 M sodium thiosulphate* used, it may be necessary to titrate with *0.1 M sodium thiosulphate*.

NOTE: there is a 15 s to 30 s delay in neutralising the starch indicator for peroxide values of 70 and greater, due to the tendency of trimethylpentane to float on the surface of the aqueous medium and the time necessary to adequately mix the solvent and the aqueous titrant, thus liberating the last traces of iodine. It is recommended to use *0.1 M sodium thiosulphate* for peroxide values greater than 150. A small amount (0.5 per cent to 1.0 per cent (*m/m*)) of high HLB emulsifier (for example polysorbate 60) may be added to the mixture to retard the phase separation and decrease the time lag in the liberation of iodine.

Carry out a blank determination (V_0 ml). If the result of the blank determination exceeds 0.1 ml of titration reagent, replace the impure reagents and repeat the determination.

$$I_p = \frac{1000\,(V_1 - V_0)\,c}{m}$$

c = concentration of the sodium thiosulphate solution in moles, per litre.

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2.5.6. SAPONIFICATION VALUE

The saponification value I_S is the number that expresses in milligrams the quantity of potassium hydroxide required to neutralise the free acids and to saponify the esters present in 1 g of the substance.

Unless otherwise prescribed, use the quantities indicated in Table 2.5.6.-1 for the determination.

Table 2.5.6.-1

Presumed value I_S	Quantity of sample (g)
<3	20
3 to 10	12 to 15
10 to 40	8 to 12
40 to 60	5 to 8
60 to 100	3 to 5
100 to 200	2.5 to 3
200 to 300	1 to 2
300 to 400	0.5 to 1

Introduce the prescribed quantity of the substance to be examined (*m* g) into a 250 ml borosilicate glass flask fitted with a reflux condenser. Add 25.0 ml of *0.5 M alcoholic potassium hydroxide* and a few glass beads. Attach the condenser and heat under reflux for 30 min, unless otherwise prescribed. Add 1 ml of *phenolphthalein solution R1* and titrate immediately (while still hot) with *0.5 M hydrochloric acid* (n_1 ml of *0.5 M hydrochloric acid*). Carry out a blank test under the same conditions (n_2 ml of *0.5 M hydrochloric acid*).

$$I_S = \frac{28.05\,(n_2 - n_1)}{m}$$

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2.5.7. UNSAPONIFIABLE MATTER

The term “unsaponifiable matter” is applied to the substances non-volatile at 100-105 °C obtained by extraction with an organic solvent from the substance to be examined after it has been saponified. The result is calculated as per cent *m/m*.

Use ungreased ground-glass glassware.

Introduce the prescribed quantity of the substance to be examined (*m* g) into a 250 ml flask fitted with a reflux condenser. Add 50 ml of *2 M alcoholic potassium hydroxide R* and heat on a water-bath for 1 h, swirling frequently. Cool to a temperature below 25 °C and transfer the contents of the flask to a separating funnel with the aid of 100 ml of *water R*. Shake the liquid carefully with 3 quantities, each of 100 ml, of *peroxide-free ether R*. Combine the ether layers in another separating funnel containing 40 ml of *water R*, shake gently for a few minutes, allow to separate and reject the aqueous phase. Wash the ether phase with 2 quantities, each of 40 ml, of *water R* then wash successively with 40 ml of a 30 g/l solution of *potassium hydroxide R* and 40 ml of *water R*; repeat this procedure 3 times. Wash the ether phase several times, each with 40 ml of *water R*, until the aqueous phase is no longer alkaline to phenolphthalein. Transfer the ether phase to a tared flask, washing the separating funnel with *peroxide-free ether R*.

Distil off the ether with suitable precautions and add 6 ml of *acetone R* to the residue. Carefully remove the solvent in a current of air. Dry to constant mass at 100-105 °C. Allow to cool in a desiccator and weigh (*a* g).

$$\text{Unsaponifiable matter} = \frac{100a}{m} \text{ per cent}$$

Dissolve the residue in 20 ml of *alcohol R*, previously neutralised to *phenolphthalein solution R* and titrate with *0.1 M ethanolic sodium hydroxide*. If the volume of *0.1 M ethanolic sodium hydroxide* used is greater than 0.2 ml, the separation of the layers has been incomplete; the residue weighed cannot be considered as “unsaponifiable matter”. In case of doubt, the test must be repeated.

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2.5.8. DETERMINATION OF PRIMARY AROMATIC AMINO-NITROGEN

Dissolve the prescribed quantity of the substance to be examined in 50 ml of *dilute hydrochloric acid R* or in another prescribed solvent and add 3 g of *potassium bromide R*. Cool in ice-water and titrate by slowly adding *0.1 M sodium nitrite* with constant stirring.

Determine the end-point electrometrically or by the use of the prescribed indicator.

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2.5.9. DETERMINATION OF NITROGEN BY SULPHURIC ACID DIGESTION

SEMI-MICRO METHOD

Place a quantity of the substance to be examined (*m* g) containing about 2 mg of nitrogen in a combustion flask, add 4 g of a powdered mixture of 100 g of *dipotassium sulphate R*, 5 g of *copper sulphate R* and 2.5 g of *selenium R*,