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EDITORIAL

Phosphorus Compounds: Their Discovery in Biological World

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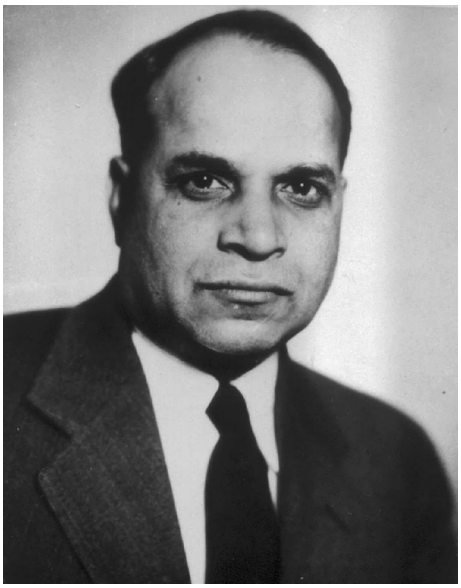

Human kind shall be indebted to Dr. Subbarao Yellapragada for his discovery of Phosphorus estimation method. The first study on discovery of Phosphorus as an element revealed its biological link, dating back to 17th century. Hennig Brand, an alchemist, in a bid to study philosopher's stone concentrated gallons of human urine and obtained an unknown substance,' which he named 'Phosphorus' meaning light bearer [1]. In 18th century, the phosphorus was recognized as an important component of bones and the association of calcium and phosphorus was linked to rickets disease [2]. In the beginning of 20th century, Phosphorus was implicated in several human and microbial metabolic pathways [1–3].

The work of phosphorus estimation was initiated by Neumann [4]. In the method, phosphate was removed from the sample by precipitation method as ammonium phosphomolybdate. The reaction was carried at high temperature, with excess ammonium molybdate and ammonium nitrate in a distinctly acid reaction. A complete washing with 50% alcohol removed the excess of ammonium nitrate, ammonium molybdate and acid, without solubilizing the required precipitate. The quantitative estimation of phosphoric acid in next step was done upon titration with alkali. The equation is as follows [4]:

$$2(NH_4)_3PO_4 \cdot 24MoO_3 \cdot 4HNO_3 + 56NaHO = 24\ Na_2MoO_4 + 2NaH_2PO_4 + 6NH_3$$

The method however showed interference with certain neutral salts as chlorides, sulphates, etc. and the results showed 1–2% higher values. Taylor and Miller [5] employed Phenylhydrazine for reduction of Mo in the Mo phosphate precipitate. This method was used for estimation of Phosphate for some years. In 1920, Bell and Doisy made an important observation that certain reducing agents could reduce phosphomolybdic acid without showing any reaction with molybdic acid [6]. Such selective reduction was useful as the molybdenum in phosphomolybdic acid could be colorimetrically determined in the presence of molybdic acid, without precipitating the ammonium phosphomolybdate from the solution. Hydroquinone was used as a more suitable substitute as reducing agent. Though the method was much convenient and sufficiently accurate, it was not reliable and had several limitations [7]. The reaction

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products showed rapid and irregular fading of color and gave an unacceptable error margins. The values did not match gravimetric estimations [6, 7]. In this scenario, the pioneering work of Subbarao with Fiskie was initiated. Subbarao started search for a reducing agent that could overcome the limitations of reported experiments and should be quick, reliable method that won't be affected by trichloacetic acid (a component of reaction) and other interfering agents. Dr. Subbarao logically selected 1,2,4-amino-napthol sulphonic acid (ANSA) [7]. The initial step (example of phosphorus estimation in urine) included treatment of the sample with ammonium molybdate prepared in 5N sulfuric acid (Molybdenum reagent) [8]. The reaction is as following [9]:

$$7NHPO_{4.}^{3-} + 12(NH_4)_6Mo_7^{VI}O_{24} + 51H^+ \rightarrow 7(NH_4)_3PO_412Mo^{VI}O_3 + 51NH_4^+ + 36H_2O \quad (1)$$

This was immediately followed by addition of aminonaphtholsulfonic acid and gentle mixing. The reducing agent (1,2,4-ANSA) was 50 times more reactive than the reagents being earlier used; the color development took less than a minute. The readings were taken colorimetrically and the unknown values of Phosphorous thus determined.

$$\left[PMo_{12}^{VI}O_{40}\right]^{3-} + 4e^- \rightleftharpoons \left[PMo_4^{V}Mo_8^{VI}O_{40}\right]^{7-} \quad (2)$$

1,2,4-ANSA was easy to prepare than its isomer 1,2,6-ANSA, which Subbarao originally worked on and moreover, it was readily available due to its use in dye manufacturing industry [9]. This reducing agent gave consistent and rapid colorimetric results. The assay could tolerate inhibitory materials at 10 times higher concentration than while using hydroquinone. On detailed experimentations and analysis, Subbarao had proudly recorded that the estimation "is correct to 1/100,000th of a grain" [9]. This important and famous discovery of colorimetric determination of Phosphorus (for both inorganic and organic phosphates) was instantly accepted and published in 'The Journal of Biological Chemistry' [8, 9]. It became part of textbooks of biological chemistry in 1925 and is one of the first procedures taught in biochemistry course. The paper of Fiske and Subbarao, "the colorimetric estimation of Phosphorus" stood the test of time and is being still used as basis for Phosphorus estimations in a variety of samples. It is one of the most highly cited papers (about 22,258 citations) in the history of biochemistry and certainly the one for which Fiske and Subbarao are most remembered [9].

This led to a major breakthrough in biochemistry with the identification of the energy currency of cells, ATP, characterized and described as "adenosinetriphosphate" in 1929. Though the text books cite Karl Lohmann as discoverer of ATP, the work of Lohmann is thought to be influenced by his earlier discussions with Fiske [9]. Ironically, Lohmann also utilized the technique of Subbarao and Fiske for the discovery of ATP.

Subbarao discovered another high energy organic molecule, Phosphocreatine. Fiske and SubbaRao demonstrated that much of the phosphate in muscle filtrates was not inorganic, as had been believed, but a compound that was equimolar creatine and phosphate. They also were able to show that phosphocreatine was hydrolyzed during muscle contraction and resynthesized during the recovery period [10–12]. The discovery of the energy currency (phosphocreatine and ATP) emphasizes the role of phosphorus in living organisms. This would not have been possible without the simple, accurate phosphate determination method. The study thus also enhanced the understanding of muscle biochemistry, the metabolism and the diseases related to it [13, 14].

With the advent of science, sensors for inorganic and organic phosphates in the form of amperometric, potentiometric enzyme electrodes, plant tissue electrode, screen printed electrodes, etc. have been developed, but in basic laboratories, Fiske and Subbarao method for phosphorus estimation is still a followed technique.

Besides this, Yellapragada Subbarao discovered 5 Medical molecules, known as Miracle Drugs: Folic acid, Aueromycin, Tetracycline, Methotrexate, which opened new methods to the treatment of Nutritional diseases, Infectious diseases, Worm-transmitted diseases and the cancer, respectively. His "Miracle Drugs" made it possible for millions of people to live longer. The drugs still have high potential and are adding new benefits to the health of society. Subbarao did not fetch the required recognition, even so, his name was wrongly put up in publications as SubbaRow. The man of Science just had huge zeal to work for humanity and never bothered for fame.

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