107. PHOTOELECTRIC ESTIMATION OF INDOLE

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INDOLE and its derivatives can be conveniently detected in small concentrations by means of the colours which they produce in the presence of acidified alcoholic *p*-dimethylaminobenzaldehyde (Ehrlich's reagent). Unfortunately, these colours are often transient and do not lend themselves readily to quantitative work. This difficulty has been overcome by using a photoelectric colorimeter. With this instrument, a measurement of the optical density [$\log$ (incident intensity/transmitted intensity)] of a solution could be made within 7 sec. of the colour being developed, and the readings so obtained were reproducible. The method has been employed for concentrations up to an equivalent of 2 mg. of indole per 100 ml. of solution, by the technique about to be described.

Instrument

The colorimeter was a Hilger 'Absorptiometer' [Hilger, 1936]. 1 cm. cells were used with a combination of colour filters (Nos. 6 and 7) giving maximum transmission at about 4400 Å., this being found to give optimum sensitivity. The instrument scale is calibrated directly in optical densities.

Ehrlich's reagent

1.5 g. of *p*-dimethylaminobenzaldehyde were dissolved in 175 ml. of ethyl alcohol, and 30 ml. of conc. HCl were added [cf. Cole, 1933].

Method

5 ml. of the solution to be tested were diluted with an equal volume of water and 5 ml. of the reagent (which could be warmed previously with advantage) were added. The mixture was rapidly brought just to boiling point, when the colour was fully developed. The coloured solution was transferred immediately to a 1 cm. absorption cell and matched in the absorptiometer. With practice, the time from boiling to the completion of matching could be reduced to 7 sec. The density was then read, using water in the comparison cell. Since this last adjustment is made independently of the coloured solution, fading cannot affect the result once the initial matching is completed.

Calibration

A solution containing 1.6 mg. of pure indole (Roche Products: M.P. 52.5°) in 100 ml. of water was employed, and a series of standards containing 5, 4, 3, 2, 1 and 0 ml. of this in 10 ml. was prepared. Each was heated to boiling point in turn with 5 ml. of Ehrlich's reagent and its colour density measured. The colour produced by indole itself is quite stable and several settings of the absorptiometer scale could be made. At these low concentrations the colours are not intense, but there is a linear relationship between concentration and optical density which breaks down in stronger solutions. The values appeared to depend

on the age of the reagent and it was desirable to prepare a new calibration curve for each set of estimations. A typical calibration is set out in Table 1; the positive density at zero concentration of indole is due to the residual colour of the reagent.

Table 1. *Calibration of absorptiometer scale against indole concentrations*

Concentration of indole mg./100 ml.	Scale reading $d_{1\text{ cm.}}$	Concentration of indole mg./100 ml.	Scale reading $d_{1\text{ cm.}}$
1.60	0.185	0.64	0.090
1.28	0.152	0.32	0.059
0.96	0.122	0.00	0.029

Accuracy

Over this range of densities the scale is divided to 0.01 and can easily be read to 0.001 by interpolation. For 8 readings at 1.60 mg./100 ml. the observed variation gives an estimated probable error less than 0.001 in density, or less than 0.5 % in concentration, for the mean value; at 0.96 mg./100 ml. the error is about 0.8 %, and for the most dilute solution, where the probable error in density, over a series of readings, is still only 0.001, the probable error in concentration is 1.6 %.

SUMMARY

Small concentrations of indole and its derivatives can be estimated quickly and accurately with Ehrlich's *p*-dimethylaminobenzaldehyde reagent by the use of a photoelectric colorimeter.

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