cent V/V) R. Add *0.1 M sodium hydroxide* until a violet-blue colour is obtained, without exceeding the end-point. Place the solution in the test-tube (*D*). Without interrupting the stream of carbon dioxide, remove the funnel (*B*) and introduce through the opening into the flask (*A*) 25.0 g of the substance to be examined (*m* g) with the aid of 100 ml of *water R*. Add through the funnel 80 ml of *dilute hydrochloric acid R* and boil for 1 h. Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little *water R* to a 200 ml wide-necked, conical flask. Heat on a water-bath for 15 min and allow to cool. Add 0.1 ml of a 1 g/l solution of *bromophenol blue R* in *alcohol (20 per cent V/V) R* and titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to violet-blue (V_1 ml). Carry out a blank titration (V_2 ml).

Calculate the content of sulphur dioxide in parts per million from the expression:

$$32\,030 \times (V_1 - V_2) \times \frac{n}{m}$$

n = molarity of the sodium hydroxide solution used as titrant.

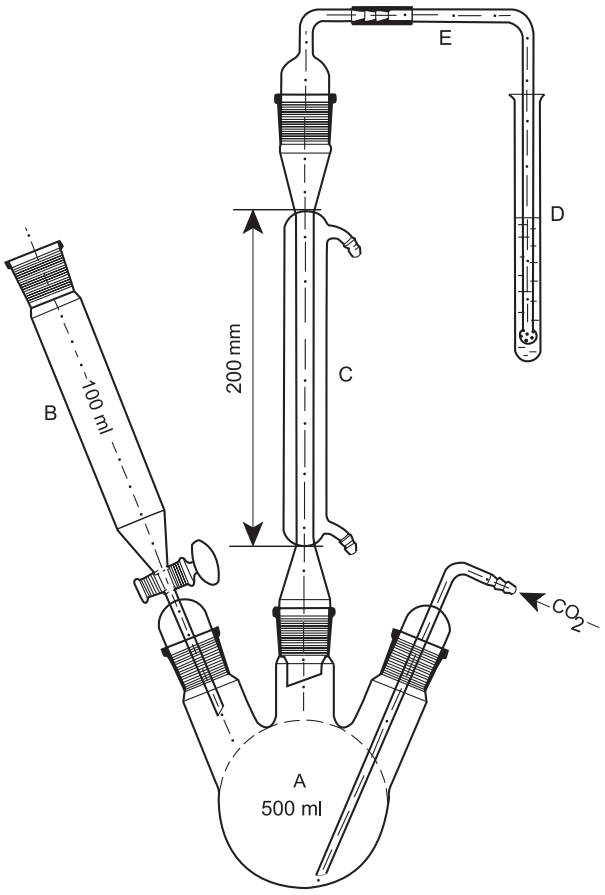

Figure 2.5.29.-1.– *Apparatus for the determination of sulphur dioxide*

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2.5.30. OXIDISING SUBSTANCES

Transfer 4.0 g to a glass-stoppered, 125 ml conical flask and add 50.0 ml of *water R*. Insert the stopper and swirl for 5 min. Transfer to a glass-stoppered 50 ml centrifuge tube and centrifuge. Transfer 30.0 ml of the clear supernatant liquid to a glass-stoppered 125 ml conical flask. Add 1 ml of *glacial acetic acid R* and 0.5 g to 1.0 g of *potassium iodide R*. Insert the stopper, swirl, and allow to stand for 25 min to 30 min in the dark. Add 1 ml of *starch solution R* and titrate with *0.002 M sodium thiosulphate* until the starch-iodine colour disappears. Carry out a blank determination. Not more than 1.4 ml of *0.002 M sodium thiosulphate* is required (0.002 per cent, calculated as H_2O_2).

1 ml of *0.002 M sodium thiosulphate* is equivalent to 34 µg of oxidising substances, calculated as hydrogen peroxide.

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2.5.31. RIBOSE IN POLYSACCHARIDE VACCINES

Test solution. Use a volumetric flask with a suitable volume for preparation of a solution containing about 5 mg per millilitre of dry polysaccharide. Transfer the contents of a container quantitatively to the flask and dilute to volume with *water R*. Dilute the solution so that the volumes used in the test contain 2.5 µg to 25 µg of ribose. Introduce 0.20 ml and 0.40 ml of the diluted solution into tubes in triplicate.

Reference solutions. Dissolve 25 mg of *ribose R* in *water R* and dilute to 100.0 ml with the same solvent (stock solution containing 0.25 g/l of ribose). Immediately before use, dilute 1 ml of the stock solution to 10.0 ml with *water R* (working dilution: 25 mg/l of ribose). Introduce 0.10 ml, 0.20 ml, 0.40 ml, 0.60 ml, 0.80 ml and 1.0 ml of the working dilution into 6 tubes.

Prepare a blank using 2 ml of *water R*.

Make up the volume in each tube to 2 ml with *water R*. Shake. Add 2 ml of a 0.5 g/l solution of *ferric chloride R* in *hydrochloric acid R* to each tube. Shake. Add 0.2 ml of a 100 g/l solution of *orcinol R* in *ethanol R*. Place the tubes in a water-bath for 20 min. Cool in iced water. Measure the absorbance (*2.2.25*) of each solution at 670 nm using the blank as the compensation liquid. Draw a calibration curve from the absorbance readings for the 6 reference solutions and the corresponding content of ribose and read from the curve the quantity of ribose in the test solution for each volume tested. Calculate the mean of the 3 values.

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2.5.32. WATER: MICRO DETERMINATION

PRINCIPLE

The coulometric titration of water is based upon the quantitative reaction of water with sulphur dioxide and iodine in an anhydrous medium in the presence of a base with sufficient buffering capacity. In contrast to the volumetric method described under (*2.5.12*), iodine is produced electrochemically in the reaction cell by oxidation of iodide. The iodine produced at the anode reacts immediately with the water and the sulphur dioxide contained in the reaction cell. The amount of water in the substance is directly proportional to the quantity of electricity up until the titration end-point. When all of the water in the cell has been consumed, the end-point is reached and thus an excess of iodine appears. 1 mole of iodine corresponds to 1 mole of water, a quantity of electricity of 10.71 C corresponds to 1 mg of water.

Moisture is eliminated from the system by pre-electrolysis. Individual determinations can be carried out successively in the same reagent solution, under the following conditions: