This Week's Citation Classic CC/NUMBER 39 SEPTEMBER 27, 1982

Allen R J L. The estimation of phosphorus. *Biochemical J.* 34:858-65, 1940.
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In the Fiske and Subbarow[1] method for estimating phosphorus by reduction of phosphomolybdic acid the intensity of the blue colour so produced varies with time and temperature. A method is described in which these difficulties are eliminated and which is applicable also to turbid and coloured solutions. [The *SCI*® indicates that this paper has been cited in over 1,410 publications since 1961.]

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April 27, 1982

"In my early days as a graduate student at the University of Melbourne I became familiar with the Duboscq colorimeter, much favoured by biochemists at that time for colorimetric analyses. In this device, the colour densities generated in test and reference samples by the chosen analytical procedure were compared by finding the depths of solution that produced equal intensities of colour when viewed by transmitted light in a split field eyepiece. The concentration of the substance under analysis present in the test solution could then be calculated.

"When I arrived in Cambridge in 1936 to work on the phosphorus metabolism of the higher plants, I found that the colorimeter was being replaced by the more refined photometer. Here the optical density of the test solution was measured directly by a photocell connected to a sensitive galvanometer and scale calibrated against a graded series of standard solutions. But when I started to use a photometer for estimating phosphorus by the popular Fiske and Subbarow[1] method I soon encountered problems. First, the colour density went on increasing for about 20 minutes after the reagents were added to the test solution. One had to wait for at least that time in order to avoid errors. This was inconvenient, but more serious was the real danger that organic phosphorus compounds in the materials I was working with would be hydrolysed in the strongly acid solution during the waiting period and thus appear as free phosphate. Secondly, colour density at any particular time depended on temperature: as the environmental temperature rose, the strength of the colour increased. Neither effect had mattered when a colorimeter was used because the test solution was compared with a reference solution prepared simultaneously at the same temperature. I eventually solved both problems by substituting 2:4-diaminophenol hydrochloride (amidol) for the 1-amino-2-naphthol-4-sulphonic acid used by Fiske and Subbarow as a reducing agent. Amidol had been considered for use by previous workers but never seriously investigated. The density of the blue colour produced with this reagent is for all practical purposes independent of time and temperature.

"I was able, by an extension of the amidol method, to overcome another vexing problem. The estimation of phosphorus in many of the plant extracts with which I was working was made difficult by the presence in these of turbidity and extraneous colour. I found that the blue colour could be extracted by *iso*butyl alcohol and measured in the photometer free from any interference.

"I have not worked in this field for more than 40 years.[2] The amidol method has been highly cited because it became available just when everyone was changing over from colorimeters to photometers and was no doubt welcomed by other investigators who were experiencing the same problems that I had encountered. But the survival of this old method into the age of analysis by black boxes and other sophisticated gadgetry is as surprising to me as it is gratifying."

1. **Fiske C H & Subbarow Y.** The colorimetric determination of phosphorus. *J. Biol. Chem.* 66:375-400, 1925.
2. No review of methods determining phosphorus has been published recently.