The anilin-alcohol-safranin is also very satisfactory for staining sections of stems and leaves. The stain may be prepared as above with the addition of one cc of 4 per cent. acetic acid. After the usual procedure, stain 3 to 10 minutes, wash with water, counterstain with ½ per cent. cotton blue in 70 per cent. alcohol for a few seconds, dehydrate, clear and mount in balsam. The entire process is carried out on the slide, staining jars being unnecessary.

Another combination of dyes that is apparently as good, or nearly as good, as safranin and cotton blue for stem and leaf sections is anilin-alcohol-basic fuchsin (3 minutes) followed by Delafield's haematoxylin.

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THE DETERMINATION OF CO_2 CONTENT OF AN ATMOSPHERE IN A CLOSED SYSTEM[1]

IN a previous publication[2] a colorimetric method was described for the determination of the CO_2 content of an atmosphere. This method was based on the estimation of pH in a standard $NaHCO_3$ solution in equilibrium with the CO_2 in the atmosphere. As described, the method was not applicable to closed systems; since many physiological studies make use of closed containers, two modifications have been devised which can be used in these studies.

Method 1: This is a modification of a method described by Osterhout for measuring the respiration of bacteria.[3] The atmosphere from the closed system is circulated through a 13 mm test-tube fitted with an inlet tube drawn to a 1 mm capillary tip and containing approximately 4 cc of $NaHCO_3$ solution plus indicator. The latter is prepared by mixing 1 cc of a given indicator with 20 cc of 0.001N $NaHCO_3$. The gas is circulated through the solution by means of an ordinary aspirator bulb and returned to the original container. About 20 aspirations of the bulb circulates enough gas to bring the solution to equilibrium with the atmosphere; its pH is determined by comparison with a standard Hellige color disk and the pCO_2 read from the standardization curve.[2] For cresol red or brom-thymol-blue the equation of this curve is:

$$\text{Log } pCO_2 = 7.30 - pH$$

This method is fairly accurate and more rapid than any heretofore suggested. However, if several determinations must be made at short intervals, a second method which automatically indicates the pCO_2 proves more satisfactory.

Method 2: An automatic determination of the pCO_2 in the atmosphere of a closed system can be obtained by suspending a 25 x 50 mm tube containing 2 cc of the $NaHCO_3$ plus indicator solution inside the closed system, with provision for addition and withdrawal of the solution. At any time the CO_2 content of the atmosphere can be determined from the color of the solution. For accurate work, the pH can be estimated by comparison with standard buffer solutions (2 cc in a 25 x 50 mm tube). With a little practise, however, the operator can judge the pH of the solution without the use of these standards. Although this device can be used to estimate the actual CO_2 content of the atmosphere in a closed system, it is of especial value for notifying the operator when the CO_2 has reached a given predetermined level. In the latter case, an indicator is used which exhibits a pronounced color change at a pH corresponding to the pCO_2 desired.

Tests of the methods: To test the methods, a known quantity of CO_2 was added to the atmosphere in a closed system. After 20 minutes, the time found to be necessary for the suspended solution to reach equilibrium with the atmosphere, the pCO_2 was estimated by the two methods; the buffer standards were used in the automatic method to insure greater accuracy. Both methods gave satisfactory results in tests of atmospheres whose CO_2 content ranged from 0.03 to 0.7 per cent. The estimations checked the actual quantity of CO_2 within 5 to 10 per cent., which is the limits of accuracy for the colorimetric method.

In connection with various physiological studies both methods have proved reliable in greenhouse experiments. The second method is particularly useful when CO_2 must be added periodically to a closed container. An indicator is selected which has a definite color change at a pH corresponding to the lowest level of CO_2 desired. For example, if it is required to keep the CO_2 level greater than 0.15 per cent., phenol red is an appropriate indicator, since at this concentration of CO_2 it turns from a definite yellow to a definite pink. If the CO_2 is not to be added until reduced to the concentration of air, cresol red is satisfactory. Greenhouse tests on the second method carried out over a period of six months show that the color change of the indicator corresponds to a quite definite pCO_2 in the atmosphere and that the method can be entirely relied upon to indicate when CO_2 shall be added to plants in physiological experiments. The indicator solution used should be changed at least every three days for highest accuracy.

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[1] Herman Frasch Foundation in Agricultural Chemistry, Paper No. 80. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

[2] P. W. Wilson, "Colorimetric Method for Determination of CO_2 in Gas Mixtures," SCIENCE, 78: 462–463, 1933.

[3] W. J. V. Osterhout, "A Method of Studying Respiration," *Jour. Gen. Physiol.*, 1: 17–22, 1918.