EMMERSON, M. F. & HOLTZER, A. (1967).   *J. phys. Chem., Ithaca*, **71**, 3320–3330.

HILDEBRAND, J. H. & SCOTT, R. L. (1964a).   *Solubility of Non-electrolytes*, 3rd Edn, p. 129, New York: Dover.

HILDEBRAND, J. H. & SCOTT, R. L. (1964b).   *Ibid.*, p. 267.

SCHICK, M. J. & GILBERT, A. H. (1965).   *J. Colloid Sci.*, **20**, 464–472.

WALKER, E. E. (1952).   *J. appl. Chem.*, **2**, 470–481.

The estimation of aqueous solutions of ω-diazoacetophenones by polarography

The involvement of the diazo group in the inhibition of purine synthesis has been demonstrated by Levenberg, Melnick & Buchanan (1957), thus making the study of this group important in biological systems.   We have investigated the effects of a group of ω-diazoacetophenones, on several biological systems both *in vivo* and *in vitro*.   During the course of this work we have found that existing methods of estimating these compounds in aqueous buffers, and in solutions containing organic material, are inadequate.

ω-Diazoacetophenones have characteristic absorption bands in the ultraviolet region of the spectrum (Leveson & Thomas, 1966), and this property has been used to determine the concentration of these compounds.   For instance, Aziz & Tillet (1968) studied the rate of hydrolysis of diazoketones by following spectrophotometrically the decrease in their characteristic absorbances.   In solutions containing protein this method is unsatisfactory, due to interference in the absorption at about 280 nm.

We have found a convenient way of assaying ω-diazoacetophenones is by polarography.   The DC polarographic behaviour of ω-diazoacetophenone has been
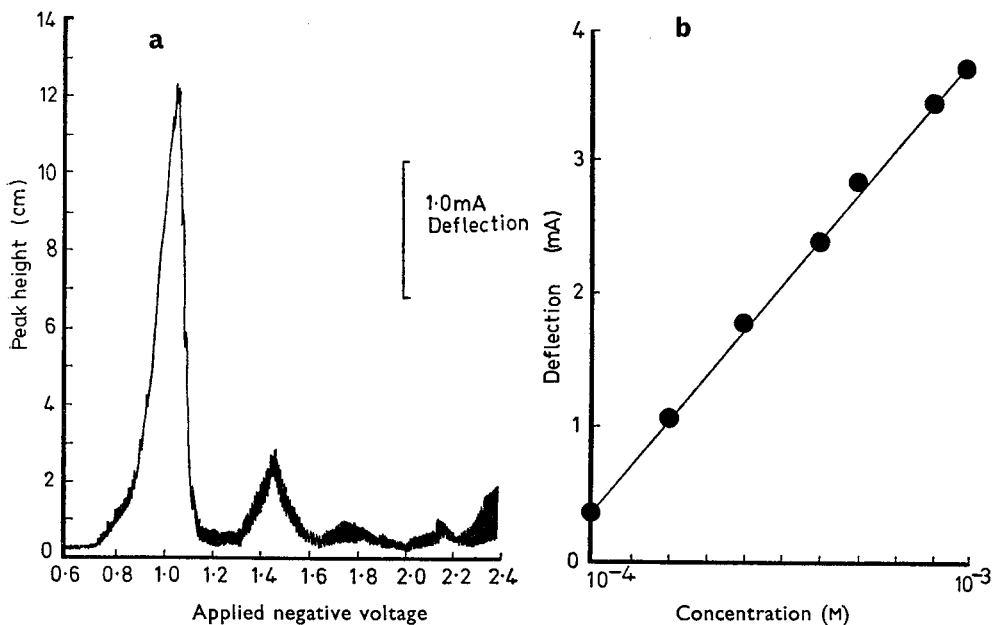

FIG. 1.   a.   AC polarogram of 10^{-3}M ω-diazoacetophenone in 0·2M phosphate buffer at pH 6·7. The height of the primary peak is dependent on the concentration of the ω-diazoacetophenone. Substituted ω-diazoacetophenones (10^{-4}–10^{-3}M) give similar traces under the same conditions.

b.   The calibration curve of ω-diazoacetophenone in 0·2M phosphate buffer, pH 6·7, at 25°, using AC polarography.

reported (Foffani, Salvagnini & Pecile, 1959; Coombs & Leveson, 1964), and the behaviour of some nuclear substituted diazoacetophenones in aqueous buffer solutions has been described (Bailes, 1968). The polarograms typically show three waves, the first of which (a six electron wave) is concentration dependent, and can be used for quantitative analysis. In our work we have used AC rather than conventional polarography. This modification gives a trace that is suitable for assay purposes (Fig. 1a). Characteristically it shows a primary well defined peak, the height of which is concentration dependent, followed by two smaller peaks, which do not interfere with the measurement of the primary peak.

For ω-diazoacetophenones which are sufficiently soluble, it is convenient for routine assay purposes to construct a calibration curve as shown in Fig. 1b. For insoluble ω-diazoacetophenones we used the calibration curve for the unsubstituted compound in the assay. This is accurate only if the diffusion current constants are the same for the unsubstituted and substituted compounds. Values of the diffusion current constant ($\mu A\ mg^{-2/3}\ s^{1/2}\ mM^{-1}$), I, for five relatively soluble ω-diazoaceto-phenones ($x-C_6H_4 \cdot CO \cdot CHN_2$) in 0·2M phosphate buffer, pH 6·7 at 25°, calculated from the results of Bailes (1968) are:

X =	H	*m*-Me	*p*-Me	*p*-OMe	*m*-Cl	*m*-F
	10·10	9·67	9·67	9·67	8·70	10·13

Although the effect of nuclear substitution on I is generally slight, it must be appreciated that there is the possibility of introducing a large error by using the technique; for example, the I value for *m*-chloro-ω-diazoacetophenone.

The polarograph used was the Cambridge general purpose model, fitted with a Univector unit. By this means a small alternating potential was superimposed upon the DC applied to the cell. The polarogram was obtained by plotting the alternating component of the current against the electrode potential. All determinations were carried out in Cambridge Instrument Co. cells. The solutions were de-oxygenated for 10 min with nitrogen before readings were taken.

Solutions of the ω-diazoacetophenones were prepared in 0·2M phosphate buffer at the pH required. Saturated solutions were prepared by shaking excess of the solid in about 10 ml of phosphate buffer at 25° for 24 h. For estimation, these solutions were transferred, with undissolved solid, to the polarographic cell, maintained at 25° by means of a water jacket.

It seems likely that polarography will prove a satisfactory method for assaying other diazo-ketones, and using AC polarography, traces in a convenient form for assay purposes can be obtained.

Pharmacology Group,   A. J. COLLINS
School of Pharmacy,   R. J. ANCILL
Bath University of Technology,
Bath, Somerset, U.K.
October 23, 1969

REFERENCES

AZIZ, S. & TILLET, J. G. (1968). *J. chem. Soc. (B)*, 1302–1307.
BAILES, M. (1968). Ph.D. Thesis Bath University of Technology, P.154.
COOMBS, D. M. & LEVESON, L. L. (1964). *Analytica chim. Acta*, 30, 209–210.
FOFFANI, A., SALVAGNINI, L. & PECILE, C. (1959). *Ann. Chim. (Rome)*, 49, 1677–1688.
LEVESON, L. L. & THOMAS, C. W. (1966). *Tetrahedron*, 22, 209–215.
LEVENBERG, B., MELNICK, I. & BUCHANAN, J. M. (1957). *J. biol. Chem.*, 225, 163–176.