ASSAY METHODS

Alginates Assay

(Carbon Dioxide Determination by Decarboxylation)

Apparatus

The apparatus required is shown in Figure 1 below. It consists essentially of a soda lime column, A, a mercury valve, B, connected through a side arm, C, to a reaction flask, D, by means of a rubber connection. Flask D is a 100-ml round-bottom, long-neck boiling flask, resting in a suitable heating mantle, E. The reaction flask is provided with a reflux condenser, F, to which is fitted a delivery tube, G, of 40-ml capacity, having a stopcock, H. On the reflux condenser is mounted a trap, I, containing 25 g of 20-mesh zinc or tin. The trap I should be connected with an absorption tower, J. The absorption tower consists of a 45-cm tube fitted with a medium-porosity fritted glass disk sealed to the inner part above the side arm and having a delivery tube sealed to it extending down to the end of the tube. A trap, consisting of a bulb of approximately 100-ml capacity, is blown above the fritted disk and the outer portion of a ground spherical joint is sealed on above the bulb. A 250-ml conical flask K, is connected to the bottom of the absorption tower. The top of the tower is connected to a soda lime tower, L, which is connected to a suitable pump to provide vacuum and air supply, the choice of which is made by a 3-way stopcock, M. The volume of air or vacuum is controlled by a capillary-tube regulator or needle valve, N. All joints are size 35/25, ground spherical type.

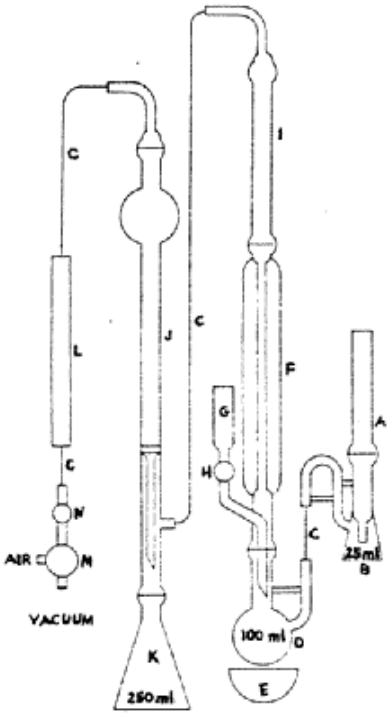

Figure 1. Apparatus for Carbon Dioxide determination by Decarboxylation

Procedure

Weigh to the nearest 0.1 mg, 250 mg of the sample, previously dried in vacuum for 4 h at 60°. Transfer into the reaction flask, D, add 25 ml of 0.1 N hydrochloric acid, insert several boiling chips, and connect the flask to the reflux condenser, F, using syrupy phosphoric acid as a lubricant.

Note: *Stopcock grease may be used for the other connections.*

Check the system for air leaks by forcing mercury up into the inner tube of the mercury valve, B, to a height of about 5 cm. Turn off the pressure using the stopcock, M. If the mercury level does not fall appreciably after 1 to 2 min, the apparatus may be considered to be free from leaks. Draw carbon dioxide-free air through the apparatus at a rate of 3,000 to 6,000 ml per h. Raise the heating mantle, E, to the flask, heat the sample to boiling, and boil gently for 2 min. Turn off and lower the mantle, and allow the sample to cool for 15 min. Charge the delivery tube, G, with 23 ml of concentrated hydrochloric acid. Disconnect the absorption tower, L, rapidly transfer 25.0 ml of 0.25 N sodium hydroxide into the tower, add 5 drops of n-butanol, and again connect the absorption tower. Draw carbon dioxide-free air through the apparatus at the rate of about 2,000 ml per h, add the hydrochloric acid to the reaction flask through the delivery tube, raise the heating mantle, and heat the reaction mixture to boiling.

After 2 h, discontinue the current of air and heating. Force the sodium hydroxide solution down into the flask, K, using gentle air pressure, and then rinse down the absorption tower with three 15-ml portions of water, forcing each washing into the flask with air pressure. Remove the flask, and add to it 10 ml of a 10% solution of barium chloride ($BaCl_2 \cdot 2H_2O$). Stopper the flask, shake gently for about 2 min, add phenolphthalein TS, and titrate with 0.1 N hydrochloric acid. Perform a blank determination.

Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2).

Cellulose Derivatives Assay

(Ethoxyl and Methoxyl Group Determination)

Apparatus

The apparatus used for the ethoxyl and methoxyl determination is shown in Figure 2. The boiling flask A, is fitted with a capillary side-arm, B, for the introduction of carbon dioxide and is connected to a column, C, which serves to separate aqueous hydriodic acid from the more volatile ethyl or methyl iodide. The volatile iodide passes through an aqueous red phosphorus suspension in a scrubber trap, D, and is finally absorbed in the bromine acetic acid solution in an absorption tube, F. The carbon dioxide is introduced from a device arranged to minimize pressure fluctuations and connected to the apparatus by a small capillary containing a small cotton plug.

Procedure

Prepare the apparatus by placing in trap D, through the funnel K or tube F and the connecting side-arm, a volume sufficient to make trap D half-full of a suspension of about 60 mg of red phosphorus in 100 ml of water. Rinse the tube F and the side-arm with water into trap D. Dry carefully the absorption tube F and pour down the funnel K 7 ml of bromine acetic acid TS. Weigh 0.05 g of the sample, to the nearest 0.1 mg, in a tared gelatin capsule, and place it in the boiling flask along with a few glass beads or pieces of porous plate. Add 6 ml of

hydriodic acid TS and attach the flask to the condenser, using a few drops of the acid to seal the junction. Bubble carbon dioxide through the apparatus at the rate of about 2 bubbles per sec. Place the boiling flask in an oil bath heated to 150°, and continue the reaction for 40 min. Drain the contents of the absorption tube F into a 500 ml conical flask containing 10 ml of a 1 in 4 solution of sodium acetate. Rinse tube F with water, adding the rinsings to the flask, and finally dilute with water to about 125 ml. Add formic acid, dropwise, with swirling, until the reddish-brown colour of the bromine is discharged, then add 3 additional drops. A total of 12 to 15 drops are usually required. Let stand for 3 min, and add 15 ml of dilute sulfuric acid TS and 3 g of potassium iodide, and titrate immediately with 0.1 N sodium thiosulfate, using starch TS as indicator near the endpoint. Perform a blank determination, including also a gelatin capsule and make any necessary correction.

Each ml of 0.1 N sodium thiosulfate is equivalent to 0.517 mg of (-OCH_3) or 0.751 mg of (-OC_2H_5).

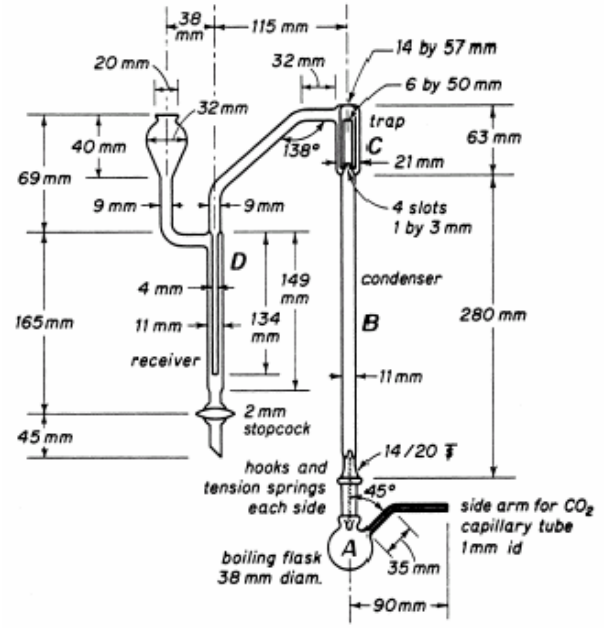

Figure 2. Apparatus for determination of ethoxyl and methoxyl groups

Polysorbates Assay

(Oxyethylene Group Determination)

Caution: *Use a safety shield and conduct the distillation in a hood.*

Principle

The oxyethylene groups are converted to ethylene and ethyl iodide which can be determined by titration. By utilizing a conversion factor determined on a reference sample, it is possible to compute the polyoxyethylene ester content.

Apparatus

An arrangement of apparatus for the analysis is shown in Figure 3. It consists in part of the reaction flasks (A), condenser, trap (B), and first absorption tube (C) of a Clark alkoxyl apparatus. These are followed by an absorption tube (D) made from a section of a spiral from a Widmer distillation column and a standard-taper (24/40) gas inlet adapter. Dimensions of

the apparatus not readily determined from the diagram are as follows: carbon dioxide inlet, capillary, 1-mm inside diameter; flask A, 28-mm diameter, 12/18 standard-taper joint; condenser, 9-mm inside diameter; inlet to trap B, 2-mm inside diameter tube; inlet to trap C, 7/15 standard-taper joint, 2-mm inside diameter tube; trap C, 14-mm inside diameter; trap D, inner tube, 8-mm outside diameter, 2-mm opening at bottom of spiral; spiral, 1.75-mm rod, 23 turns, 8.5 rise per turn; trap D, outer tube, approximately 12.5-mm inside diameter, with side-arm 7 cm from top of spiral; side-arm, 3.5-mm inside diameter, 2 mm opening at bottom. The stopcock is lubricated with silicone grease. The absorption tubes may be conveniently suspended by a series of properly spaced sheet-metal clips attached to a stick clamped at an angle of about 60°.

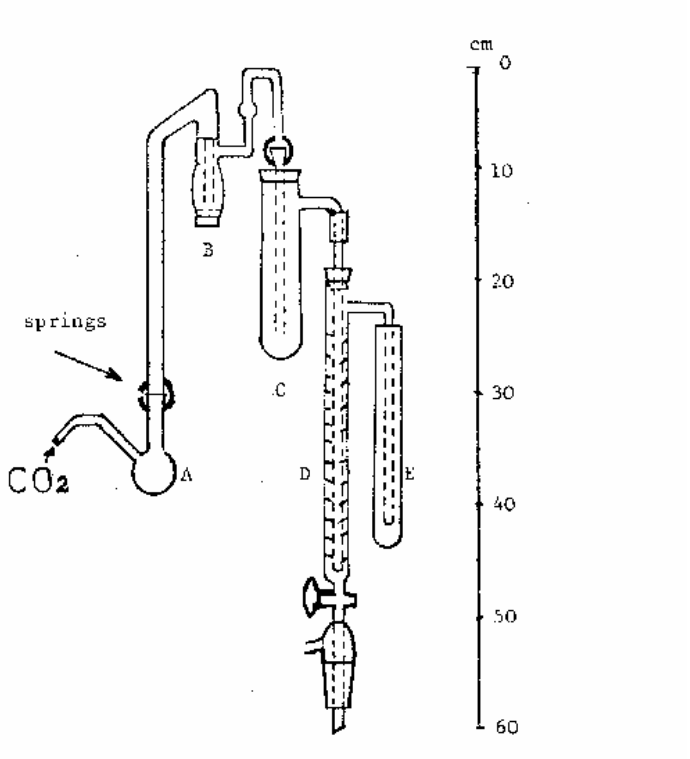

Figure 3. Apparatus for determination of oxyethylene groups

Procedure

Fill trap B with a suspension of a small amount of red phosphorus in enough water to cover the inlet tube. Pipet 10 ml of acid silver nitrate TS into tube C, pipet 15 ml of bromine-bromide TS into tube D, and place 10 ml of a 10% potassium iodide solution in trap E. Place about 0.05 g of the sample, accurately weighed, in the reaction flask A, together with a Hengar boiling granule and 10 ml of hydriodic acid TS. Connect the flask to the apparatus, pass a slow stream of carbon dioxide through (about 1 bubble per sec), and heat the flask slowly in an oil bath to 140-145°.

Maintain the flask at this temperature for at least 40 min, until there is no longer any cloudy reflux in the condenser above the reaction flask, and until the supernatant liquid in the silver nitrate trap C has clarified almost completely. Five min before the completion of the reaction, heat the silver nitrate trap C to 50-60° in a hot water bath to drive out any dissolved olefin.

On completion of the decomposition, disconnect tubes D and C cautiously in that order. Then disconnect the carbon dioxide source and remove the oil bath from flask A. Connect the spiral absorption tube, D, by its lower adapter to a 500-ml iodine-titration flask containing 10 ml of 10% potassium iodide solution and 150 ml of water. Remove the potassium iodide tube, E, and rinse the side-arm into it. Allow the bromine solution to run into the titration flask through the stopcock and rinse the tube and spiral with a few ml of water. Add the contents of the potassium iodide tube to the titration flask, stopper and allow to stand 5 min. Add 5 ml of dilute sulfuric acid TS and titrate at once with 0.05 N sodium thiosulfate, using 2 ml of starch TS as indicator.

Rinse the contents of the silver nitrate trap C into a flask, dilute to 150 ml with water, heat to boiling, cool to room temperature, and titrate with 0.05 N ammonium thiocyanate, using 3 ml of ferric ammonium sulfate TS as indicator.

Perform a blank determination omitting the sample.

Calculation

The volumes of sodium thiosulfate solution (S ml) of normality N and ammonium thiocyanate solution (S' ml) of normality N' used to titrate the contents of the bromine and silver nitrate traps are subtracted from the corresponding blank titrations (B and B' ml, respectively) and the following calculations made:

$$\% C_2H_4O = [(B - S) \times N \times 2.2] / \text{wt. of sample in g}$$

$$\% C_2H_4O = [(B' - S') \times N' \times 4.4] / \text{wt. of sample in g}$$

The sum of the values obtained from these calculations represents the total oxyethylene content of the sample. The % of polyoxyethylene ester can be estimated from the ratio of the % of oxyethylene in the unknown sample to that in a reference sample of known purity.