115. *The Absorptiometric Determination of Traces of Metals. Reversion : A New Procedure.*

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Factors influencing the accuracy with which metals can be determined absorptiometrically after extraction as complexes into an immiscible organic phase are discussed. Existing procedures are shown to fail when adventitious light-absorbing materials derived from the analytical sample are absent in the control determination. A new procedure (" reversion ") designed to overcome these difficulties does not require reagents of known or constant composition. By reversion procedure a single calibration curve serves for the determination of a number of metals with dithizone.

MANY metals can be determined absorptiometrically by shaking their aqueous solutions with an appropriate reagent, HR, dissolved in an immiscible organic solvent into which the metal

$$n\mathrm{HR} + \mathrm{M}^{n+} \rightleftharpoons \mathrm{MR}_n + n\mathrm{H}^+ \quad . \; . \; . \; . \; . \; . \; . \; . \quad (1)$$

complex formed in accordance with equation (1) is completely extracted at the pH employed. If $C_r{}^\circ$ is the (fixed) original concentration of the reagent and C_c the concentration of metal complex formed, the optical density, E_m, of a sample of the organic phase measured in a cell of length 1 cm. will be given by

$$E_m = \varepsilon_c C_c + \varepsilon_r(C_r{}^\circ - nC_c)k = (\varepsilon_c - nk\varepsilon_r)C_c + \varepsilon_r C_r{}^\circ k \quad . \; . \; . \; . \quad (2)$$

where ε_c and ε_r are the molecular extinction coefficients of the complex and reagent respectively at the wave-length used. The factor k $(0 < k < 1)$ arises from the fact that excess of reagent will be partitioned between the two phases to an extent dependent upon the pH and the ratio of phase volumes employed.

When varying amounts of metal are shaken with a constant (and excess) amount of reagent, the ratio of phase volumes, the pH, and other factors such as time of shaking, composition and strength of buffer solutions, etc., being kept constant, a series of values for E_m are obtained which, when plotted against the amount of metal taken (a quantity to which C_c will be directly proportional), provide a " standard " or " calibration " curve. The metal content of an unknown can now be obtained from this curve by interpolation. Traces of the metal being determined in any of the solvents or reagents used would naturally introduce an error which is allowed for by carrying out a " blank " and subtracting the amount of metal found by reference again to the standard curve. Now, if this " direct " procedure is to be reliable, the terms $C_r{}^\circ$ and k of equation (2) cannot be allowed to vary. Over the range where Beer's law is obeyed, the standard curve will be linear, and its slope (which is a measure of the sensitivity of the procedure) will be greatest when $|\varepsilon_c - nk\varepsilon_r|$ has its maximum value. For reagents colourless in the visible region maximum sensitivity is commonly obtained by measuring E_m at the wave-length where the coloured complex has its maximum absorption. Where $\varepsilon_c \gg \varepsilon_r$ the term $\varepsilon_r C_r{}^\circ k$ makes a negligible contribution to E_m, so that small variations in k or $C_r{}^\circ$ are of little moment. The situation is, however, more complicated if the reagent [*e.g.*, dithizone (diphenylthiocarbazone)] absorbs appreciably over the region where the absorption band of the complex rises to its maximum. Furthermore, the notorious impermanence of solutions of this reagent and the possibility of losses being incurred during extraction (*q.v.*) make it troublesome to ensure that $C_r{}^\circ$ has the same value when an unknown sample is being analysed as it had when the standard curve was set up.

The difficulty is simply overcome in many cases by carrying out a control experiment simultaneously with the determination of the unknown. If $E_m{}'$ be the optical density of such a " blank " we have, since in equation (2), $C_c = 0$:

$$E_m{}' = \varepsilon_r C_r{}^\circ k, \text{ whence } E_m - E_m{}' = (\varepsilon_c - nk\varepsilon_r)C_c \quad . \; . \; . \; . \; . \quad (3)$$

In this case the standard curve is obtained by plotting ΔE_m, the difference between the optical density of blank and unknown, against the amount of metal taken : it will be independent of the concentration of reagent taken provided that k remains the same. The advantages of this " compensation " procedure, which both corrects for the presence of impurities in the materials used and absolves the analyst from the necessity of using reagent solutions of known or constant strength, are not sufficiently well known or appreciated.

However, it is not always possible to ensure that the conditions obtained in the determination of the unknown are exactly reproduced when carrying out the blank. To particularise, the value of k will depend upon the ratio of phase volumes, whilst dissolved salts will affect the partition coefficient of the reagent, and small variations of pH may greatly influence the extent to which it passes out of the organic phase. For example, for equal volumes of aqueous buffer and a solution of dithizone in carbon tetrachloride :

pH of aqueous phase	8·25	8·50	8·75	9·00	9·25
Dithizone % in organic phase	84	72	58	42	26

The position is even more complex with amphoteric reagents like oxine and its analogues. If the aqueous sample (*e.g.*, a biological digest) contains hydrogen peroxide, nitrous acid, halogens, or permanganate, or kations such as tervalent iron or thallium, the reagent may be partly destroyed by oxidation, though this can generally be avoided by the addition of, *e.g.*, hydroxylamine before extraction. However, the possibility of the reagent forming water-soluble complexes (as, *e.g.*, aluminium does with 8-hydroxy-2-methylquinoline) which would reduce its concentration in the organic extract must not be overlooked. Apart from variation in $C_r°$ and in k there is, however, a more insidious source of potential error should the unknown (but not the blank) contain one or more additional metals capable of forming complexes under the experimental conditions used, or should the masking agent added to prevent the extraction of unwanted metal complexes prove inadequate to the demands made upon it. If C_i is the concentration of such a complex, or that of any pigment or extractable impurity which would likewise augment the optical density of the organic extract, equation (2) must be amended to

$$E_m = \varepsilon_c C_c + \varepsilon_r(C_r^{\circ} - nC_c - x)k + \Sigma\varepsilon_i C_i \quad . \quad . \quad . \quad . \quad . \quad (2a)$$

where ε_i is the extinction coefficient of the impurity, nC_c the number of equivalents of reagent used in complex formation, and x the amount lost in other ways. The difference between the optical density of blank and unknown will no longer be directly proportional to the amount of metal being estimated. Even the more elaborate procedures (cf. Kozelka and Kluchesky, *Ind. Eng. Chem. Anal.*, 1941, **13**, 484; Brown, *ibid.*, 1946, **18**, 493) must likewise fail if adventitious light-absorbing species are present. To meet such contingencies we have been led to devise the following new procedure.

If E_m is the optical density of a " mixed-colour " organic extract in a cell of length l, containing concentrations C_c of metal complex MR_n, C_r of excess reagent, and C_i of various coloured impurities of extinction coefficient ε_c, ε_r, and ε_i, we have

$$E_m = (\varepsilon_r C_r + \varepsilon_c C_c + \Sigma\varepsilon_i C_i)l \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3a)$$

If now the organic phase is shaken with a reagent which will decompose only the metal complex MR_n in the sense of equation (1) right to left, permitting the return of metal ion into the aqueous layer, whilst liberating an equivalent amount (nC_c) of reagent in the organic phase, the optical density will change to a new value, E_r, given by

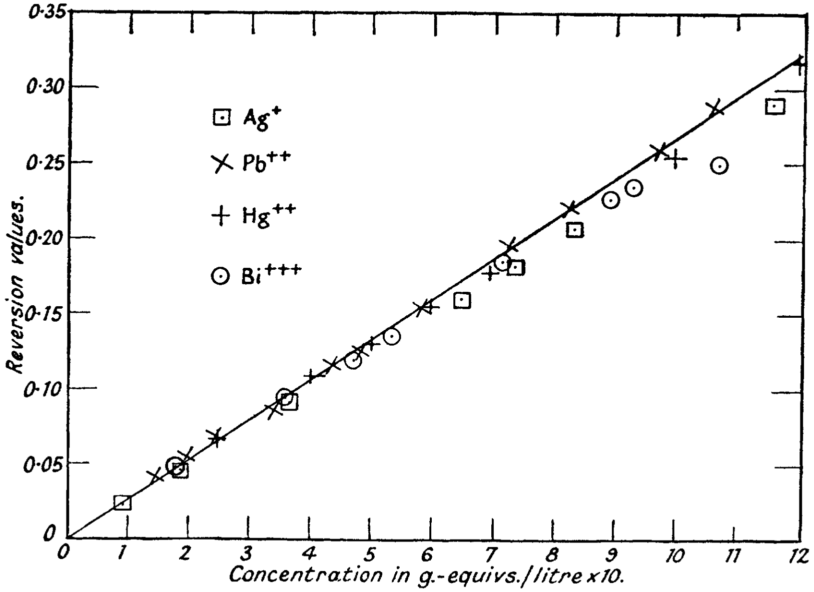
$$E_r = (\varepsilon_r C_r + n\varepsilon_r C_c + \Sigma\varepsilon_i C_i)l \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

We shall refer to this process as " reversion ". The change in optical density, which we term " the reversion value ", R, is given by

$$R = E_r - E_m = l(n\varepsilon_r - \varepsilon_c)C_c \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

It follows that a plot of reversion value against the amount of metal extracted should be linear, independent of the actual concentration of reagent used, and unaffected by the presence of other light-absorbing components. This treatment assumes that the composition of the complex remains unchanged over the range of values of C_c involved, so that the number n is constant (but not necessarily integral), that Beer's law is obeyed by the reagent and its metal complexes, and that the reversion reagent is truly specific. The choice of reversion agent will naturally depend upon the metal being determined and the nature of other revertible materials present. Qualitative data concerning the stability of various metal complexes in the presence of hydrogen ions, or anions, or organic reagents capable of competitive complex formation are often available and these, together with a knowledge of the analytical chemistry and pH–extractability curves for the elements concerned, provide a basis for developing a rational reversion procedure. Its value when determining mercury in the presence of much copper is demonstrated in the following paper.

Both E_m and E_r are measured at the same wave-length, which for maximum sensitivity should be that for which $|n\varepsilon_r - \varepsilon_c|$ is greatest. For most metal–dithizone systems this condition will be satisfied by choosing the wave-length at which the reagent itself absorbs most strongly ($\sim$6000 A.). A point of some interest arises from the fact that most metal "dithizonates" transmit freely at this wave-length: since ε_c can be neglected in comparison with ε_r, equation (5) reduces to $R = nC_cl\varepsilon_r$, and a single calibration curve can be used for the determination of a variety of metals provided the concentration axis is graduated in units of gram-equivalents. Indeed, since all measurements of optical density involve essentially the same species (dithizone), the same lamp-filter combination being used throughout, the slope e_r of the reversion curve will be identical with that of the graph relating the concentration and optical densities of solutions of pure dithizone. A Hilger "Spekker" absorptiometer being used with a tungsten lamp and Ilford orange filter No. 607, the value of e_r (effective) for dithizone was 30·5 × 10^3 and 26·75 × 10^3 l./g.-mol./cm. for chloroform and carbon tetrachloride, respectively. Higher sensitivities could, of course, be obtained with a more suitable filter, or with a spectrophotometer. Reversion data for the representative uni-, bi-, and ter-valent metals silver, lead, mercury, and bismuth, plotted in the figure together with a line of slope 26·75 × 10^3, demonstrate the correctness of our predictions and establish the formulæ AgDz, $PbDz_2$, $HgDz_2$, and $BiDz_3$ for the absorbing species (Dz = $C_{13}H_{11}N_4S$)—at least when an excess of dithizone is present.



2 : 3-Dimercaptopropanol ("BAL") appears to have great possibilities as a reversion reagent, for it will revert all metal dithizonates we have so far examined over a wide range of pH. The determination of several constituents in the same solution is made possible by using "BAL" to determine the total metal content, or the amount remaining after selective reversion of individual constituents. Reversion procedure is not, of course, limited to the dithizone system, for the principle is equally applicable to other suitable systems, *e.g.*, metals and *o*-nitrosophenols or oxine, etc., or to measurements on mixtures of compounds absorbing in the ultra-violet or infra-red.

EXPERIMENTAL.

Materials.—Fresh distilled water, chloroform, carbon tetrachloride, and acetic acid were redistilled from Pyrex vessels shortly before use. Solutions of ammonia and hydrochloric acid were prepared by passing the pure gases into metal-free water. Dithizone was purified several times as recommended by Sandell ("Colorimetric Determination of Traces of Metals", New York, 1944, p. 89). The procedure followed in cleaning glassware, storing dithizone solutions, preparing metal-free buffers, etc., followed the recommendations of Bambach and Burkey (*Ind. Eng. Chem. Anal.*, 1942, **14**, 904).

Standard Curve for Dithizone in Carbon Tetrachloride.—The following optical densities of solutions obtained by diluting a standard containing 8·376 mg. of highly purified dithizone in 1 l. of carbon tetrachloride were measured in a 1-cm. cell by using the absorptiometer, lamp, and filter specified above. The drum was set to read 0·870 with the comparison cell containing pure solvent.

Optical density	0·086	0·178	0·266	0·353	0·437	0·524	0·606	0·686	0·772	0·856
Concn., mg./l.	0·838	1·675	2·513	3·350	4·188	5·026	5·863	6·701	7·538	8·376

The plot is linear up to at least 6·5 mg./l., with a slope of $26·7 \times 10^3$ l./g.-mol./cm. A recalibration carried out independently a year later on another sample of dithizone gave ε_r (effective) = $26·8 \times 10^3$.

Standard Curve for Dithizone in Chloroform.—Dilute solutions were prepared from a standard containing 16·8 mg./l. The plot is linear up to about 6·5 mg./l. with ε_r (effective) = $30·5 \times 10^3$ l./g.-mol./cm. :

Optical density ...	0·102	0·205	0·410	0·605	0·790	0·935	1·100	1·215	1·32	1·42	1·50
Concn., mg./l. ...	0·84	1·68	3·36	5·04	6·72	8·40	10·08	11·76	13·44	15·12	16·8

Reversion Curve for Lead.—10 Ml. of citrate buffer (50 g. of citric acid in 100 ml. of water brought to pH 8·5—9·0 with ammonia), 5 ml. of 10% w/v potassium cyanide solution, *x* ml. of standard lead solution (9·986 mg. of "Spekpure" lead in 1000 ml. of 1 : 100 nitric acid), and (25 − *x*) ml. of nitric acid (1 : 100) were shaken for 2 minutes in a pear-shaped separating funnel with 20 ml. of dithizone solution (5 mg./l.). After settling, a portion of the organic phase was withdrawn through a dry pledget of (metal-free) cotton wool, and its optical density, E_m, determined in the usual manner. A further portion (about 10 ml.) was withdrawn into another separating funnel and reverted by shaking for 2 minutes with an approximately equal volume of 5N-sulphuric acid. After the layers had separated, a portion of the organic phase was withdrawn as before for the measurement of its optical density, E_r. The plot of reversion values against concentration is linear up to 1·25 p.p.m., a reversion value of 0·1 unit of optical density corresponding to $3·76 \times 10^{-6}$ g.-equiv./l. or 0·388 μg. of lead/ml.

x, ml.	Concn., g.-equiv./l. × 10^6.	E_m.	E_r.	*R*.	*x*, ml.	Concn., g.-equiv./l. × 10^6.	E_m.	E_r.	*R*.
0·0	0·0	0·265	0·274	0·009	1·20	5·79	0·183	0·347	0·164
0·295	1·45	0·237	0·288	0·051	1·50	7·23	0·195	0·400	0·205
0·405	1·93	0·247	0·312	0·065	1·70	8·20	0·156	0·387	0·221
0·50	2·41	0·240	0·318	0·078	2·00	9·64	0·120	0·392	0·272
0·70	3·38	0·228	0·320	0·092	2·19	10·60	0·115	0·404	0·289
0·90	4·34	0·202	0·329	0·127	2·50	12·05	0·113	0·438	0·325
0·98	4·81	0·192	0·326	0·134	2·95	14·5	0·035	0·385	0·350

No special care was taken in measuring the cyanide solution, so the pH of extraction may have varied somewhat from point to point. The determinations were not carried out in the order quoted above and no special pains were taken to ensure the constancy of the strength of reagent. These (deliberate) sources of variation are the cause of the irregularities in E_m and illustrate some of the drawbacks of the "direct" procedure (p. 537). The reversion values, however, are free from such irregularities, as expected. The "blank" reversion value of 0·009 may be due to impurities or to the fact that the solvents used were not completely immiscible. It was subtracted from all reversion values before the figure was plotted; but since its magnitude does not affect the slope of the reversion curve this refinement is not a practical necessity. Repetition of these experiments with a sample of dithizone which had not been specially purified gave a reversion curve of substantially the same slope.

The 1-cm. Spekker cells require about 7 ml. of solution. Where it is desirable to use only 10 ml. of dithizone for extraction no serious error due to evaporation is incurred if, after measurement of E_m, the contents of the cell are transferred to a separatory funnel and reverted together with sufficient of the remaining mixed-colour extract to give at least 7 ml. for the measurement of E_r. The scale can be still further reduced if 1-cm. micro-cells are available.

Reversion Curve for Bismuth.—40 Ml. of a citrate–cyanide buffer of pH 8·8 containing known amounts of bismuth were extracted with 20 ml. of a solution of dithizone in chloroform as described for lead. Reversion was effected with 2N-sulphuric acid :

Concn., g.-equiv./l. × 10^6	1·78	3·57	4·69	5·35	7·14	8·92	9·28	10·71	12·85
Reversion value	0·049	0·095	0·119	0·134	0·184	0·227	0·235	0·251	0·291

Reversion Curve for Silver.—Varying known amounts of silver in 10 ml. of N/100-sulphuric acid (pH 3·0—3·1) were extracted with 10 ml. of dithizone solution (E_0 = 0·49). Reversion was effected by shaking the mixed-colour extract with an equal volume of 6% w/v potassium iodide in 0·25N-sulphuric acid :

Concn., g.-equiv./l. × 10^6	0·925	1·85	3·70	6·48	7·35	8·34	11·57
Reversion value	0·023	0·046	0·091	0·160	0·182	0·208	0·291

Reversion Curve for Mercury.—Mercury was extracted from 10 ml. of N/4-sulphuric acid by 10 ml. of dithizone (E_0 = 0·64). Reversion of a portion was effected with an equal volume of acidified potassium iodide solution (2% w/v in N/100-hydrochloric acid containing sufficient sodium thiosulphate to decolourise the mixture; cf. Morrison and Paige, *Ind. Eng. Chem. Anal.*, 1946, **18**, 211) :

Concn., g.-equiv./l. × 10^6	2·49	3·99	4·99	6·98	9·97	11·96
Reversion value	0·068	0·109	0·130	0·179	0·255	0·319

The reversion curve in chloroform has been studied in greater detail (see following paper) and is strictly linear over the range $0 \leqslant R \leqslant 0·73$.

Reversion by 2 : 3-*Dimercaptopropanol.*—Metal complexes formed by this reagent ("BAL") are much more stable than metal dithizonates under comparable conditions. The addition of one drop of 0·5% w/v solution in chloroform caused immediate and quantitative reversion of solutions of mercury dithizonate in chloroform or carbon tetrachloride. That the reagent is equally effective in reverting dithizonates of copper and lead was demonstrated as follows. 20 Ml. of a solution of dithizone in chloroform (5·27 mg./l., $E_0 = 0{\cdot}634$) were vigorously shaken for ½ minute (100 shakes) with 20 ml. of (*a*) a borax–sodium hydroxide buffer of pH $\sim$10 previously freed from all extractable metallic impurities and left saturated with chloroform, (*b*) the same buffer containing known amounts of copper or lead, and (*c*) the mixtures used for (*b*) with the addition of a factitious excess of the dimercaptopropanol. The amount of dithizone remaining in each organic phase was then determined absorptiometrically :

		Optical density (orange filter) :				Optical density (orange filter) :	
Copper added, μg.	pH.	without BAL.	with 400 μg. of BAL.	Lead added, pH.	pH.	without BAL.	with 400 μg. of BAL.
0	10·13	0·465	0·466	0	10·11	0·468	0·468
4	10·13	0·390	0·463	4	10·11	0·441	0·469
8	10·14	0·291	0·467	8	10·11	0·394	0·469
20	10·13	0·168	0·467				

Though dithizone was partitioned into the aqueous phase in each case, values of optical densities below 0·465 could only be due to its reaction with copper or lead, since the pH (measured after extraction) did not vary. The identical values of series (*a*) and (*c*) show that these metal complexes were reverted quantitatively by BAL.

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