

(0.55 gm.), boiling point  $84^\circ$ ,  $n_D^{20}$  1.3834 (found: C, 35.6;  $C_5F_5N$  requires C, 35.5 per cent): mass-spectrometry, principal mass peaks: 169,  $C_6F_5N$ ; 150,  $C_5F_4N$ ; 138,  $C_4F_4N$ ; 124,  $C_4F_4$ . The compound had  $\lambda_{max}$  at  $254 m\mu$  ( $\epsilon$ , 2,700 in ethanol). The presence of strong bands in the infra-red spectrum at 1,650, 1,537, and 1,505  $cm^{-1}$  was also consistent<sup>6</sup> with a pyridine structure, and the absence of strong bands at frequencies higher than 1,650  $cm^{-1}$  showed that no olefinic bonds were present.

The other three components from the pyrolysis have not yet been identified, but are believed to be unsaturated.

Pentafluoropyridine is a colourless and almost odourless liquid. It is immiscible with water but miscible with organic solvents. We have been unable to detect any basic properties of the compound. A potentiometric titration in aqueous ethanol against *N*/20 acid showed no end-point. Hydrogen chloride in dry ether gave no hydrochloride, and no picrate was formed in ethanol solution. This behaviour is different from that of the primary amine pentafluoroaniline<sup>7</sup>, which was weakly basic and formed an easily hydrolysable hydrochloride in ethereal solution. It is noteworthy that pyridine is a stronger base than aniline.

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### A Modification for washing the Benzidine Sulphate Precipitate in the Determination of Sulphate

AMONG the numerous procedures that have been developed for determining sulphate with benzidine, that reported by Kent and Whitehouse<sup>1</sup> is most commonly employed as a micromethod. The reliability of the method has also been confirmed by me.

In this, as in all the other procedures, difficulty is experienced in removing the excess of benzidine from the benzidine sulphate precipitate without losing any of the latter. The difficulty was previously overcome by collecting the benzidine sulphate precipitate in a porous glass filter crucible and washing the unreacted soluble benzidine thoroughly. The method yields accurate and reproducible results provided that the filter is of standard porosity and the filtering time is held constant. Owing to the difficulties associated with the use of the filter crucible, the method is, however, time-consuming.

I have developed the following procedure for washing the benzidine sulphate after its precipitation by the method of Kent and Whitehouse. The contents of the precipitation tube are transferred to a centrifuge tube of about 10-ml. capacity which tapers to a sharp point. One drop of an aqueous suspension of barium sulphate which has been carefully rinsed free of excess barium or sulphate ions is then added and it is centrifuged for 5 min. at 3,000 r.p.m. Two more drops of the barium sulphate suspension are added, and the tube centrifuged for a further 15–20 min. The upper clear layer is then removed and the mouth of the tube dried with cellulose tissue. About 9 ml. of an alcohol-acetone (1 : 1) mixture is added to the tube, and the precipitate is broken up with a stiff steel wire. The tube is centrifuged about 5 min., two drops of the barium sulphate suspension are added, and the centrifugation continued for about 10 min. It should be noted that the total amount of barium sulphate added forms a cone 1–1.5 mm. high at the bottom of the tube when tightly packed. The upper clear layer is decanted, about 9 ml. of the alcohol-acetone mixture is added and the tube is centrifuged for about 10 min. After the supernatant has again been removed, 3 ml. of *N* hydrochloric acid is added to dissolve benzidine sulphate precipitate, which is broken up with a steel wire. After  $\frac{1}{2}$  hr., 2 ml. of water is added to the tube, and the latter is centrifuged for 5 min. The solution containing the benzidine is transferred to another tube for diazotization by the method of Kent and Whitehouse.

The microprecipitate of benzidine sulphate is always enclosed between two tightly packed layers of barium sulphate during the decantations. After the first centrifuged precipitate has been broken up, the benzidine adsorbed on the barium sulphate is removed by washing with the alcohol-acetone mixture. After the centrifuged precipitate has been broken up a second time, the pure benzidine sulphate is dissolved in the hydrochloric acid. The solubility of barium sulphate is so slight that it cannot cause any significant error.

In spite of its apparent complexity, the method can be readily employed in routine analyses. One hundred analyses can be performed daily with a centrifuge that takes 50 tubes and a larger throughput can be achieved by using a larger centrifuge. The accuracy of the method is about the same as reported by Kent and Whitehouse. The absorbance reading for the blank is 0.020 when a Beckman *DU* spectrophotometer is employed.

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### Freezing-Point Depressions of Hoagland's 'Carbowax' Systems

SOLUTIONS of various osmotic pressures have been widely used in biology. Sugars and their derivatives, commonly used to maintain osmotic pressures, have the drawback that many, if not all, of these compounds can be metabolized by the test tissue or organism. McClenden and Blinks<sup>1</sup> described the use of 'Carbowax 4000' (polyethylene glycol) in maintaining various osmotic concentrations in a study of the Hill reaction. Excellent results were reported. This