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Note

Sensitive method for the detection of phosphates and polyphosphates on paper chromatograms

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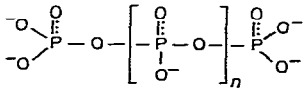
In previous work[1], we demonstrated that the spots produced by phosphates and arsenates at pH 3–4 are stained red by acetic acid that contains toluidine blue or methylene blue and uranyl acetate. We have found that this colour change ("metachromasia") is due to the formation of the uranyl phosphate and uranyl arsenate.

Further work[2,3] showed that all oxy acids of phosphorus(V) and arsenic(V) that contain at least two ionizable atoms of hydrogen and that are capable of producing insoluble uranyl salts at pH 3–4 yield red spots. We have termed this phenomenon "metachromasia through uranyl ions"[4] in order to differentiate it from classical metachromasia, which occurs in the absence of the uranyl ions with some anionic macromolecules, most of which are produced by living cells[5,6]. Of these anionic macromolecules, special attention has been paid to the "metachromasis bodies", which were found by Babeş[7] in the cells of some microorganisms. Wiame[8] established that the metachromatic bodies are polyphosphates with a linear structure of the type



$n = 2$–10: oligopolyphosphates
$n > 10$: macropolyphosphates

Ebel and Müller[9] studied the mechanism of the metachromatic reaction produced by these linear polyphosphates and found that the metachromatic colour is given only by polyphosphates with a chain length corresponding to a degree of condensation not less than 8 (octaphosphates).

As the molecules of both oligopolyphosphates and macropolyphosphates contain two phosphate groups, end, both containing two ionizable atoms of hydrogen, we decided to study procedures based on this new type of metachromasia in order to permit the localization of phosphates and polyphosphates by paper chromatography.

EXPERIMENTAL

The behaviour of sodium and potassium orthophosphate (PO_4^{3-}), pyrophosphate ($P_2O_7^{4-}$), tripolyphosphate ($P_3O_{10}^{5-}$), tetrapolyphosphate ($P_4O_{13}^{6-}$), hexapolyphosphate ($P_6O_{19}^{8-}$) and decapolyphosphate ($P_{10}O_{31}^{12-}$) was studied for the individual salts and their mixtures. A Graham salt sample containing 20 phosphate groups, and which can be considered to be an icosapolyphosphate ($P_{20}O_{61}^{22-}$), was also tested.

The solutions were obtained by dissolving 0.025–0.050 g of the salts in 6–7 ml of 0.2 *M* acetic acid and diluting to 10 ml with propanol-2. For the hexapolyphosphates and above, the solution turns turbid, in these instances 0.2 *M* acetic acid was used for dilution to 10 ml.

Procedures

Two procedures were adopted: (a) a direct method using chromatographic paper strips, and (b) a circular chromatographic method.

Direct chromatographic paper strip procedure. This method proved satisfactory for studying the chromotropic behaviour of the phosphates in the presence of uranyl salts. This technique consists in pipetting 0.0025–0.005 ml of each solution on to rectangular Whatman No. 1 chromatographic paper strips. The strips are dried at 50°, then divided into two series. One series is kept for 1 min in solution A, consisting of 50 ml of 0.2 *M* acetic acid, 20 ml of distilled water, 30 ml of acetone and 0.025 g of toluidine blue 0 (Merck), and the second series in solution B, consisting of solution A + 0.100 g of uranyl acetate. The pH of both solutions is 3 before the acetone is added. The strips are then immersed in two or three acetone baths for 2–3 min each and then examined.

Circular chromatographic procedure. This procedure, which has been described previously, is intended to establish whether or not the uranyl-containing solution (B) stains the spots and permits the accurate localization of each substance, both separately and in mixtures. Whatman No. 1 paper discs, 10–12 cm in diameter, were used and the behaviour of the phosphates both separately and in mixtures was studied. In the former instance, 0.0025–0.005 ml of each solution was pipetted on to the paper discs, and in the latter, 0.0025–0.005 ml of each solution was pipetted successively on to the same disc. After each pipetting, the liquid was evaporated at 50°. The developing solvent recommended by Ebel[10] was used, which consists of 70 ml of propanol-2, 30 ml of distilled water, 4 g of trichloroacetic acid and 0.4 ml of ammonia solution (20%). After development, each disc was immersed for 1–2 min in two successive diethyl ether or acetone baths. Subsequently, each disc was kept for 1 min in the staining solution which contained uranyl ions (solution B), then immersed in three or four acetone baths for 2–3 min per bath. The chromatograms thus obtained were finally examined.

RESULTS AND DISCUSSION

On examining the chromatographic strips treated with solution A, only two red-mauve metachromatically coloured spots were revealed, which obviously contrasted with the blue (orthochromatic) background of the strip. One of the spots

corresponded to the decapolyphosphates and the second to Graham's salt. The other five anions tested, from the orthophosphate to the hexaphosphate, did not produce any spots. Although the procedure we used was not similar to that used by Ebel and Müller[9], our results confirmed those reported by the latter workers, who concluded that the metachromatic polyphosphate-induced effect depends upon the degree of condensation of the polyphosphates.

However, the chromatographic strips obtained after treatment with solution B demonstrated that all seven anions investigated produced metachromatic spots, irrespective of their degree of condensation.

The examination of the chromatographic discs demonstrated that this method provides a clear localization of phosphates, oligopolyphosphates and macropolyphosphates as red spots as soon as the disc is immersed in solution B (Fig. 1). After washing the disc with acetone the red colour turns red-mauve.

Fig. 1. Paper chromatographic separation of phosphates and polyphosphates. Detection with an acetic acid solution of toluidine blue and uranyl acetate. Materials spotted: PO_4^{3-}(P_1), $P_2O_7^{4-}$(P_2), $P_3O_{10}^{5-}$(P_3), $P_4O_{13}^{6-}$(P_4), $P_6O_{19}^{8-}$(P_6), $P_{10}O_{31}^{12-}$(P_{10}) and $P_{20}O_{61}^{22-}$(P_{20}).

On washing the chromatograms with acetone, dehydration of the spots takes place simultaneously with removal of solution B retained by the chromatographic paper. Water plays an important part in inducing the metachromatic effect[11].

Sensitivity studies showed that the method can detect 1–2 μg of phosphorus containing spots on the chromatograms. This level is comparable to those provided by methods based on the conversion of orthophosphates and polyphosphates (after hydrolysis) into molybdophosphates and their subsequent reduction to molybden blue either with hydrogen sulphide[12] and exposure to ultraviolet light[13] or by other procedures[14].

In addition to its sensitivity, the method is rapid and does not require the additional operations that are necessary in previous methods.

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