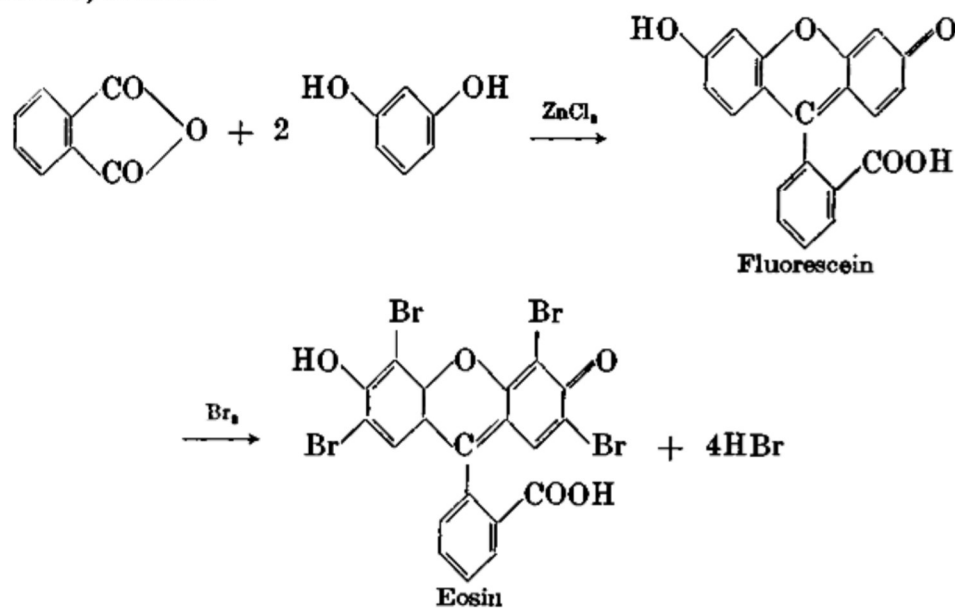


To a mixture of pure phenol and 25 g. of phthalic anhydride contained in a 250 ml. round-bottomed flask, add 20 g. (11 ml.) of concentrated sulphuric acid. Heat the flask in an oil bath at 115–120° for 9 hours. Then pour the reaction mixture whilst still hot into 1 litre of hot water contained in a 2 litre beaker, and boil until the odour of phenol has disappeared; add water to replace that lost by evaporation. When cold, filter the yellow, granular precipitate at the pump and wash it with water. Dissolve the solid in dilute sodium hydroxide solution, filter from the undissolved residue (the by-products of the reaction). Acidify the filtrate with dilute acetic acid and a few drops of dilute hydrochloric acid, and allow to stand overnight. The crude phenolphthalein separates as a pale yellow, sandy powder; filter and dry. Purify the crude product by dissolving it in six times its weight of absolute alcohol, add decolourising carbon and reflux on a water bath for 1 hour. Filter the hot solution through a preheated Buchner funnel, wash the residue with 2 parts by weight of boiling absolute alcohol and concentrate the combined filtrate and washings to two-thirds of its bulk on a water bath. Dilute the cooled solution with eight times the weight of cold water (it will become turbid), stir the mixture well and, after standing for a few seconds, filter through a wet filter to remove the resinous oil which separates. Heat the filtrate on a water bath to evaporate most of the alcohol; the turbidity disappears and the phenolphthalein separates out in the form of a white powder. Filter this off and dry. The yield of pure phenolphthalein, m.p. 256–258°, is 18 g.

### VIII.7. FLUORESCEIN AND EOSIN

Fluorescein is obtained by condensing phthalic anhydride (1 mol) with resorcinol (2 mols) in the presence of anhydrous zinc chloride. The tetrabromo derivative, readily prepared by the addition of the calculated quantity of bromine, is eosin.



Dibromofluorescein is prepared by treating fluorescein in 80 per cent. acetic acid solution with the theoretical quantity of bromine.

**Fluorescein.** Grind together in a mortar 15 g. of phthalic anhydride and 22 g. of resorcinol, and transfer the mixture to a 350 or 500 ml. conical flask. Support the flask in an oil bath and heat to 180° (internal temperature). While the oil bath is being heated, weigh out rapidly 7 g. of anhydrous zinc chloride, immediately grind it to a coarse powder in a mortar and place it in a stoppered tube. (The zinc chloride should not be exposed to the air longer than is absolutely necessary; if the contents of the stock bottle appear moist, dry a 10–15 g. portion by fusing it in a porcelain dish.) Add the zinc chloride in small portions, with stirring by means of a thermometer, to the mixture in the flask. Continue the heating at 180° with stirring at intervals of 2–3 minutes until the solution becomes so viscous that further stirring is not practicable (45–90 minutes). The resulting dark red mass consists largely of a mixture of fluorescein and zinc chloride together with basic zinc salts. Allow the oil bath to cool to about 90°, and add 200 ml. of water and 10 ml. of concentrated hydrochloric acid to the reaction mixture, and then raise the temperature of the oil bath until the water boils. Stir the mixture from time to time when the temperature of the oil rises above 110°: great care should be taken to prevent the dilute acid from boiling over. Continue the boiling until the reaction mixture has disintegrated and all the zinc salts have dissolved. Filter the insoluble residue of fluorescein at the pump, grind it with water in a mortar, and filter again. Dry at 100°. The yield is 30 g. This product is pure enough for the preparation of eosin.

The fluorescein may be purified by dissolving it in dilute sodium hydroxide solution, filtering if necessary, precipitating with dilute hydrochloric acid (1:1), filtering, washing and drying.

**Eosin (Tetrabromofluorescein).** Place 16.5 g. of powdered fluorescein and 80 ml. of rectified (or methylated) spirit in a 250 ml. flask. Support a small dropping funnel, containing 36 g. (12 ml.) of bromine, above the flask: make sure that the stopcock of the funnel is well lubricated before charging the latter with bromine. Add the bromine dropwise during about 20 minutes. When half the bromine has been introduced, and the fluorescein has been converted into dibromofluorescein, all the solid material disappears temporarily since the dibromo derivative is soluble in alcohol: with further addition of bromine the tetrabromofluorescein (sparingly soluble in alcohol) separates out. Allow the reaction mixture to stand for 2 hours, filter off the eosin at the pump, wash it with alcohol, and dry at 100°. The yield of eosin (orange-coloured powder) is 25 g.

**Sodium salt of eosin.** Grind together in a mortar 12 g. of eosin with 2 g. of anhydrous sodium carbonate. Transfer the mixture to a 250 ml. conical flask, moisten it with 10 ml. of rectified spirit, add 10 ml. of water and warm on a water bath, with stirring, until the evolution of carbon dioxide ceases. Add 50 ml. of ethyl alcohol, heat to boiling, and filter the hot solution through a fluted filter paper (supported in a short-stemmed funnel) into a beaker, and allow to stand overnight. Filter off the brownish-red crystals of sodium eosin, wash with a little alcohol, and dry. The yield is 10 g.

**Dibromofluorescein.** To a suspension of 33 g. of fluorescein in 125 ml. of 80 per cent. acetic acid, warmed to 80° and stirred mechanically,