CXLVI. THE COLORIMETRIC ESTIMATION OF LYCOPIN.

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PALMER [1922] states that "No method has yet been devised for estimating lycopin quantitatively. A suitable standard must first be found, and a method discovered for separating lycopin from carotene." It has recently been shown by Coward [1924] that it is possible to separate lycopin from carotene by filtration through a column of dry chalk, and it only remained to find a suitable colour standard.

A number of easily purified inorganic salts were examined but none was found whose colour changes, on successive dilution, matched those of lycopin. It was therefore decided to adopt the dilution colorimeter of Stanford [1923] and to make up an artificial standard whose colour and intensity should correspond to that of a dilute solution of lycopin of known concentration.

A crystalline sample of lycopin was prepared from fresh tomatoes by the method described by Willstätter and Escher [1910]. A portion of this was dissolved in light petroleum (B.P. below 40°) and its purity tested by the method of chromatographic fractionation as described by Coward [1924]. No trace of any other colouring matter could be detected spectroscopically in any of the fractions.

The strength of lycopin solution chosen was 0.5 mg. per litre, which was considered to be as dilute as any which would be obtained in analytical experiments. A few milligrams of the lycopin were therefore weighed out by means of a micro-balance, with an accuracy of 1 part in 200, and dissolved in the amount of light petroleum necessary to make a solution of this strength. A layer of this solution 30 mm. thick is exactly matched by a standard solution prepared in the following way. Two stock solutions are made up (1) potassium dichromate 0.02 %, (2) cobalt sulphate (nickel free) 0.5 %. Of these, quantities equivalent to (1) 6.9 mg. and (2) 135.0 mg. respectively are taken, mixed, and the volume made up to 100 cc. This standard is stable for at least 24 hours, and the two solutions can be kept separately for a considerably longer period.

The apparatus used is essentially that of Stanford, the source of light being a Sheringham Daylight Lamp, and the comparison eyepiece that of a Duboscq colorimeter. Since the solvent used is light petroleum it is advisable to use

plain open cells as described by Stanford. A rectangular glass cell $30 \times 30 \times 100$ mm. high was therefore obtained, and graduated on one side to measure up to 80 cc. A thick (8 mm.) plate glass lid was made for this, centrally drilled and a stopper ground in. The lid is held in place by rubber bands, the joint being smeared with a little glycerol which, since it is insoluble in light petroleum, prevents leakage. For the standard solution a plain cell $30 \times 30 \times 30$ mm. is used¹.

In order to carry out an estimation, the lycopin solution of unknown strength is diluted in a measuring cylinder until it is only slightly darker than the standard, and the degree of dilution noted. 25 cc. of this are then measured into the glass cell and light petroleum added until the two solutions match. The volume of the solution is then read off. Each cubic centimetre of liquid in the cell contains 0.0005 mg. of lycopin, and from this reading and a knowledge of the previous dilution the total lycopin present is calculated.

A series of tests with lycopin solutions of various strengths has shown that the concentration of 0.5 mg. per litre, arbitrarily chosen, is actually more easily compared than those which are either stronger or weaker than this.

It has been found possible for a practised observer to carry out a series of estimations with an error of considerably under 1 %.

I am indebted to Miss Katharine H. Coward for suggesting this piece of work and for help in determining the colour standard.

¹ The glass cells used in this estimation were obtained from Messrs J. J. Griffin and Sons, Ltd., Kingsway, London.

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