

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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K. NEELAKANTAM.

Department of Chemistry,
Andhra University,
Waltair,
July 11, 1940.

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.

M. G. SASTRY.

Andhra University,
Waltair,
July 14, 1940.

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

being 750-570 $\mu\mu$ (Fig. 1). It has been found by us that Beer's law holds for these mixtures and a straight line relation is obtained between the change in the logarithms of the extinction coefficient K of the mixture and the percentage of carotin, at a wave-length of 470 $\mu\mu$. Curves 1 and 2 in Fig. 1 have been obtained with

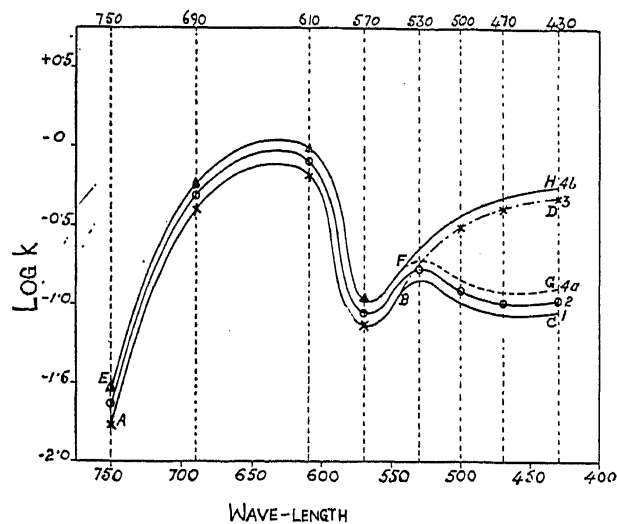


FIG. 1

Typical colour curves of chlorophyll solution in 80 per cent. methyl alcohol. Curve 1, (A B C) 4 mg. in 100 c.c. Curve 2, 6 mg. in 100 c.c. Curve 3, (A B D) 4 mg. of chlorophyll in 100 c.c. with 1.2 per cent carotin as an impurity. Curve 4b (E F H) drawn from observational readings of a test sample of chlorophyll. Curve 4a (E F G) the ideal curve for the same sample after comparing with the normal curve.

chlorophyll solutions in 80 per cent. methyl alcohol, containing in 100 c.c. 4 mg. and 6 mg. respectively. As Beer's law holds, the two curves are parallel. Curve 3 has been obtained with a solution of 4 mg. of chlorophyll in 100 c.c. and contains 1.2 per cent. carotin as an added impurity. Curve 4b is plotted from the observations on a test sample of chlorophyll. From a perusal of the region of deviation from the normal shape (Fig. 1, A B C), an ideal Curve 4a can be drawn and the actual change in log K caused by the carotin in this absorption region can be measured and the percentage of carotin read off from a calibration curve.

By the above procedure, it has been possible to detect carotenoid impurities in chlorophyll solutions down to a concentration of 0.05 per cent. The method of colour measurements and

purity tests described here for chlorophyll solutions is applicable to other substances following Beer's law. The method has been specially useful in the laboratories in maintaining the purity of certain culture solutions by comparing their colour curves at regular intervals.

B. N. SINGH.

N. K. ANANTHA RAO.

Institute of Agricultural Research,
Benares Hindu University,
July 25, 1940.

¹ B. N. Singh and N. K. Anantha Rao, *Plant Physiology*, April 1937.

Utility of Leaf-tip Smear Technique

FOLLOWING the method of J. T. Baldwin,¹ tips of very young leaves about 1 mm. in length from the apex were selected from the youngest terminal bud, they were put in Carnoy's fixative (chloroform-acetic acid-alcohol) for about 10 minutes, were passed through a mixture of 95 per cent. alcohol and concentrated HCl in equal proportion for not more than 5 minutes, they were again dipped in Carnoy's for 5 minutes. Now the leaf-tips were put in a drop of aceto-carmine on a slide, with the help of a scalpel these leaf-tips were torn and put under a coverglass. With uniform and gentle pressure of the scalpel on the coverglass the leaf-tips were crushed uniformly and the slides were then passed over a flame three or four times, taking care not to boil the aceto-carmine, and each time a slight pressure was applied on the coverglass to spread the smear uniformly. The slides were ringed carefully and stored in a cool place in the dark for three or four days when the nuclear stains were found to have developed satisfactorily. The slides were then made permanent according to Barbara McClintock's² method. The smears showed all the phases of the somatic mitosis as in Fig. 1, and in some cases the chromosome-counts were possible.

The advantage of this leaf-tip-technique is that in a short time all the phases of the nuclear division can be demonstrated to beginners in