

Analyze 1 ml. aliquots from each of the above three extracts by the colorimetric method.

**Colorimetric Method for Pectic Substances.**—Pipette 1 ml. aliquots of the extract into each of 2 large test tubes (25 x 200 mm.) and add 0.5 ml. of the 0.1 percent alcoholic carbazole to the sample tube and 0.5 ml. of purified ethyl alcohol to the blank tube. A white flocculent precipitate will form in the sample tube.

Add 6 ml. of concentrated sulfuric acid to each of the tubes with constant agitation. An automatic acid burette is advantageous for adding the acid and should be set to deliver the 6 ml. of acid in seven sec. to obtain a temperature of 85°C. (heat of solution). The test tubes are immediately placed in a water bath heated to 85°C.; otherwise varying intensities of red color may be produced in duplicate samples. The tubes should remain in the bath for five min. During this waiting period the temperature need not be maintained. Remove tubes from water bath, allow to cool for 15 min., and transfer the solutions to microabsorption cells. Read immediately the percent transmittance in an electrophotometer with a 525 millimicron ( $m\mu$ ) filter, after nulling the instrument against the blank. Refer to the standard curve to obtain the concentration of anhydrogalacturonic acid (AGA) in micrograms ( $\mu g.$ ). Calculate the percent AGA in the samples using the formula:

$$\text{Percent AGA} = \frac{\mu g. \text{ AGA} \times \text{dilution volume (ml.)} \times 100}{1,000,000 \times \text{sample weight (g.)}}$$

### PREPARATION OF STANDARD CURVE WITH AGA

Weigh accurately 120.5 mg. of galacturonic acid monohydrate, vacuum dried five hr. at 30°C. or dried over phosphorus pentoxide at room temperature, and transfer to a liter volumetric flask. Add 0.5 ml. N sodium hydroxide and dilute to volume with distilled water. Mix thoroughly and allow the solution to stand overnight. This standard solution contains 100  $\mu g.$  of AGA per ml. Molecular weight ratio of AGA to galacturonic acid monohydrate is 176/212.

Prepare working standards covering the range of 10 to 70  $\mu g.$  of AGA per ml. Develop the color standards as described for sample extract using 1 ml. aliquots of each working standard, and record the percentages of transmittance. For the curve using the Fisher electrophotometer, plot the log percent trans-