116. *Studies with Dithizone. Part I. The Determination of Traces of Mercury.*

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The influence of acidity, halide-ion concentration, and time of shaking upon the extractability of mercury and copper by dilute solutions of dithizone in both chloroform and carbon tetrachloride has been studied. The quantitative determination of mercury in the presence of copper is best effected by extraction with a solution of dithizone in chloroform from a sulphuric acid solution of pH $\sim$1, as free from halide ions as possible. Even under these optimum conditions some copper is co-extracted as " dithizonate " and mercury is seriously over-estimated when an equal or greater weight of copper is present. By a sensitive new absorptiometric procedure (" reversion "), mercury can be determined without error in the presence of at least 20 times as much copper : a change of optical density of 0·1 unit (1-cm. cell) corresponds to 0·32 p.p.m. of mercury. The *mercury–dithizone complex* has been prepared as a scarlet solid and shown to possess the formula $Hg(C_{13}H_{11}N_4S)_2$.

MANY procedures have been elaborated in the past decade for the determination of mercury in biological material with diphenyl- and di-β-naphthyl-thiocarbazone (*e.g.*, Reith and van Dijk, *Chem. Weekblad*, 1940, **37**, 186; Laug and Nelson, *J. Assoc. Offic. Agric. Chem.*, 1942, **25**, 399; Cholak and Hubbard, *Ind. Eng. Chem. Anal.*, 1946, **18**, 149), for these reagents are not very selective and interference from a number of metals, *e.g.*, manganese, iron, copper, and zinc, must be minimised. To overcome interference by copper, Barnes (*Analyst*, 1946, **71**, 578; *J. Marine Biol. Assoc.*, 1946, **26**, 343) has used potassium cobalticyanide successfully as a complexing agent, whilst Milton and Hoskins (*Analyst*, 1947, **72**, 6) have revived the principle of extractive titration. Maren (*J. Lab. Clin. Med.*, 1943, **28**, 1511), on the other hand, goes so far as to state that " copper need not interfere if the proper conditions of pH are maintained." Should this be true irrespective of the relative amounts of copper and mercury present, a very simple and quick procedure could be devised for the quantitative determination of the latter. We could not, however, confirm the statement (*loc. cit.*) that " in 50 c.c. [of] 0·25N-hydrochloric acid, 1000 mg. of copper does not change the colour of dithizone reagent " and since Maren does not state explicitly the " proper " pH for extraction, we thought it desirable, not only to determine the optimum pH for the determination of mercury in the presence of a variable excess of copper, but also to ascertain what latitude is permissible in this adjustment of pH and in the composition of the aqueous phase undergoing extraction. Some workers have used chloroform, others carbon tetrachloride, as the solvent for the dithizone, seldom indicating the reasons for their choice. We have therefore made a study of the pH–extractability curves for mercury and copper, using both of these organic solvents.

Dilute solutions of dithizone in chloroform (6·5 mg./l.) being used, the percentage of mercury extracted from solutions in hydrochloric acid was found to be significantly smaller than from sulphuric acid of the same normality (Fig. 1). Extractability should, however, be a function of ion activity rather than concentration, and data for the activity of hydrogen ions in hydrochloric and sulphuric acid (Harned and Owen, " The Physical Chemistry of Electrolytic Solutions," Reinhold, 1943) being used, the percentage of mercury extracted, plotted against p{H} is shown in Fig. 2. The difference between the two acidic media is now less marked, but the extractability of mercury from hydrochloric acid is consistently less than from sulphuric acid of the same pH, the divergence increasing with concentration in a manner which suggests that ions of the type $HgCl_3'$ or $HgCl_4''$ are involved. Consistently with this hypothesis, the

FIG. 1.

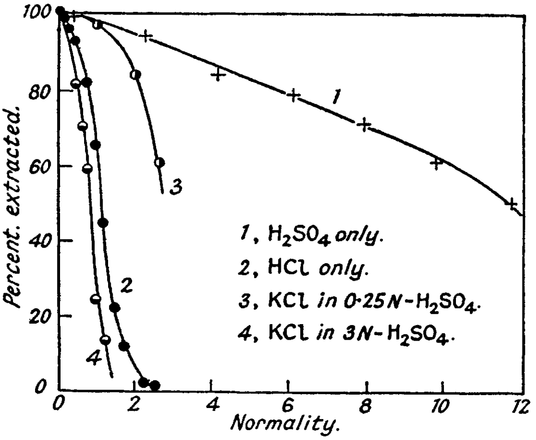
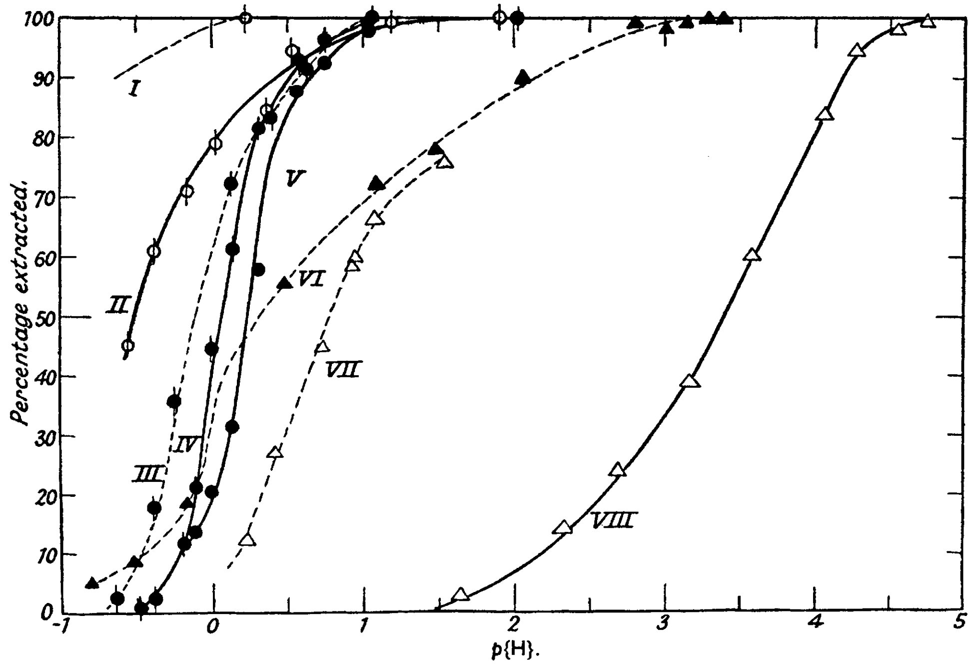


FIG. 2.



Solutions in carbon tetrachloride ------ ; *solutions in chloroform* ————. *Copper in sulphuric acid* △, *curves VII and VIII. Copper in hydrochloric acid* ▲, *curve VI. Mercury in sulphuric acid* ○, *curves I and II ; mercury in hydrochloric acid* ●, *curves III, IV, and V.*

Curves I—IV refer to a variable excess of dithizone ; curves V—VIII are for a constant excess of this reagent.

extractability of mercury from 0·25N- and 3N-sulphuric acid is progressively reduced by addition of potassium chloride (Fig. 1) : equivalent concentrations of bromide or iodide are still more effective (p. 546). Curves for the extractability of mercury by solutions of dithizone in carbon tetrachloride were found to lie to the left of those for chloroform, but experimental difficulties limited the number of observations in the more strongly acid solutions.

If a fixed amount of dithizone is used to extract increasing amounts of metal, it is obvious that the excess of reagent remaining in the organic phase will gradually decrease. Since extractability increases as the square of this excess concentration (provided that the equation tested by Kolthoff and Sandell, *J. Amer. Chem. Soc.*, 1941, **63**, 1906, for the zinc–dithizone system is applicable here), the resulting extractability curves will lie to the left of those obtainable when the concentration of excess dithizone is maintained constant throughout. Curves plotted for both " variable " and " constant " excess of reagent are reproduced in Fig. 2, whence it will be seen that their difference is less significant than the positional displacement along the pH axis caused by a change in the metal being extracted.

Discussion.—From an inspection of the mutual disposition of the extractability curves shown collectively in Fig. 2, it is clear that carbon tetrachloride (broken lines) is a less suitable solvent than chloroform (continuous lines), although even at the lowest pH permissible for the quantitative extraction of mercury some copper will still be extracted. There is, however, a more cogent reason for preferring chloroform. The actual *rate* of attaining partition equilibrium in the two-phase system comprising initially a solution of dithizone in an organic solvent and an aqueous solution of a metal depends upon the organic solvent as well as the pH and the concentration of the reacting species. The following data show that from solutions of the same acidity the initial rate of extraction of copper is greater with a solution of dithizone in carbon tetrachloride than with one in chloroform.

Copper (μg.) extracted with dithizone dissolved in :

Time of shaking, mins.	(a) CCl_4.			(b) $CHCl_3$.		
	pH = 0·40.	1·07.	2·05.	2·60.	3·66.	4·68.
1	1·8	4·26	4·8	0·6	3·2	4·4
1½	—	—	—	1·2	4·0	5·0
3	1·8	4·4	5·5	—	—	—
5	1·8	4·5	5·8	1·4	4·2	5·3
8	—	—	5·8	1·5	4·4	5·5

[6 μG of copper in 10 ml. of buffer ; 10 ml. of organic phase : (*a*) 6·3, (*b*) 6·0 mg./l.]

This effect had been noted qualitatively by Greenleaf (*J. Assoc. Offic. Agric. Chem.*, 1942, **25**, 385), and Barnes (*loc. cit.*) quotes data which suggest that extraction equilibrium is attained in carbon tetrachloride in 5 minutes' shaking at pH 1·6 and 2·8, but more than 15 minutes' shaking are needed at pH 4·2 when chloroform is used. Subsequent work (Irving and Williams, unpublished), whilst correlating this effect with the smaller partition coefficient of dithizone in the latter solvent, has confirmed the difficulty of reaching true partition equilibrium in this and certain other metal–dithizone systems. On this account values given here for the percentage of copper extracted by solutions of dithizone in chloroform may well be underestimated in the lower pH range. But since the extraction of mercury at pH ~1 is complete within 1 minute's shaking, it is clear that the amount of copper simultaneously extracted will be (i) less than corresponds to equilibrium conditions, and (ii) much less than would have been the case if carbon tetrachloride had been employed.

A further choice may now be exercised in respect of the pH and composition of the aqueous phase undergoing extraction. Chloroform being used, extraction of mercury is complete at pH ~1 and above; but since halide ions reduce the amount extracted, it would appear advisable to avoid the addition of hydrochloric acid or even large quantities of hydroxylamine hydrochloride to biological digests (cf. Maren; Laug, and Nelson, etc.; *locc. cit.*). There is a positive advantage in using sulphuric acid for, as the following data show, more latitude is possible when diluting a biological digest. Excessive dilution would increase the danger of interference from copper with either acid, but insufficient dilution could more easily lead to gross errors with hydrochloric acid.

	Sulphuric acid.					Hydrochloric acid.				
Normality	2·00	1·00	0·50	0·10	0·01	2·00	1·00	0·50	0·10	0·01
pH	0·56	0·91	1·0	1·15	1·45	−0·31	0·09	0·42	1·10	2·04
Hg extracted, % ...	96	98·5	99·5	100	100	5	55	86	99·5	100

Interference from copper will naturally become increasingly serious as the extraction medium becomes more alkaline. The following data show that, contrary to Maren's statement (*loc. cit.*), errors due to copper (which is returned as mercury) must be expected even under optimum conditions when the copper/mercury ratio is large.

Copper taken, μg.	0	100	500	1000
Mercury found, μg.	10·0	11·3	12·2	14·9

[10 μG. of mercury in 10 ml. of N/4-sulphuric acid containing varying amounts of copper were shaken for one minute with 10 ml. of dithizone solution (6 mg./l. in chloroform).]

Reversion Procedure.—To eliminate such unavoidable interference from copper we make use of the reversion procedure described in the preceding paper. If E_m is the optical density of the mixed-colour organic phase containing mercury dithizonate, excess of dithizone, and copper dithizonate—with possibly other adventitious coloured materials—and E_r is the optical density (measured at the same wave-length and cell-thickness) after treatment with a suitable reagent which reacts with ("reverts") the mercury dithizonate (but not the copper complex or any other species present), transferring mercuric ions into the aqueous phase and setting free n equivalents of dithizone (HDz) in the organic phase, we have for the reversion value:

$$R = E_r - E_m = l(n\varepsilon_d - \varepsilon_c)[\mathrm{HgDz}_n] = k(\text{amount of mercury extracted})$$

Here ε_d, ε_c are the (effective) extinction coefficients of dithizone and mercury dithizonate respectively with a Spekker absorptiometer–tungsten lamp–Ilford orange filter combination, l is the cell length, and n the (constant) number of equivalents of dithizone associated with one atom of mercury. The appropriate reversion agent to use in this system follows from the effect of halide ions in reducing the extractability of mercury by dithizone noted above. The reversion of a solution of mercury dithizonate in chloroform by 1% (w/v) potassium iodide solution amounted to 89% at pH 4, 97·5% at pH 2, and became quantitative at pH 1·43: with 0·2% potassium iodide the corresponding figures were 6%, 84%, and 89%. The reversion of mercury dithizonate by iodide ions thus becomes increasingly effective as the acidity increases. But it is under just such conditions that the decomposition of copper dithizonate (in effect, reversion by hydrogen ions, cf. Fig. 2) becomes increasingly important. As might be expected, the initial *rate* of decomposition of copper dithizonate is proportional to the hydrogen-ion activity and at pH ~4 significant reversion of a solution of the complex in chloroform can be avoided if the time of shaking is not much longer than that needed to revert the mercury complex. The extent to which copper and mercury dithizonates are reverted under comparable conditions by shaking with 6% (w/v) potassium iodide solution is shown below, where the figures in parentheses refer to the number of experiments carried out, and the probable error of a single determination, respectively:

	Reversion, %, of	
pH.	copper dithizonate.	mercury dithizonate.
1·86	5·4 (8; ±0·5)	—
3·12	2·4 (4; ±0·4)	99·5 (3; ±0·2)
3·97	0·0 (3; —)	99·6 (3; ±0·2)

A further test of 6% potassium iodide as a reversion reagent was made by carrying out a series of determinations of mercury in the absence, and in the presence of copper. At pH 1·45 the estimates of mercury are about 10% too high, but at pH 3·97 the average reversion values are statistically identical and demonstrate that mercury can be determined accurately in the presence of at least 50-fold atomic excess of copper.

Metals taken, μg.		Average reversion values ($E_r - E_m$) at:	
Hg.	Cu.	pH 1·45.	pH 3·97.
11·6	0	0·355 (8; ±0·002)	0·356 (3; ±0·002)
11·6	200	0·391 (4; ±0·004)	0·358 (5; ±0·004)
12·4	0	0·380 (5; ±0·004)	0·381 (9; ±0·003)
12·4	200	0·417 (4; ±0·015)	0·384 (10; ±0·004)

The standard reversion curve for mercury was constructed by extracting known amounts of the metal from N/4-sulphuric acid with a solution of dithizone in chloroform: after measurement of E_m, a portion of the organic phase was reverted by shaking with 6% potassium iodide in a phthalate buffer of pH 3·97 and E_r was measured. The graph was linear for changes of optical density $0 \leqslant R \leqslant 0{\cdot}7$ and over the range 0—2·4 p.p.m. of mercury: a reversion value of 0·1 unit of optical density corresponds to 0·32 p.p.m. of mercury. Independent determinations of the concentration of the same mercury solution by two different observers gave reversion values of 0·375 (9; ±0·003) and 0·380 (5; 0·004), respectively, showing that 18·6 µg. of mercury can be determined with a probable error not greatly exceeding 1% when 15 ml. of dithizone solution are employed. The presence of copper does not materially reduce this precision (see above), and determinations of 20 µg. of mercury in the presence of 20,000 µg. of bismuth, lead, or ferrous iron, and 100,000 µg. of ferric ion were effected with a maximum error of 3%.

The reversion method has been successfully employed in many determinations of mercury in urine, fæces, and organs of experimental rats, and detailed procedures will be published elsewhere. No difficulty should, however, be experienced in applying the basic analytical principles to any other specific problem.

Mercury–Dithizone Complex.—Bamberger, Padova, and Omerod (*Annalen*, 1926, **446**, 288) obtained a red compound, m. p. 220° (decomp.), from solutions of mercuric oxide and dithizone in glacial acetic acid, but did not analyse it. Wöbling and Steiger (*Z. angew. Chem.*, 1933, **46**, 279) obtained a red-brown substance, m. p. 146—148°, by adding a concentrated solution of dithizone in sodium hydroxide to one of mercuric chloride in hydrochloric acid : the formula $C_{13}H_{11}N_4SHg$ was proposed but no analytical data were given. Our own preparation (p. 547) crystallised from warm pyridine in fine scarlet needles, m. p. 215·5°, of formula $C_{26}H_{22}N_8S_2Hg$. That this is the substance formed in the absorptiometric estimation of mercury with dithizone is confirmed by a study of the slopes of the mixed-colour or reversion curves, from which it can be shown (p. 547) that the formula of the *complex* present is $HgDz_2$ ($Dz = C_{13}H_{11}N_4S$).

Working in a room with indifferent west lighting, we experienced none of the inconveniences due to the photosensitivity of the mercury complex (remarked by previous workers) until March 1946, during the year's first spell of bright sunlight, when a solution of mercury dithizonate in chloroform turned to an intense royal-blue. The orange-yellow colour returned slowly in the dark, and immediately upon shaking with an aqueous solution of mercuric chloride in N-sulphuric acid. Upon exposure to bright sunlight the blue colour developed again, and the sequence of colour changes could be repeated at will.

EXPERIMENTAL.

Data for the curves of Fig. 1 and 2 were obtained by extracting a constant amount of mercury (or copper) buffered to a suitable pH with a solution of dithizone in chloroform or carbon tetrachloride as desired, an adequate excess being used in all cases. The amount of metal–dithizone complex formed and the amount of unreacted dithizone were then read off from a standard " mixed-colour " curve obtained by extracting various known amounts of metal with a fixed (and excess) amount of dithizone at a pH found from previous determinations to be the optimum for complete extraction. In some cases the estimation of the amount of metal extracted was more conveniently obtained by a reversion procedure (*loc. cit.*). The symbols E_0, E_m, and E_r denote the optical density of the initial dithizone solution, that of a mixed-colour extract containing the complex and unreacted dithizone, and the product of a reversion, respectively. All such measurements were made with a Spekker absorptiometer fitted with a tungsten lamp and using Ilford orange filter No. 607 with a 1-cm. cell. The acidity of buffered aqueous phases was measured after extraction with dithizone by using a Cambridge pH meter. The purification of reagents and apparatus, and details of manipulation followed current practice with dithizone (see preceding paper).

The Standard Mixed-colour Curve for Mercury Dithizonate.—(*a*) *In chloroform.* 10 Ml. of dithizone solution ($E_0 = 0·755$) were shaken for 2 minutes with *x* ml. of standard mercury (20 mg./l. of N/4-sulphuric acid) and (10 − *x*) ml. of N/4-sulphuric acid.

Hg taken, µg.	6·0	10·0	12·8	16·0	20·0	21·2	22·0	22·8
E_m	0·574	0·470	0·384	0·277	0·156	0·109	0·086	0·059
Hg taken, µg.	24·0	24·4	24·8	32·0	40·0	60·0	80·0	
E_m	0·035	0·016	0·016	0·012	0·013	0·007	0·008	

(*b*) *In carbon tetrachloride.* Each phase, 10 ml.; $E_0 = 0·645$.

Mercury taken, µg.	4·0	8·0	12·0	16·0	20·0	22·0	24·0
E_m	0·536	0·420	0·316	0·192	0·083	0·043	0·014

Extractability of Mercury by Solutions of Dithizone in Chloroform.—(a) *Hydrochloric acid solutions.* When 20 µg. of mercury in 10 ml. of hydrochloric acid of various concentrations were extracted with 10 ml. of dithizone ($E_0 = 0·769$) the excess of reagent increased from 22% to about 10,000% as the percentage of metal extracted diminished. To secure data based on a constant excess of dithizone, a similar series of extractions were carried out in which the amount of mercury taken was varied systematically at each pH studied until the organic extract had approximately the same E_m of 0·153. Since E_0 was 0·764, this corresponded to a constant 25% excess of dithizone.

Acid, N	0·012	0·120	0·205	0·365	0·629	0·894	1·15	1·43	1·68	2·21	2·49
% Extracted at											
(i) variable	100	99·0	96·3	92·9	81·4	64·6	44·9	21·6	12·1	2·1	1·1
(ii) constant excess of dithizone	100	98·0	92·4	87·7	58·0	31·6	20·5	13·5	—	—	—

(b) *Sulphuric acid solutions.* Each phase, 10 ml.; E_0 = 0·769.

Acid, N	0·025	0·25	2·16	4·08	5·97	7·91	9·82	11·72
% Extracted	100	99·6	94·4	84·3	78·8	70·9	60·8	45·3

Extractability of Mercury by Solutions of Dithizone in Carbon Tetrachloride.—(a) *From hydrochloric acid.* 10 Ml. each phase; E_0 = 0·619.

pH	2·00	1·03	0·64	0·41	0·14	−0·22	−0·36	−0·61
% Extracted	100	99·2	91·5	83·2	72·3	36·2	18·1	2·7
% Excess of free dithizone	26	27	28	38	63	250	5000	—

(b) *From sulphuric acid.* Extraction from 10N-acid was apparently as complete as from N/4-sulphuric acid. Extraction data could not be obtained from more concentrated acid as the reagent began to decompose or partition into the aqueous phase, colouring it blue.

Effect of Halide Ions in Reducing the Extractability of Mercury.—(a) *Chloride ions.* The following percentages of mercury were extracted by 10 ml. of dithizone dissolved in chloroform (E_0 = 0·75) from 10 ml. of (i) 3N-sulphuric acid, or (ii) N/4-sulphuric acid, containing 20 μg. of mercury and various amounts of potassium chloride.

KCl, N		0	0·4	0·6	0·75	1·0	1·2	2·0	2·6
Hg, %, extracted	(i)	100	81·5	70	59	24	13	—	—
	(ii)	100	—	—	—	97	—	84	61

(b) *Bromide ions.* 25 Ml. of sulphuric acid–sodium acetate buffer of pH ~2, containing potassium bromide were extracted with 10 ml. of dithizone:

KBr, N	0	0·25	0·51	0·70	0·91
pH	1·99	1·96	1·90	1·89	1·84
Hg, %, extracted	100	67·3	29·0	13·0	8·0

(c) *Iodide ions.* The percentage of mercury extracted from (i) 20 ml. or (ii) 25 ml. of sulphuric acid–sodium acetate buffer of pH 1·99, and (iii) 30 ml. of a similar buffer of pH 1·43, all containing potassium iodide in various amounts, by 10 ml. of dithizone (E_0 = 1·1) was:

KI, % (w/v)		0·01	0·02	0·04	0·08	0·1	0·18	0·30	1·00	1·80
Hg, %, extracted	(i)	23	—	8	6	—	—	—	—	—
	(ii)	—	—	—	—	7	—	3	2·5	1·5
	(iii)	—	11	—	—	3	—	0·5	0	0

Extractability of Copper by Solutions of Dithizone in Chloroform.—The standard "mixed-colour" curve was obtained by extracting 10 ml. of a sodium acetate–acetic acid buffer of pH 5·3 containing 0—5 μg. copper with 10 ml. of dithizone (E_0 = 0·678): 10 minutes' shaking was used in every case. The extractability curves were determined by shaking acetate buffers containing amounts of copper ranging from 3·9 to 50 μg., chosen so that with E_0 = 0·68, there should be a constant excess of dithizone as indicated by values of E_m = 0·396 ± 0·010 at every point on the curve.

pH	5·39	4·76	4·48	4·28	4·08	3·60	3·14	2·75	2·34	1·63
% Extraction	100	99·6	99·5	95·9	83·7	60·0	38·9	24·7	14·8	3·4

Extractability of Copper by Solutions of Dithizone in Carbon Tetrachloride.—The standard "mixed-colour" curve was obtained by shaking 10 ml. of dithizone (E_0 = 0·65) for 10 minutes with 10 ml. of a sulphuric acid–sodium acetate buffer of pH 2·4 containing varying amounts (0—6 μg.) of copper (cf. Morrison and Paige, *Ind. Eng. Chem. Anal.*, 1946, **18**, 213).

(a) *In hydrochloric acid solutions.* Buffers of dilute hydrochloric acid and sodium acetate were used to cover the whole pH range; E_0 was 0·670 and E_m was adjusted to 0·403 ± 0·025 by varying the amount of copper in the aqueous phase so as to achieve a reasonably constant excess of dithizone throughout.

pH	−0·78	−0·45	−0·17	0·20	0·50	1·10	1·50	2·07	2·78	3·02	3·31	3·40
% Extraction	5·0	9·0	19·0	39·0	55·8	72·8	78·4	90·5	99·8	99·3	100	100

(b) *In sulphuric acid solutions.* Sulphuric acid–sodium acetate buffers were used with E_0 = 0·679 and E_m = 0·373 ± 0·025.

pH	0·08	0·25	0·43	0·76	0·94	0·97	1·10	1·79
% Extraction	6·2	11·2	23·5	43·0	60·0	60·5	67·0	80·7

In all extraction experiments enough organic solvent was added to the aqueous phase before extraction with dithizone: the dithizone solutions were already saturated with water.

Reversion Curve for Mercury in the Presence of Copper.—20 Ml. of N/4-sulphuric acid containing various known amounts of mercury were shaken for one minute (60—100 vigorous shakes by hand) with 20 ml. of a chloroform solution of dithizone (E_0 = 0·755). After 2 minutes' standing to allow the phases to separate, the organic layer was drawn off through a small plug of cotton-wool inserted into the shortened stem of the separating funnel. The first runnings were rejected, and enough was then taken to rinse and fill a 1-cm. absorptiometer cell (about 7 ml.). The bulk of the remainder was transferred to a second clean separating funnel and shaken for one minute with an approximately equal volume of the reversion mixture described below. After standing for 2 minutes (during which E_m

is conveniently measured), a portion of the organic phase is removed as before for the measurement of E_r. Pure chloroform is placed in the comparison cell with the iris diaphragm on the left adjusted to give a balance with the drum set at an arbitrary value just greater than E_0: drum readings are thus made on the most open part of the scale when the concentration of mercury is smallest. The constancy of E_r during a series of measurements with dithizone solution of identical E_0 affords a valuable check on non-systematic errors.

The organic extracts necessarily contain dissolved water: since they often become heated above room temperature during extraction, the excess sometimes separates on cooling as a translucent cloud in the absorptiometer cell and gives rise to erroneous high readings of optical density. Warming the cells slightly (*e.g.*, on the lamp-house) will avoid this error, but a more satisfactory procedure was found to be the addition of one drop of ethyl (or better methyl) alcohol to the absorption cell. This changes the concentration of the extract by less than 1%, but this can be allowed for if required.

Hg taken, μg.	6·0	10·0	12·0	20·0	25·6	28·0
E_m	0·669	0·609	0·576	0·467	0·382	0·338
E_r	0·760	0·759	0·765	0·760	0·758	0·759
$R = E_r - E_m$	0·091	0·150	0·189	0·293	0·376	0·421
Hg taken, μg.	32·0	40·0	44·0	48·0	50·0	60·0
E_m	0·277	0·155	0·083	0·035	0·015	0·012
E_r	0·762	0·760	0·757	0·760	0·761	0·757
$R = E_r - E_m$	0·485	0·605	0·674	0·725	0·746	0·745

The reversion mixture was prepared by dissolving 10·2 g. of potassium hydrogen phthalate and 30 g. of potassium iodide in 500 ml. of doubly distilled water, a few drops of concentrated sodium thiosulphate solution being added if necessary to reduce any iodine liberated. Metallic impurities were then removed by shaking with successive portions of a solution of dithizone in chloroform (10 mg./l.) until the final extract remained green. So prepared, the reversion reagent is saturated with chloroform and it should be kept in the dark, preferably in contact with few ml. of dithizone solution which serves as an indicator, the green colour being discharged by any iodine liberated or by adventitious contamination by metals.

Mercury Dithizone Complex.—When increasing amounts of mercury react with a constant amount of dithizone, the E_m values fall linearly, as can be seen from the data for the standard mixed-colour curve (p. 545) or the reversion curve (see above): but the optical density never reaches zero even when a large excess is available. This residual optical density may be due to the absorption of (*a*) mercury dithizonate itself at the wave-length used, (*b*) excess of dithizone which must theoretically be present always in equilibrium with a metal complex, or (*c*) impurities in the reagent. Additional data exclude (*c*) but do not permit any assessment of the relative contributions of (*a*) or (*b*). However, knowing the relationship between the optical density and concentrations of solutions of dithizone in chloroform (preceding paper), and the quantity of mercury which reacts completely with the amount of reagent taken on the basis of (*a*) or (*b*) as limiting possibilities, the number of molecules of dithizone reacting with one atom of mercury is found to be $2 \pm 0·02$. Assuming the reagent to be monobasic (evidence for which will be presented elsewhere) the formula of the complex may be written $HgDz_2$ ($Dz = C_{13}H_{11}N_4S$).

Preparation. Dithizone (1 g.) in 100 ml. of chloroform was stirred vigorously with mercuric chloride (1 g., a two-fold excess) dissolved in N-sulphuric acid. The red flocks which rapidly separated were allowed to stand for 10 minutes, collected on a coarse sintered-glass funnel, and washed free from excess of dithizone by acetone followed by chloroform. The product was very sparingly soluble in ethyl acetate, *cyclo*hexane, nitrobenzene, or ether, but dissolved in carbon tetrachloride, chloroform, acetone, and ethyl alcohol to the extent of about 100, 200, 200, and 25 mg./l., respectively. Warm quinoline or pyridine proved better solvents and the *mercury–dithizone complex* separated from the latter in tufts of scarlet needles, m. p. 215—216°. For analysis they were washed free from adhering pyridine with acetone and dried to constant weight over sulphuric acid: direct light was carefully excluded throughout (Found: C, 42·9; H, 3·0; N, 15·9; S, 8·7; Hg, 28·0. $C_{26}H_{22}N_8S_2Hg$ requires C, 43·9; H, 3·1; N, 15·8; S, 9·0; Hg, 28·2%).

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