RESEARCH PAPERS

THE PHOTOMETRIC DETERMINATION OF 2:3:5:6-TETRACHLORONITROBENZENE

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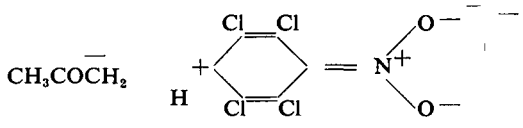
THE current interest in 2:3:5:6-tetrachloronitrobenzene as a prominent inhibitor of sprouting and rotting of potatoes during storage has made a rapid method for the determination of small amounts of this substance a topical problem. The toxicity of the compound has recently been discussed in this journal by Buttle and Dyer[1].

This substance was first prepared by Jungfleisch[2] and later by Beilstein and Kurbatow[3] by nitration of 1:2:4:5-tetrachlorobenzene. Page[4] prepared the compound by chlorination of nitrobenzene in presence of iron chloride. It was studied later by Holleman[5], Berckmans and Holleman[6], Dyson, George and Hunter[7], and by Hüffer[8]. Berckmans and Holleman[6] were able to demonstrate that when treated with sodium methylate it was hydrolysed to 2:3:5:6-tetrachloroanisol and nitrite ions.

The benzene ring is heavily loaded with negative substituents. In this case the single nitro group is enough to stabilise a *p*-quinoid structure of the addition product (see below) when tetrachloronitrobenzene is allowed to react with active methylene groups in alkaline solution. This reaction may be formulated in the same way as the general reaction between aromatic nitro compounds and active methylene groups, see Canbäck[9]. Thus when tetrachloronitrobenzene is dissolved in acetone and alcoholic potassium hydroxide is added a brilliant red-bluish colour is produced. When the colour has faded and the solution is acidified with nitric acid, chloride ions are not present in the solution while nitrite ions are easily shown to be present. The structure of the coloured anion might thus be formulated in the following way:—



We have tried to use this reaction in a quantitative way. If the amount of the reagents, the reaction time, the water content of the acetone, etc., are rigidly controlled it is possible to use the reaction for the determination of small amounts. However, the shape of the standard curve indicates that the destruction of the compound is rather rapid and not always uniform. We tried, therefore, to find a better method. If tetrachloronitrobenzene is hydrolysed quantitatively to 2:3:5:6-tetrachloroanisol and nitrite ion the latter may be easily determined by any of the classical methods for the determination of nitrite, see Allport[10] and Snell and Snell[11]. We have preferred to use the reagents recom-

mended by Jendrassik and Falcsik-Szabó[12], who used procain and α-naphthylamine. After some trials the following method was adopted:—

50 to 300 μg. of tetrachloronitrobenzene is dissolved in 5 ml. of acetone and 5·0 ml. of 0·5N alcoholic potassium hydroxide is added. The solution is refluxed on a boiling water bath for 15 minutes. After cooling to room-temperature 5·0 ml. of diazo reagent I (see below) and 5·0 ml. of diazo reagent II are added. After standing for 30 minutes the coloured solution is transferred to a 25 ml. volumetric flask by the aid of ethyl alcohol (95 per cent.) and made up to the volume with ethyl alcohol (95 per cent.). The extinction is measured in a photometer with a filter with maximum transmission at about 500 mμ. A blank is made on 5 ml. of acetone.

Diazo reagent I: 3·0 g. of procain hydrochloride is dissolved in a mixture of 15 ml. of glacial acetic acid and 85 ml. of distilled water.

Diazo reagent II: 0·20 g. of α-naphthylamine is dissolved in 30 ml. of boiling distilled water and filtered through a warm funnel. The filter is washed with 2 × 30 ml. of hot water. To the filtered solution 30 ml. of glacial acetic acid and water to 150 ml. are added.

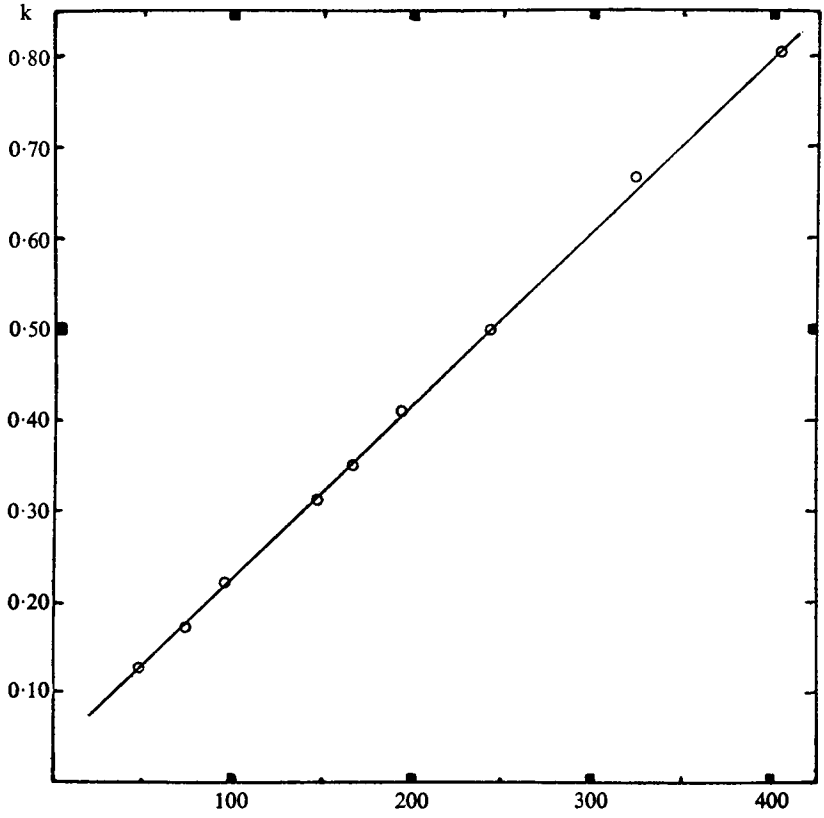


FIG. 1. Standard curve.

The reagents are stable for at least 1 week.

In Figure 1 the standard curve is shown (Lumetron photo-electric Colorimeter, filter M 515). The absorption curve of the azo dye is given in Figure 2. Figures 3 and 4 give respectively the rates of hydrolysis (expressed as extinction coefficient *versus* time) when treated (as described in the method) with 0·5N alcoholic potassium hydroxide at room-temperature and when refluxed on a boiling water-bath. In Figure 5 the rate of development of the colour after the addition of the diazo reagents is shown.

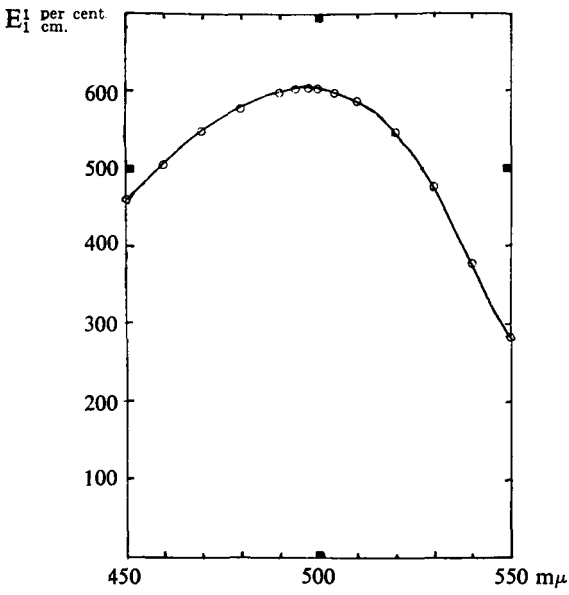


FIG. 2. Absorption curve (Beckman Quartz spectrophotometer model DU).

To extract the compound from potatoes, etc., acetone, light petroleum (e.g., Skellysolve F) or other organic solvents which can be removed by distillation may be used. This extraction increases the specificity of the method as the number of organic compounds giving nitrite ions when treated with alkali is relatively small. Aliphatic nitrates and some nitro benzene derivatives may interfere.

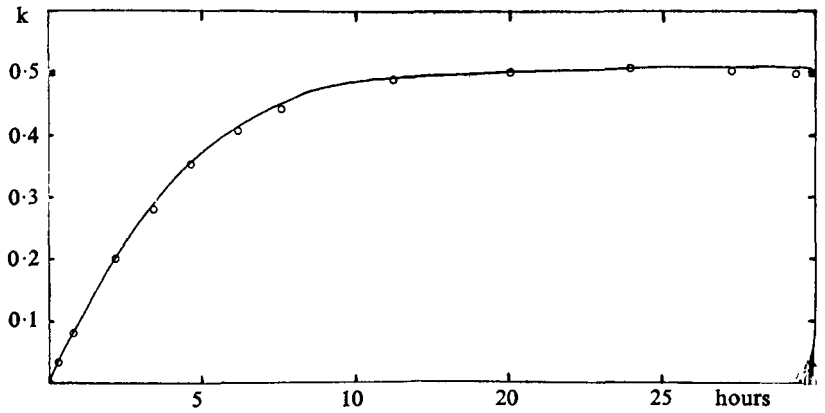
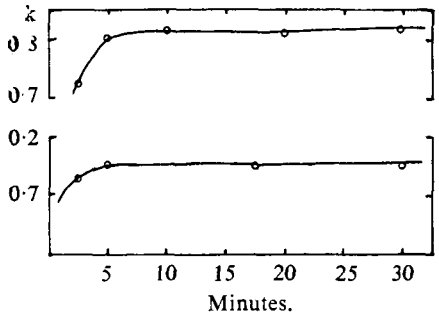


FIG. 3. Hydrolysis at 20°C.



If large quantities of fat are present in the acetone or light petroleum extract (e.g., when testing rat fæces) the final coloured solution may be turbid. However, the coloured solution can be filtered clear, but this operation must be included in the operations leading to the standard curve and in the blank.

FIG. 4. Hydrolysis on the water-bath. Lower curve about 60 μg. Upper curve about 400 μg.

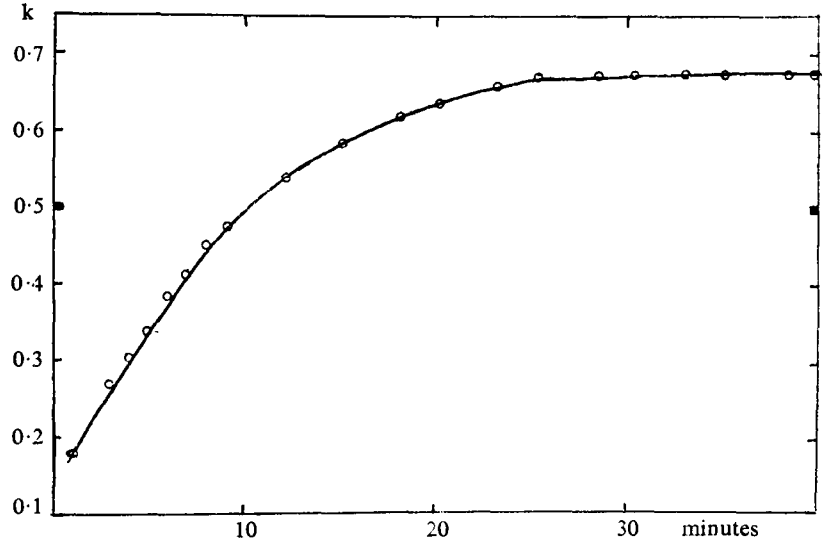


FIG. 5. The development of the colour.

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