

acetic acid buffer (pH. 3.5 — 3.65), (b) enzyme solution and (c) 1 c.c. H₂O₂ (1.8 mg.). The reaction is carried out at 30° C. for 5 minutes, and then stopped by addition of a 25 per cent. solution of NaOH till the solution changes from a blue to orange red colour (about 2 c.c. of alkali are required). On saturating the inactivated reaction mixture with NaCl, the dye is thrown out. This is extracted with 5 c.c. butyl alcohol. The alcohol layer containing the dye is separated and after washing once with saturated brine is drawn into a 25 c.c. flask through a filter of cotton wool (to keep out undissolved, mechanical impurities, if any). The separator is washed several times with small quantities of ethyl alcohol to complete extraction of the dye and each washing passed through the original filter. The combined filtrates are made up to 25 c.c. with ethyl alcohol. A control is run under identical conditions but with the boiled enzyme. The amount of purpuro-benzidine formed is determined in the Pulfrich Photometer (cell 20.06 mm., filter

S 53) and evaluated by reference to a previously constructed standard-graph: Purpuro-benzidine vs. Extinction Coefficient.

The peroxidase activity of *Chow-Chow* (*Sechium edule*) enzyme extract has been determined by this method.

The graph (Fig. 1) proves that under the conditions of the experiment the amount of dye formed is strictly proportional to enzyme concentration. Consequently, for the range of enzyme concentration studied, the quantity of purpuro-benzidine which is determined, gives us an exact measure of peroxidase activity.

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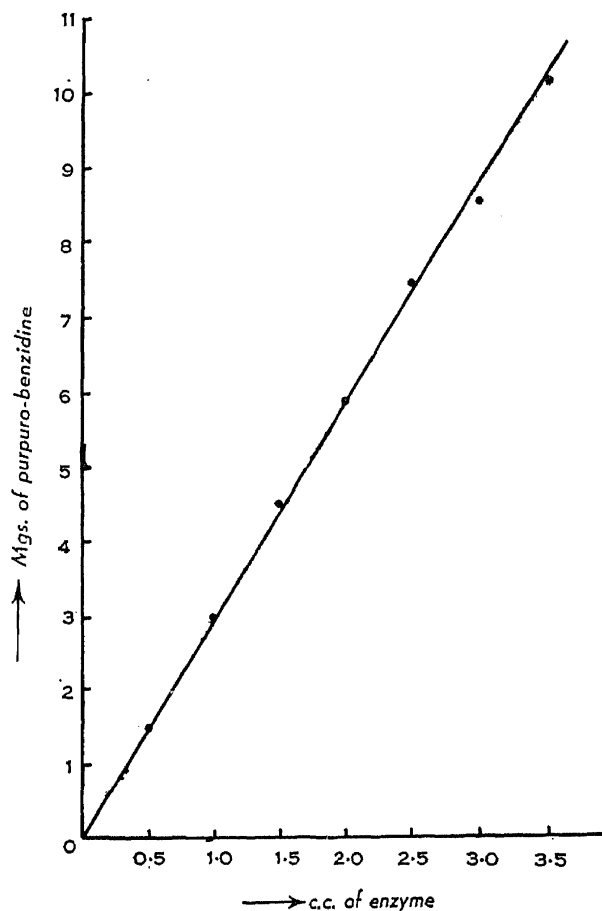


FIG. 1

¹ Srinivasan, M., *Biochem. J.*, 1936, **30**, 2077.

² Willstätter and Weber, *Annalen der Chemie.*, 1926, **449**, 156.

³ Zirm, K. L., Reuter, F., and Willstardt, H., *Biochem. Z.*, 1932, **245**, 290.

A Note on the Determination of Lead Permanganometrically (Low's Method)

HEMPEL'S method¹ as modified by Low² has been in use for a long time for the determination of lead permanganometrically in technical analysis. Low's method has also been adopted by the British Pharmacopea. This method which consists in precipitating the lead as oxalate from acetic acid solution, dissolving the precipitate in dilute sulphuric acid and titrating with permanganate has been criticised by Morris³ and Wetherell⁴ on the grounds that precipitation is incomplete unless carried out from a 60 per cent. acetic acid solution, and that the liberation of oxalic acid from the precipitate by dilute sulphuric acid is not quantitative. Other investigators have frequently suggested variations in procedure such as filtering on asbestos and using dilute nitric acid for the solution of the lead oxalate before the

addition of sulphuric acid. All these modifications very frequently give low results.

Coppock and Coppock⁵ attributed the low results to the greater solubility of lead oxalate than calcium oxalate. However, it is evident from the data given by Riesenfeld,⁶ and Kohlthoff and Furman⁷ that the reverse is the case and hence the low results obtained by Low's method could not be due to this cause. Further, though the instability of dilute solutions of oxalic acid has been pointed out by several investigators, contradictory statements are found in the literature regarding the oxidisability of oxalic acid by chlorine, and nitric acid.

It has now been found that 60 per cent. acetic acid is unnecessary for quantitative precipitation of the lead oxalate, and that the use of nitric acid for solution of the lead oxalate is inadmissible as it oxidises the oxalic acid. Regarding the non-quantitative liberation of oxalic acid from the precipitate by dilute sulphuric acid, it has been found that this acid is best replaced by hydrochloric acid not only for the solution of the precipitate but also for the subsequent titration. As long as the concentration of the hydrochloric acid is well below 1N, no appreciable oxidation of this acid occurs under the conditions of an oxalate-permanganate titration. A detailed account of the investigation will be published later.

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¹ Hempel, *Jahresbericht*, 1853, 627.

² Low, *J. Am. Chem. Soc.*, 1893, **15**, 550; cf. A. H. Low, *Technical Methods of Ore Analysis*; and Scott, *Standard Methods of Chemical Analysis*.

³ Morris, *Chem. and Drug.*, 1919, **91**, 52.

⁴ Wetherell, *Quart. J. Pharm.*, 1935, **8**, 453.

⁵ Coppock and Coppock, *Volumetric Analysis*, 1934.

⁶ Riesenfeld-Ray, *A Manual of Practical Inorganic Chemistry*, 1933, p. 449.

⁷ Kohlthoff and Furman, *Volumetric Analysis*, 1928, p. 271.

Structure of the (3, 0) Band $\lambda 2569$ of the OD Molecule

IN continuation of the work on the structure of the OD bands of Heavy Water reported previously¹ the (3,0) band at $\lambda 2569$ has been photographed and measured. On account of the relatively small intensity of the band, exposures for about four hours have been found necessary using the medium Hilger Quartz Spectrograph (dispersion 10 Å per mm. approx. at $\lambda 2600$). The band corresponds to the one at $\lambda 2447$ of OH, due to the electronic transition ${}^2\Sigma^+ \rightarrow {}^2\pi_{inv}$. The rotational structure has been analysed and the six main P, Q, R, branches are derived. The values of the constants have been calculated to be (in cm.^{-1})

$$B'_3 = 8.13 \quad B''_0 = 9.94$$

Details of the structure will be published elsewhere.

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¹ *Curr. Sci.*, 1940, **9**, 172; 1940, **9**, 225.
Nature, 1940, **145**, 778.

Typical Colour Curves and their Application for Purity Tests in Physiological Researches

IN a recent communication¹ from these laboratories a new Photoelectric Photometer was described for chemical analysis, based on the measurement of light absorption of the solution of substance occasioning a colour reaction, within a narrowly defined region of the spectrum with the aid of a quantitatively variable light diminution; photoelectric cells (Caesium Becker & Co.) being used to indicate equivalence of light.

A successful application of the above instrument for purity tests based on colour measurements has been in an examination of chlorophyll solutions for carotenoid impurities. Carotin has a characteristic absorption in the region 560-430 $\mu\mu$, the corresponding band for chlorophyll