395. *Studies with Dithizone. Part II. Dithizone as a Monobasic Acid.*

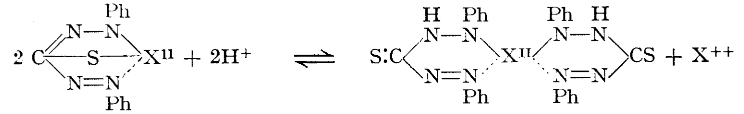
By H. Irving, S. J. H. Cooke, S. C. Woodger, and R. J. P. Williams.

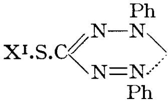
The assumption implicit in all previous work that metal complexes of dithizone result from the replacement of one (or more) of its hydrogen atoms is proved true for the silver complex. Attempts to determine the basicity of dithizone by conventional methods failed owing to its virtual insolubility in water. However, dithizone and the yellow " dithizonate " ion obey Beer's law, and spectrophotometric measurements in a water–alcohol–chloroform monophase showed that its acid strength was intermediate between that of methyl-red and bromothymol-blue with pK = 5·25 ± 0·15. The partition coefficients of dithizone between aqueous buffers and carbon tetrachloride, chlorobenzene, and chloroform severally were measured over the range pH 4—13. Combined with measurements of p_0, the partition coefficient of undissociated dithizone, they demonstrate that it is a monobasic acid with pK 5·77 ± 0·25 : no indication of a second stage of ionisation was obtained. An explicit relationship of general applicability is deduced between the percentage of material extracted and the pH of the aqueous phase in terms of pH$_{\frac{1}{2}}$, the pH at which the partition coefficient is unity (50% extraction). This magnitude (which is simply related to p_0 and pK) is shown to be a characteristic of extraction curves all of which have the same form and are completely superimposable by lateral displacement. Molecular-weight determinations by ebullioscopic and isopiestic methods confirm the absence of association in a dilute solution of dithizone. Attention is drawn to the possibilities of internal hydrogen bonding in dithizone, its anion, and metal complexes.

Although " dithizone " (diphenylthiocarbazone) (IV) is undoubtedly one of the most valuable and widely used organic reagents for the colorimetric determination of trace metals, few of its metal complexes have been obtained in a state of purity or completely analysed and nothing is known of their structure. Of the structural formulæ proposed by Fischer (*Angew. Chem.*, 1934, **47**, 685; 1937, **50**, 919) and extensively quoted (cf. Sandell, " Colorimetric Determination of Trace Metals," New York, 1944, pp. 74, 397), it may be said that some are stereochemically impossible (*e.g.*, I) and others are improbable (II; cf. Pfeiffer and Glaser, *J. pr. Chem.*, 1938, **151**, 134), whilst confirmatory evidence is lacking in all cases. It should be emphasised that spectrophotometric studies of dilute solutions of dithizone and its metal complexes cannot do more than demonstrate how many molecules of dithizone reagent have interacted with one equivalent of metal. Thus the formula PbDz for lead " dithizonate " (Liebhavsky and Winslow, *J. Amer. Chem. Soc.*, 1937, **59**, 1966), later revised to $PbDz_2$ (Clifford, *J. Assoc. Offic. Agric. Chem.*, 1943, **26**, 26), or the formulæ AgDz, $HgDz_2$ . . . . $BiDz_3$ (Irving, Andrew, and Risdon, this vol., p. 539) assume implicitly that dithizone is behaving as a monobasic acid in forming these complexes.*

Owing to the low percentage of hydrogen in the metal dithizonates, the difficulty of obtaining them as pure solids, and the fact that they may contain water (*e.g.*, $ZnDz_2,H_2O$; Fischer, *Annalen*, 1882, **212**, 316; $ZnDz_2,\frac{1}{2}H_2O$, Irving and Williams, unpublished observation), it is difficult to obtain formal proof that one (or more) hydrogen atom is replaced by a metal in complex formation and the possibility of the reagent being simply co-ordinatively attached (cf. thioacetamide in its argentous complex, $[Ag \longleftarrow (S{:}CMe{\cdot}NH_2)_4]$; Cox, Wardlaw, and Webster, *J.*, 1936, 776). That dithizone itself displays acidic character follows from the fact that, though very insoluble in water, it dissolves readily in aqueous alkali to give a yellow solution from which it is re-precipitated on acidification. The decrease in pH when a neutral solution of a silver salt is shaken with dithizone (p. 1853) is a direct proof that metal atoms displace hydrogen in the formation of the complex. The influence of pH upon the extractability of metals by dithizone (cf. Sandell, *op. cit.*) confirms this, and the successful quantitative treatment of the zinc–dithizone system (Kolthoff and Sandell, *J. Amer. Chem. Soc.*, 1941, **63**, 1906; Irving and Williams, unpublished) depends *inter alia* on the assumption that the reagent behaves as a monobasic acid.

Now Fischer postulates that all metal dithizonates can exist in so-called keto-forms in which the hydrogen atom of one phenylimino-group is replaced by metal (as II). Such complexes form preferentially in acid or neutral solution, but in basic solutions or with a deficiency of dithizone they pass over into " enol " complexes (as I or III) derived from the thiol form of the reagent by the loss of two hydrogen atoms. The evidence for such changes of composition (erroneously referred to as tautomerism) is qualitative and scanty and would appear to be based largely upon observations of the copper–dithizone system (Fischer and Leopoldi, *Wiss. Veröff. Siemens-Konzern*, 1933, **12**, 44) though neither the violet-red " keto " nor the yellow-brown " enol " complex has been obtained pure and characterised. The implication that dithizone has *two* dissociable hydrogen atoms is, however, inevitable and in connection with an extended study of metal–dithizone systems we decided to examine the behaviour of dithizone as an acid and to evaluate at least its first dissociation constant, which is of importance when considering the extent of partition of the reagent between aqueous buffers and an immiscible organic phase (p. 1850; cf. Irving, Andrew, and Risdon, this vol., p. 541) and the extent to which the extraction of a metal–dithizone complex is influenced by pH (Irving and Williams, preceding paper).


(I; " enol.") (II; " keto.")


(III; " enol.")

(X^I and X^{II} are univalent and bivalent metals, respectively.)

Krebs and Speakman (*J.*, 1945, 593) have shown that the dissociation constant of a sparingly soluble acid can be calculated from its solubility, *S*, in various buffer solutions and the solubility, S_0, of the undissociated acid. The method failed with dithizone for in the time required to reach saturation considerable decomposition had taken place in the more alkaline solutions, whilst in neutral or acid solutions the solubility was too small to be measured accurately.

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* Most authors (cf. Sandell, *op. cit.*) write Dz as the symbol for dithizone itself and Dz′ for the *uncharged* residue $C_{13}H_{11}N_4S$. We suggest that this confusing and ambiguous nomenclature should be abandoned in favour of HDz for the acidic reagent dithizone ($C_{13}H_{12}N_4S$), reserving Dz for the uncharged residue $C_{13}H_{11}N_4S$, and Dz′ for the singly charged dithizonate anion.

Graphical extrapolation did not give a reliable value for S_0, and although it proved possible to measure $(S - S_0) \propto [\text{Dz}']$ absorptiometrically, a direct determination of S_0 failed owing to the virtual insolubility of the reagent in dilute acids or neutral solutions. For the same reason conventional physical methods (pH titration, conductivity measurements, etc.) were equally inapplicable.

Preliminary measurements having shown that the ionisation of dithizone was complete at about pH 10·5, and that the yellow solution of its ions ($\lambda_{\text{max.}} = 405$ mμ) obeyed Beer's law up to $E(0{\cdot}25 \text{ cm.}) = 0{\cdot}5$, we proposed to measure the optical density in a series of buffers of decreasing pH and thus to obtain a measure of the variation in [Dz′] whence the dissociation constant could be calculated (cf. Michaelis and Gyemant, *Biochem. Z.*, 1920, **109**, 165; 1921, **119**, 307). But when even the smallest quantity of an alcoholic solution of dithizone which was sufficient to give a measurable optical density when dissolved in dilute ammonia (complete ionisation) was added to a buffer of pH 7 or 8·5, the resulting yellow solution had a definite pink hue and gave a positive Tyndall cone test showing that part of the dithizone had been thrown out of solution as a colloid.

When ammonia gas is passed into a chloroform solution of dithizone a deep red solid separates which must be an unstable ammonium salt since it readily loses ammonia and regenerates dithizone. Similarly the addition of *n*-butylamine to a solution of dithizone in chlorobenzene changed the colour from green to red, the change being more or less reversed by adding varying amounts of acetic acid (or phenol), the exact hue depending upon the relative amounts of green reagent and red dithizonate ion. Attempts to follow the equilibrium spectrophotometrically and hence to determine the relative acidities of dithizone and acetic acid (or phenol) in chlorobenzene, and thence the dissociation constant of the former in water (Griffiths, *J.*, 1938, 818), were fruitless since the optical density of the mixed solutions varied with time and in such a way as to preclude any simple and reliable extrapolation to equilibrium conditions.

It is well known that solutions of dithizone in chloroform give two absorption bands with $\lambda_{\text{max.}}$ 605 and 445 mμ (in carbon tetrachloride, 615 and 446 mμ), the first being the more intense. Though insoluble in water and but sparingly soluble in alcohol, dithizone dissolves well in a monophase consisting of 20% water, 30% chloroform, and 50% ethyl alcohol, and when acid is added there results a green solution with $\lambda_{\text{max.}}$ 595 and 440 characteristic of the (presumably) undissociated molecule. When alkali is added the colour changes to red and the absorption spectrum now shows a single absorption band with $\lambda_{\text{max.}}$ 485 corresponding with that of the dithizonate ion already observed in aqueous solution ($\lambda_{\text{max.}}$ 465 mμ) or in chlorobenzene ($\lambda_{\text{max.}}$ 490). By additions of drops of an appropriate buffer a series of solutions were prepared containing the same total concentration of dithizone but varying proportions of the undissociated acid and its ion. The transmittancy of these solutions was measured at 485 and 598 mμ, and the pH at which the dithizone was 50% dissociated was found to be 6·3 and 6·4, respectively. The pH of the lightly buffered solutions was measured with a glass electrode–standard calomel cell combination, and it need scarcely be pointed out that neither they nor the average value of 6·35 for the p*K* of dithizone in the mixed solvent can be directly correlated with similar measurements in aqueous solution. However, evidence from another direction (p. 1850) suggested that the dissociation constant of dithizone in water was of the order of 10^{-6}, whilst the indicators bromothymol-blue and methyl-red are known to be somewhat weaker and stronger acids with p*K* values of 7·1 ± 0·05 and 5·10 ± 0·05, respectively. Using the same mixed solvent and procedures as in the case of dithizone, spectrophotometric measurements gave the values 8·45 ± 0·05 and 6·2 ± 0·1 for their acid exponents in this medium. There is no reason to suppose that the order of acid strengths in the mixed solvent, *viz.*, methyl-red > dithizone > bromothymol-blue, will not be preserved in water. Since the mixed solvent is markedly protophilic, containing as it does 70% of aqueous alcohol, we may further assume that the relative strengths of these acids will not be seriously affected by change of solvent, whence by linear interpolation of p*K* values we calculate the dissociation constant of dithizone in water to be $5{\cdot}6 \times 10^{-6}$ (±40%) or $pK = 5{\cdot}25 \pm 0{\cdot}15$.

The extent to which aqueous alkaline solutions will extract dithizone is of great importance in trace-metal analysis (Irving, Andrew, and Risdon, *loc. cit.*), but no systematic study has yet been undertaken though several qualitative observations and a few measurements by Clifford and Wichmann with chloroform and carbon tetrachloride have been recorded (*J. Assoc. Offic. Agric. Chem.*, 1936, **19**, 130). Apart from their intrinsic interest such data should be capable of demonstrating whether dithizone can behave as a polybasic acid and, as the following treatment will show, should afford data for the respective dissociation constants. Though we shall specify dithizone as the solute, the treatment is generally applicable to other acids which fulfil the necessary conditions and, with obvious modifications, to the partition of bases between aqueous buffers and an immiscible organic phase.

If dithizone, HDz, exists in both water and organic solvents as unassociated molecules and ionises only in the former, its partition coefficient, p, will be given by

$$p = \frac{\text{total concentration of dithizone in water}}{\text{total concentration of dithizone in organic layer}}$$

$$= \frac{[\mathrm{HDz}]_w + [\mathrm{Dz'}]_w}{[\mathrm{HDz}]_o} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where the subscripts w and o refer to the aqueous and the organic phase respectively. When the acidity is sufficiently high to repress the ionisation of the acid completely, the partition coefficient becomes $p_0 = [\mathrm{HDz}]_w/[\mathrm{HDz}]_o$, the constant value for undissociated dithizone. $K_1 = \{\mathrm{H}\}_w[\mathrm{Dz'}]_w/[\mathrm{HDz}]_w$ being written for the Brønsted (first) dissociation constant, equation (1) reduces to

$$p = p_0 + p_0K_1/\{\mathrm{H}\}_w \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

A plot of p against $1/\{\mathrm{H}\}_w$ should thus be a straight line with slope p_0K_1 and intercept p_0. The corresponding equation for a dibasic acid would be

$$p = p_0 + p_0K_1/\{\mathrm{H}\}_w + p_0K_1K_2/\{\mathrm{H}\}_w^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

so that a plot of p against $1/\{\mathrm{H}\}$ would be curved, the curvature becoming more pronounced as the pH rises, though the deviation from linearity may be small if K_2 is very small.

Chlorobenzene was found to be an excellent solvent for dithizone, yielding stable green solutions ($\lambda_{\max.}$ 618 and 442) in which Beer's law was obeyed up to at least E(1 cm.) = 0·75. The concentration of dithizone in this solvent and in chloroform and carbon tetrachloride (cf. Irving, Andrew, and Risdon, *loc. cit.*) could readily be determined absorptiometrically before and after shaking with an equal volume of an aqueous buffer, and in this way the partition coefficient was determined for each of these solvents over the range of pH 4—13. Changes of phase ratio on mixing were eliminated by pre-saturating the aqueous buffers with organic solvent and the dithizone solution with water. Scrupulous care was taken to remove all traces of metal from the solvents, buffer solutions, and apparatus used, and the successful avoidance of metal contamination was demonstrated in several cases by obtaining identical partition coefficients in the presence of, and in the absence of 2 : 3-dimercaptopropanol (BAL) (cf. Irving, Andrew, and Risdon, *loc. cit.*). Plots of p against $1/\{\mathrm{H}\}$ (not reproduced) were linear; the long graphical extrapolation, however, could scarcely be expected to give reliable values for the partition coefficients of the undissociated dithizone but at least showed that they were very small. On the other hand, the slopes of the graphs gave excellent values for the characteristic product p_0K_1 (*q.v.*).

So long as p_0 is small compared with p, equation (2) can be expressed in the logarithmic form

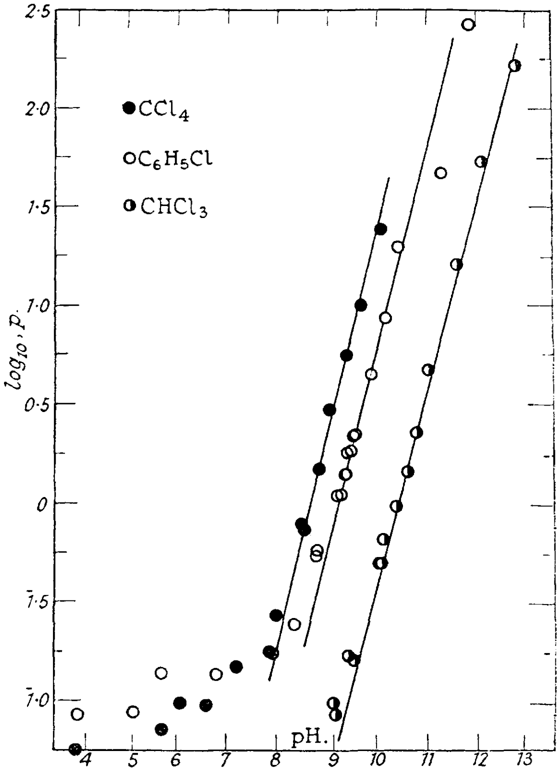
$$\log_{10} p = \log_{10} p_0 - \mathrm{p}K_1 + \mathrm{p}H \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

so the graph of $\log_{10}p$ against pH should be a straight line of unit slope. With increasing acidity of the aqueous phase the slope should diminish and the value of the ordinate will approach $\log_{10}p_0$ asymptotically. Were dithizone a dibasic acid, it follows from equation (3) that the $\log_{10}p$–pH plot would be segmented, and the slope would change from 1 to 2 as the influence of the second stage of acid dissociation becomes more pronounced. The graph of the experimental data shown in Fig. 1 shows conclusively that dithizone behaves as a purely monobasic acid, and in confirmation of the absorption spectra obtained in aqueous solutions of various alkalinities it affords no indication of a second stage of ionisation at least over the range pH 4—12. If effective at all in aqueous solution, the second stage of ionisation of dithizone must be that of a very weak acid with K_2 10^{-12}.

At low pH values the experimental points show a marked scatter (Fig. 1) and extrapolation to obtain values of p_0 is again impracticable. Since, however, the magnitude of the partition coefficient must be independent of phase volumes, a direct determination of p_0 was attempted by partitioning dithizone between the organic solvent and 50 and 75 times its volume of N/100-hydrochloric acid. The values $6{\cdot}4 \pm 1{\cdot}7 \times 10^{-4}$ and $3{\cdot}3 \pm 0{\cdot}3 \times 10^{-4}$ were obtained for carbon tetrachloride and chlorobenzene, respectively. Combining these with the corresponding values of the products p_0K_1 derived from plots of equation (2) or (4), *viz.*, $1{\cdot}26 \times 10^{-9}$ and $3{\cdot}4 \times 10^{-10}$, we arrive at values of 5·61 and 5·94 ± 0·15 for the acid exponent of dithizone. The average value 5·77 ± 0·25 is in satisfactory agreement with that obtained spectrophotometrically (p. 1849) and we adopt $pK = 5{\cdot}5 \pm 0{\cdot}3$ as a working value. All measurements were made at room temperature (20° ± 5°). The value of p_0K_1 for chloroform was found to be $2{\cdot}75 \times 10^{-11}$, whence p_0 is calculated as $1{\cdot}3 \times 10^{-5}$, a value below the range of direct measurement.

Although equation (2) successfully represents the variation of partition coefficient with hydrogen-ion activity, a simple relationship between the pH of the aqueous phase and the percentage of dithizone extracted into it would be of greater practical value. Writing equation (2) in the logarithmic form

FIG. 1.



$$\log_{10}(p - p_0)/p_0 = \log_{10}K_1 + \mathrm{pH}$$

and defining $\mathrm{pH}_{\frac{1}{2}}$ as that hydrogen-ion exponent of the aqueous phase when the partition coefficient is unity (50% extraction) we have

$$\log_{10}(1 - p_0)/p_0 = \log_{10}K_1 + \mathrm{pH}_{\frac{1}{2}}$$

Subtracting and rearranging

$$\Delta\mathrm{pH} = (\mathrm{pH}_{\frac{1}{2}} - \mathrm{pH}) = \log_{10}\left(1 + \frac{1 - p}{p - p_0}\right)$$

Since $\Delta\mathrm{pH}$ is a function of $(1 - p)/(p - p_0)$, we should be able to construct its inverse with p as a function of $\Delta\mathrm{pH}$. Explicity

$$\frac{p - p_0}{1 - p} = \frac{\coth\,(2{\cdot}3026\Delta\mathrm{pH}/2) - 1}{2}$$

and since the percentage of extraction $y = 100p/(1 + p)$, we have

$$y = \frac{50\,(\coth\,1{\cdot}1513\Delta\mathrm{pH} + 2p_0 - 1)}{(\coth\,1{\cdot}1513\Delta\mathrm{pH} + p_0)} \quad . \; . \; . \; . \; . \; . \; . \; . \quad (5)$$

So long as $\Delta\mathrm{pH}$ is large and positive ($\mathrm{pH} \ll \mathrm{pH}_{\frac{1}{2}}$), $\coth\,1{\cdot}1513\Delta\mathrm{pH} \rightarrow 1$, and equation

(5) correctly predicts the limiting percentage extraction as $100p_0/(1 + p_0)$. If, however, $|(\coth 1{\cdot}1513\Delta pH - 1)| \gg p_0$ equation (5) reduces to

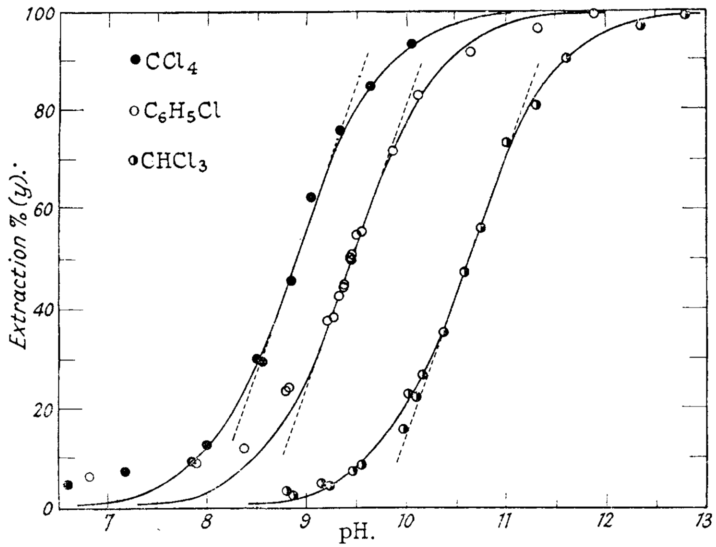
$$y = 50(1 - \tanh 1{\cdot}15\Delta pH) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad 6)$$

which reproduces values of y to within 0·1% when $p_0 = 0{\cdot}001$, and within 1% even when p_0 is as large as 0·01. By differentiation

$$dy/d(pH) = 57{\cdot}57(1 - \tanh^2 1{\cdot}1513\Delta pH) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

so that the slope at the midpoint where $y = 50\%$ and $\Delta pH = 0$ should be 57·6. Fig. 2 presents the experimental data in the form of plots of percentage extraction against pH. The curves actually drawn are those calculated by equation (6) for systems with $pH_{\frac{1}{2}}$ of 8·90, 9·45, and 10·58, respectively. The agreement is remarkably satisfactory especially in the case of chloroform where p_0 is exceptionally small. Too much emphasis need not be laid upon the discrepancies in the more acid solutions in the cases of chlorobenzene and carbon tetrachloride to which both the approximations in the use of equation (6) and the experimental difficulties attaching to measurements of very small partition values contribute. It is not without interest that the simple linear equation $y = 57{\cdot}6[(0{\cdot}87 - pH_{\frac{1}{2}}) + pH]$ is quite adequate for any curve over the range 25—75% extraction.

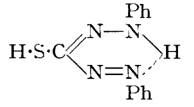
FIG. 2.



It will be appreciated from equations (6) and (7) and Fig. 2 that *all* extractability curves based on equation (2) are identical in form and can be superimposed by a mere lateral displacement along the abscissa by an amount equal to the difference between their values of $pH_{\frac{1}{2}}$. Since the most accurate measurements of partition coefficients are naturally those for which p equals, or is not far removed from, unity, values of $pH_{\frac{1}{2}}$ are more readily determinable than those of the constants p_0 or K_1 to which it is related by the expressions $pH_{\frac{1}{2}} = \log_{10} (1 - p_0)/p_0K_1 \cong pK_1 - \log_{10}p_0$. In a subsequent paper we shall discuss the extent to which the characteristic $pH_{\frac{1}{2}}$ values for dithizone and the pH values for 50% extraction in a metal–dithizone system preserve the same sequence throughout a series of solvents.

Its high melting point (163°) and sparing solubility in hydrocarbon solvents suggests that dithizone might be associated through S–H–N bonds which are known to be of considerable stability (cf. Hopkins and Hunter, *J.*, 1942, 638). Attempts to determine the molecular weight cryoscopically in benzene, naphthalene and azobenzene proved abortive on account of its low solubility, whilst Rast's method failed owing to the decomposition of dithizone at the melting point of camphor. Indeed, preparative studies (Grummitt and Stickle, *Ind. Eng. Chem. Anal.*, 1942, **14**, 953; Nillman and Cleland, *J. Amer. Chem. Soc.*, 1943, **65**, 1300) generally stress the inadvisability of heating dithizone for long above 50°; but when it was found that the optical density of a solution in chloroform was unchanged after some 10 minutes' heating at 60° an ebullioscopic method seemed practicable. In a Cottrell aparatus the elevation of the boiling point of a solution containing 1·7 g./l. was but 0·014° (calc. for monomer 0·017°) whilst in a nearly half-saturated solution (4·5 g./l.) the rise was 0·040° (calc. 0·044°). Using Barger's micro-method (*J.*, 1903, **83**, 121), a 0·018M-dithizone solution in chloroform was found to be isopiestic with azobenzene of concentration 0·017 ± 0·002 g.-mol./l. Evidence of association in these strong and obviously non-ideal solutions is thus conflicting. At the most it might amount to 20%, whence we calculate that the association cannot exceed 0·05% in solutions of the concentration (8 mg./l. or less) used in absorptiometric work. This is confirmed by the linear relationship between concentration and optical density found in a wide range of solvents, and by the successful application of equation (2) to problems of dithizone equilibrium, since in its derivation it was postulated that dithizone was unassociated in both water and organic solvent.

Dithizone has been prepared by the reduction of diphenylnitroformazyl with ammonium sulphide (Bamberger, Padova, and Ormerod, *Annalen*, 1926, **446**, 288) and in its thiol form may be formulated as *NN'*-diphenylformazylthiol (" Beilstein," Vol. XVI, p. 26). Like the diarylformazylbenzenes which Hunter and Roberts (*J.*, 1941, 820) showed to be completely unassociated, it may well be stabilised by internal hydrogen bonding (IV*a* and IV*b*). If further, as seems reasonably certain, ionisation of dithizone involves the hydrogen of the thiol group, the dithizonate anion will be similarly constituted and stabilised. Previous workers have not considered the part which resonance may play in the structure of metal–dithizone complexes. Mercury forms strong bonds with sulphur and its dithizonate could be formulated as (V).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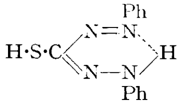
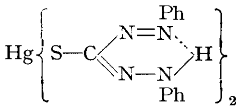

(IV*b*.)


(V.)

EXPERIMENTAL.

Materials.—All solvents, reagents, buffer solutions, and apparatus were carefully freed from trace metals, and precautions customary in work with dithizone were scrupulously observed (cf. Irving, Andrew, and Risdon, *loc. cit.*). Commercial chlorobenzene was redistilled from an all-Pyrex apparatus, the fraction of b. p. 131—133°/760 mm. being used. When kept in the dark, solutions of dithizone in this reagent showed little loss of strength over a period of some weeks. The solvent is appropriately recovered by the method recommended for chloroform by Biddle (*Ind. Eng. Chem. Anal.*, 1936, **8**, 99). Cotton-wool used in the partition work was freed from metal by repeated extraction with a solution of dithizone in chloroform, washed thoroughly with solvent, and dried in a dust-free atmosphere.

The Formation of the Silver–Dithizone Complex.—20 Ml. of 0·1N-silver nitrate (pH 6·0) were shaken with 10 ml. of dithizone solution (0·5% w./v. in chloroform). The red silver complex formed passed into the organic phase, which was then separated. The extraction was repeated with successive portions of dithizone until the persistence of the green colour showed that removal of silver had been completed. The pH of the aqueous phase was now measured and found to be 1·05, indicating a hydrogen-ion concentration of 0·1N, equivalent to that of the silver ions displaced.

The Yellow Dithizonate Ion.—(a) *Absorption spectra in aqueous solution.* The yellow solutions obtained by adding 5 ml. of a dilute solution of dithizone in alcohol to 35 ml. of 0·15N- or 1·5N-ammonium hydroxide were examined rapidly with a Hilger–Nutting visual spectrophotometer using a 4-cm. cell. The absorption spectra were indistinguishable and showed a single band with $\lambda_{max.}$ 4650 A., the position of which did not change with dilution. A series of solutions were prepared by diluting an arbitrary solution of dithizone in a borax–sodium hydroxide buffer of pH 10·5. The readings (1-cm. cell) on a Spekker absorptiometer using the tungsten lamp with an Ilford Spectrum Blue Filter No. 602 were directly proportional to concentration up to a density of 0·9. With stronger solutions in 0·25-cm. cells the calibration curve was linear up to a density reading of 0·5 and even up to 1·1 the curvature was slight and regular.

(b) *Decomposition in aqueous alkaline solution.* Successive measurement on the same solution of dithizone in 1·5N-ammonium hydroxide at 4650 A. showed that the absorbing species was decomposing at the rate of 20—30%/hour. This rate was not much affected by increased illumination or total exclusion of light, by exclusion of air, or by steady introduction of bubbles of oxygen. Hydroxylamine and traces of metals caused an increase in the rate of decomposition, but sulphite ions appeared to have no influence. The fact that the change was very slow in 0·15N-ammonium hydroxide and accelerated with the alkalinity of the aqueous buffer suggests that a hydrolysis or base-catalysed dismutation is responsible for the decomposition of such solutions.

Spectrophotometric Determination of the Dissociation Constant of Dithizone.—A mixture of 20% water, 50% ethyl alcohol, and 30% chloroform was made from freshly prepared specimens of the purified solvents. Stock solutions of dithizone, methyl-red, and bromothymol-blue were prepared by dissolving sufficient of the pure recrystallised materials to give solutions of an appropriate optical density. By adding 0·05 ml. of an appropriate acid buffer the pH of some of the dithizone solution was adjusted to 2·0 and its absorption spectrum measured with a Beckman Model D.U. Spectrophotometer using a 1-cm. cell and a nominal band width of 1 A. Absorption maxima were noted at 5950 and 4360 A. With a solution of dithizone at pH 9·5 there was a single band with a maximum at 4850 A. Similar measurements with bromothymol-blue at pH 2·4 and 9·2 gave absorption maxima at 6250 and 4250 A., respectively. Methyl-red showed a maximum at 4150 A. at pH 8·8, but in the acid solutions the absorption band in the green was relatively weak and no determination was made of its position. Buffers (approx. M/50) covering the pH range 2—10 at intervals of about 0·5 unit were prepared from suitable mixtures of hydrochloric acid, potassium hydrogen phthalate, ammonium or sodium acetate, and ammonium hydroxide. 0·05 Ml. of one of these buffers was added to 10 ml. of the stock dithizone solution, and after determination of the optical density (1-cm. cell) at 4850 and 5980 A., the pH was measured with a Cambridge pH-meter. Similar measurements with other buffer mixtures and with methyl-red and bromothymol-blue are reported in the following table.

Optical density of indicators in the mixed solvent.

pH	2·0	3·5	4·6	4·8	5·5	6·0	6·2	6·5	7·1	7·5
Dithizone at 4850 A.	0·165	0·175	0·192	0·205	0·220	0·244	0·268	0·284	0·326	0·350
„ 5980 A.	0·680	0·645	0·582	0·532	0·475	0·414	0·360	0·318	0·217	0·149

pH	8·4	9·0	10·0
Dithizone at 4850 A.	0·378	0·385	0·390
„ 5980 A.	0·102	0·059	0·045

pH	3·5	4·0	5·2	5·4	5·9	6·2	6·5	6·8	7·5	8·0
Methyl-red at 4150 A.	0·635	0·640	0·730	0·800	1·04	1·17	1·46	1·72	1·82	1·84

pH	6·0	7·5	7·9	8·2	8·4	8·5	9·0	9·7	10·0
Bromothymol-blue at 4250 A.	0·410	0·400	0·392	0·373	0·362	0·354	0·319	0·305	0·303
„ 6250 A.	0·010	0·031	0·067	0·107	0·161	0·180	0·285	0·305	0·307

The Partition of Dithizone between Aqueous Buffers and Organic Solvents.—(a) *General method.* 20 Ml. of a solution of dithizone in chloroform (or chlorobenzene or carbon tetrachloride as desired), saturated with water and of an optical density of 0·7 or less, were added to 20 ml. of a metal-free aqueous buffer presaturated with the same solvent and contained in a 125-ml. Pyrex separatory funnel. After being shaken for 1 minute or longer (100 shakes or more by hand, *q.v.*), it was allowed to stand for 2 minutes for the phases to separate. A pledget of metal-free cotton-wool was then inserted into the shortened stem of the funnel, and after rejection of the first few ml. a 1-cm. absorptiometer cell was filled with the organic phase and the optical density (E) was measured with a Spekker absorptiometer using the Ilford Orange filter No. 607. A similar measurement was made on a portion of the original dithizone solution (E_0) and the partition coefficient calculated from the quotient $p = (E_0 - E)/E$.

All optical-density readings lay well within the range over which it had previously been shown that the concentration–Spekker reading calibration curve was strictly linear. After separation of the organic phase, the pH of the aqueous phase was measured with a Cambridge pH-meter, corrections for sodium-ion concentration being made (Dole, "The Glass Electrode," Wiley, 1941) where necessary in the more strongly alkaline solutions. At least two measurements were made at each pH and the data given below are the mean of at least two, and sometimes as many as six, concordant measurements.

(b) *Rate of attaining equilibrium.* In this system partition equilibrium is reached very quickly as shown by the following absorptiometer readings:

Solvent.	pH of buffer.	Number of shakes.					
		0.	30.	50.	100.	200.	400.
$CHCl_3$	~10	0·650	—	—	0·533	0·539	0·535
$CHCl_3$	~ 9	0·650	—	—	0·618	0·614	0·616
CCl_4	8·50	0·540	0·386	0·376	0·376	0·378	—
C_6H_5Cl	9·46	0·691	0·356	0·351	0·351	—	0·352

In the case of chlorobenzene the solutions were equilibrated in a thermostat at 20°, but with the other solvents measurements were made at room temperature which seldom deviated from this by more than a few degrees. The buffer solution used were those recommended by Britton ("Hydrogen Ions," Vol. I, Chapman and Hall, 1942), *viz.*, pH 7·6—9·2, 0·05M-borax and 0·1N-hydrochloric acid; 8·5—9·5, 0·1N-acetic acid and 0·1N-ammonium hydroxide; 9·2—10·0, 0·05M-borax and 0·1N-sodium hydroxide; 10·1—11·4, 0·1M-sodium carbonate and 0·1N-hydrochloric acid; 11·0—12·1, 0·15M-disodium hydrogen phosphate and 0·1N-sodium hydroxide. They were all freed from heavy metals and other impurities which might react with dithizone by methods previously described.

(c) *Results of partition measurements.*

Chloroform.

pH	12·8	12·1	11·6	11·3	11·0	10·77	10·58	10·38	10·11	10·07	10·02
p	90·4	31·1	9·19	4·24	2·69	1·29	0·90	0·546	0·374	0·286	0·300
pH	9·97	9·53	9·47	9·18	9·13	8·85	8·80	3·97			
p	0·264	0·093	0·095	0·050	0·053	0·029	0·036	0·001			

Carbon tetrachloride.

pH	10·03	9·66	9·34	9·05	8·84	8·55	8·50	8·00	7·85	7·18	6·59
p	14·6	5·67	3·16	1·66	0·840	0·421	0·433	0·151	0·102	0·084	0·052
pH	6·07	5·67	4·99								
p	0·054	0·037	0·024								

Chlorobenzene.

pH	11·87	11·30	10·41	10·12	9·86	9·53	9·51	9·46	9·42	9·37	9·33
p	156	26·6	10·9	4·89	2·52	1·26	1·23	1·05	1·01	0·805	0·745
pH	9·28	9·20	8·82	8·80	8·36	7·90	6·81	5·69	5·15	3·98	
p	0·632	0·612	0·333	0·307	0·139	0·101	0·075	0·077	0·047	0·044	

The consistency obtained in such measurements is illustrated by the following data for the distribution of dithizone between carbon tetrachloride and a buffer of pH 8·50 :

E_0	0·540	0·538	0·538	0·540	0·538	0·286	0·286
E	0·373	0·373	0·374	0·379	0·380	0·200	0·200
Percentage extraction	30·93	30·67	30·48	29·82	29·37	30·07	30·07
Partition coefficient	0·448	0·442	0·439	0·425	0·416	0·430	0·430

The presence of adventitious traces of metals capable of reacting with dithizone would diminish the concentration of the green reagent in the organic phase and so lead to incorrect high values for the partition coefficients. Their absence was confirmed by the identity of measurements made in the presence and absence of 2 : 3-dimercaptopropanol using the technique previously described (Irving, Andrew, and Risdon, *loc. cit.*). As a further check, distribution measurements were made in the presence of this reagent (BAL) after varying amounts of copper and lead had been added deliberately to the aqueous buffers. They led to substantially the same partition coefficients as shown by the following representative data :

Measured partition coefficient.	Buffer of pH 10·11 containing Cu (μg.).				Buffer of pH 10·13 containing Pb (μg.).		
	0.	4.	8.	20.	0.	4.	8.
Without BAL	0·363	0·636	1·179	2·774	0·381	0·474	0·667
With 400 μg. BAL	0·360	0·353	0·365	0·353	0·382	0·379	0·380

(d) *The partition coefficient of undissociated dithizone.* If E_0, the initial optical density of a solution of dithizone, is reduced to E on shaking with an equal volume of buffer, the partition coefficient, p, is given by $E = E_0/(1 + p)$. When the phase ratio is increased n-fold, the resulting optical density $E_n = E_0/(1 + np)$, whence $p = (E - E_n)/(nE_n - E)$. Values for p_0 can thus be obtained if the pH of measurement is sufficiently low. 10 Ml. of a solution of dithizone in carbon tetrachloride saturated with water were shaken with 10 ml. of N/100-hydrochloric acid which had previously been freed from all metal impurity and saturated with carbon tetrachloride. Five experiments gave the mean value $E = 0{\cdot}492 \pm 0{\cdot}003$. Extraction of 10 ml. of the same dithizone solution with 500 ml. of the hydrochloric acid gave $E_n = 0{\cdot}477 \pm 0{\cdot}003$, whence $p_0 = 6{\cdot}4 \pm 1{\cdot}7 \times 10^{-4}$. With chlorobenzene $E = 0{\cdot}516 \pm 0{\cdot}001$, and with a phase ratio of 75 : 1, E_n was $0{\cdot}504 \pm 0{\cdot}0003$, whence $p_0 = 3{\cdot}1 \pm 0{\cdot}3 \times 10^{-4}$. Even with a phase ratio of 100 : 1 the values of E_n and E were not significantly different when solutions of dithizone in chloroform were studied.

The Molecular Weight of Dithizone.—Isopiestic measurements using Barger's micro-technique (*loc. cit.*) were carried out in chloroform solution. Pyrex capillaries of about 0·05 mm. diameter were used and incubation at 37° served to hasten the distillation process. In introducing drops of the very concentrated solutions of dithizone alternately with pale yellow solutions of azobenzene, the latter became tinged faintly green and served as most sensitive indicators of the absence of metallic impurities : discoloration led to the rejection of one set of experiments only.

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