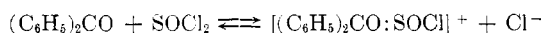


solvent benzophenone, being such a weak base, is slightly ionized. This slight ionization might be represented by the following equation:



In such a strongly acidic solvent as thionyl chloride the curves are, as one would predict on the basis of other information, typical of the titration of a weak acid against a weak base.

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Determination of Oxalate Ion and Calcium Ion by Indirect Colorimetry

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An attempt has been made to apply the decrease in optical density when the ferric-salicylate complex is treated with oxalate to determine oxalate ion by indirect colorimetry. Beer's law is followed. The influence of the acidity and presence of tartrate and citrate ions has been studied. This method can be applied to the determination of small amounts of calcium even in the presence of magnesium, and results compare satisfactorily with those obtained by volumetric methods. Calcium is precipitated in the presence of an excess of oxalate, and oxalate is determined colorimetrically. The method is reliable in the concentration range 2.5×10^{-6} to 2.5×10^{-5} mole of oxalate. An acceptable precision is achieved in the range 1.2 to 2.2 mg. of calcium.

SEVERAL colorimetric methods have been advanced recently, in which the intensity of color of a particular system is reduced by addition of the ion being determined.

Because little work has been done on the colorimetric determination of oxalate ion (11, 12), an attempt has been made to utilize the fading in color when the ferric-salicylate complex is treated with oxalate, and at the same time to apply this method to the determination of small amounts of calcium.

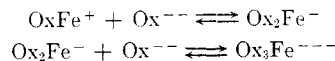
The ferric-salicylate complex has been utilized in the colorimetric

determination of iron (3, 10). Mehlig (5) has studied the influence of several factors, especially cations and anions, in this type of colorimetric work. Ions such as oxalate, citrate, and tartrate must be avoided in the determination of iron with salicylate (8). In acetic acid medium, ferric iron in presence of salicylate yields complexes whose constitution depends on the pH of the medium. According to Monnier, Rusconi, and Wenger (6) and Babko (1), three complexes may be formed, violet, red, and yellow, in accordance with the theoretical and experimental findings of Bertin (2).

The pH recommended by Mehlig (5) is the most suitable for the present method. At a pH of about 5 an equilibrium is reached between salicylate and acetate complexes. At higher pH these complexes may be destroyed by formation of colloidal ferric hydroxide.

In acetic acid medium, salicylate, oxalate, and ferric ions being present, the equilibrium depends on the amount of oxalate added to the system, since when oxalate ion increases, ferric ion decreases, as the complex OxFe^+ is formed from the complex RFe^+ .

Moreover, from the complex OxFe^+ , the following complexes may be formed:



SOLUTIONS AND APPARATUS

1. Iron standard solution, prepared from ferrous ammonium sulfate. 1 ml. = 0.1 mg. of ferric oxide.
2. Colored reagent. Mix 500 ml. of solution 1 with 250 ml. of 1% sodium salicylate, add ammonium hydroxide (1 to 1) dropwise until a yellow color appears, then add 10 drops of ammonium hydroxide (1 to 1) in excess. Make up to 1000 ml. with acetic acid (1 to 1). 20 ml. = 1 mg. of ferric oxide.
3. Oxalic acid solution. 1 ml. = 10^{-6} mole of oxalic acid.
4. Oxalate solution. 1 ml. = 10^{-6} mole of sodium oxalate.
5. Tartrate solution. 1 ml. = 2×10^{-6} mole of sodium tartrate.

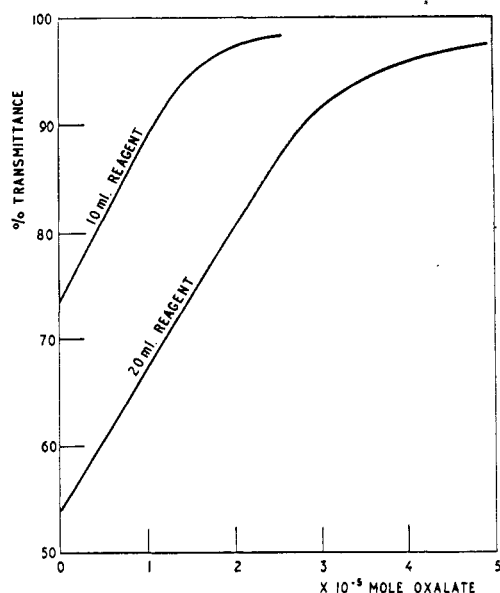


Figure 1. Effect of Amount of Reagent

6. Citrate solution. 1 ml. = 2×10^{-5} mole of sodium citrate.

The present work was done with the ETCO 308 B photo-colorimeter, using the green filter.

PROCEDURE

Calibration. In each test, 20 ml. of the colored reagent was employed. When the concentration of iron is too small, the method is less precise. Figure 1 shows the comparative curves obtained when increasing amounts of oxalate are added to samples containing 10 and 20 ml. of colored reagent. These curves give an inflection and approach asymptotically at the value of the optical transmittance which corresponds to the ferric-oxalate complex.

Twenty milliliters of colored reagent is transferred to several measuring flasks, followed by increasing amounts of the standard oxalate solution, making up to 50 ml. with distilled water. The curve is prepared at 18° C., plotting mole $\times 10^{-5}$ of oxalate as abscissas and optical density as ordinates.

It is very convenient to carry out a duplicate check of two or three points of the calibration curve (Figure 2) in each liter of the colored solution.

In the range 0.25×10^{-5} to 2.5×10^{-5} mole, the curve obtained with increasing amounts of oxalic acid coincides with that from the standard solution of oxalate.

Analytical Procedure. Transfer a volume of unknown solution (always less than 20 to 25 ml.) containing from 0.25×10^{-5} to 2.5×10^{-5} mole of oxalate to a measuring flask containing 20 ml. of colored reagent, shake, dilute with distilled water to 50 ml., shake again, measure the optical density, and read off on the standard curve.

In determinations on a micro scale, it is permissible to take portions of 1 ml. or less from the unknown solution, if in this volume the same range of oxalate ion is contained.

As the measurements must be done in an acetic acid medium, the unknown solutions added to the colored reagent must be neutral, weakly acid with acetic acid, or slightly alkaline with ammonium hydroxide. If the acidity is not due to acetic acid, it must be neutralized. An excessive increase in the hydrogen ion concentration shifts the equilibrium of the system, giving a partial destruction of the ferric-salicylate complex. The pH of the unknown solution may be estimated by the use of a univer-

Table I. Oxalate Determinations

Oxalate, Mole $\times 10^{-5}$		Error, %
Taken	Found	
0.25	0.24	-4.0
0.25	0.26	4.0
0.50	0.52	4.0
0.50	0.51	2.0
1.00	1.02	2.0
1.00	0.99	-1.0
1.50	1.51	0.67
1.50	1.54	2.6
2.00	2.02	1.0
2.00	2.00	0.0
2.50	2.48	-0.8
2.50	2.47	-1.2

sal indicator. The influence of free sulfuric and perchloric acids has also been studied.

RESULTS

In Table I, the results found for known amounts of oxalate are summarized.

The effect of the tartrate and citrate ions has also been studied, by adding to known amounts of oxalate increasing amounts of these ions up to concentrations of 2×10^{-5} mole of tartrate and 3.2×10^{-5} mole of citrate. While tartrate does not interfere even when in greater concentration than oxalate, citrate gives positive errors by decrease in the optical density, which

Table II. Influence of Tartrate and Citrate

Taken, Mole $\times 10^{-5}$			Oxalate Found, Mole $\times 10^{-5}$	Error, %
Oxalate	Tartrate	Citrate		
1.00	0.50	...	1.01	1.0
1.00	1.00	...	0.98	-2.0
1.00	...	0.50	1.02	2.0
1.00	...	1.00	1.03	3.0
2.00	1.00	...	2.01	0.5
2.00	2.00	...	1.97	-1.5
2.00	...	1.00	2.03	1.3
2.00	...	2.00	2.05	2.5

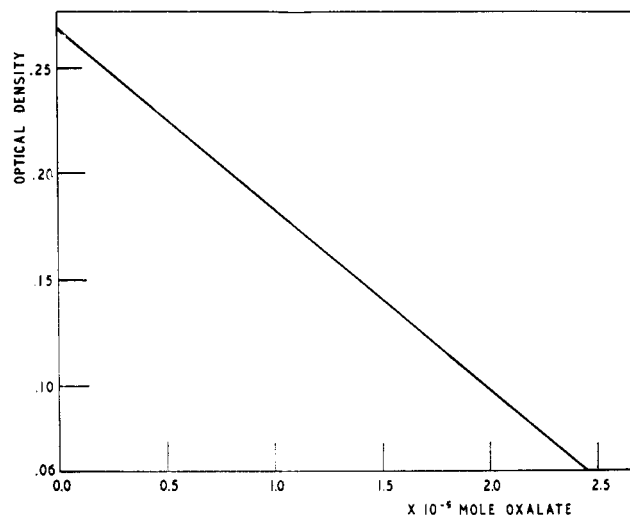


Figure 2. Calibration Curve

reach approximately 7% when the concentration of citrate is 1.6 times that of oxalate. In Table II, the results obtained in the presence of citrate and tartrate are compared.

If the transmittance readings are made three times on the same sample and the average is taken, the method is perfectly reliable in the concentration range 2.5×10^{-5} to 2.5×10^{-5} mole of oxalate and it is possible to determine even 1×10^{-6} mole of oxalate, equivalent to a concentration of 1.76 micrograms of oxalate per ml. of the 50-ml. solution prepared for the colorimetric determination.

APPLICATIONS TO DETERMINATION OF CALCIUM

The results obtained with the preceding method were sufficiently satisfactory to extend it to the determination of calcium, by precipitation as oxalate followed by indirect colorimetric determination of the oxalate which remains in the solution. Scott and Johnson (9) also utilize the precipitation as oxalate. If the precipitate is dissolved in sulfuric or perchloric acid, some free acid remains, which interferes with the color of the complex. Accordingly, the free oxalate is determined after the precipitation is effected in the presence of excess oxalate. This procedure is considered to be more rapid than the McGregor method (4) because it avoids the operation of washing, drying, and dissolving the precipitate. The correction for the fading of color due to free acid is also avoided and deviations are much less when the determination is effected with the oxalate in excess, without dissolving the precipitate.

Analytical Procedure. From the acid solution of calcium an aliquot of not more than 3.5 ml. is taken, a volume of 2 ml. being preferable. It is transferred to a centrifuge tube which contains 6 ml. of the standard oxalate solution (1×10^{-5} mole of oxalate per ml.). A few drops of ammonium hydroxide (1 to 1) are added until the solution is weakly alkaline. The amount of

Table III. Calcium Determinations

Oxalate, Mole $\times 10^{-4}$	Calcium, Mg.	
	Taken	Found
2	0.10	0.12
	0.20	0.21
	0.20	0.22
	0.40	0.38
6	0.20	0.21
	0.40	0.42
	0.40	0.41
	0.40	0.39
	0.80	0.83
	0.80	0.82
	1.20	1.25
	1.20	1.23
	1.60	1.60
	1.60	1.64
	2.00	2.06
	2.20	2.25

ammonium hydroxide to be added, once established, does not have to be calculated in each case. The solution is made up to 10 ml. with distilled water, agitated gently, and left standing for at least 0.5 hour. It is then centrifuged for 15 minutes in an electrically driven centrifuge.

From the supernatant solution, 5 ml. is removed and transferred to a 50-ml. measuring flask which contains 20 ml. of colored reagent. It is made up to 50 ml. with distilled water and shaken. The optical density is measured and compared with the standard curve. When the amount of calcium is very small, it is preferable to take less than 6 ml. of oxalate—for instance, 2 ml.—particularly when the amount of calcium is less than 0.4 mg.

Table IV. Effect of Magnesium

Oxalate, Mole $\times 10^{-5}$	Mg. Mole $\times 10^{-4}$	Calcium, Mg.		Error, %
		Taken	Found	
6	0.5	1.00	0.98	-2.0
		1.60	1.58	-1.2
	1.0	1.00	0.95	-5.0
		1.60	1.58	-1.2
	2.0	1.00	0.96	-4.0
		1.60	1.52	-5.0
	3.0	1.00	0.90	-10.0
		1.60	1.45	-9.3
12	...	1.00	0.98	-2.0
		1.60	1.00	0.0
		1.60	1.58	-1.2
		1.60	1.62	1.2
	0.5	1.00	0.98	-2.0
		1.60	1.62	1.2
		1.60	0.98	-2.0
		1.60	1.60	0.0
	1.0	1.00	0.98	-2.0
		1.60	0.98	-1.2
		1.60	1.58	-1.2
		1.60	0.97	-3.0
	3.0	1.60	1.56	-2.5

Results. Several tests made with amounts of calcium between 0.2 and 0.02 mg. show that, for these low concentrations, the errors are appreciable because the fading of the color is very small and of the same magnitude as the method deviations. Working in the presence of 6×10^{-5} mole of oxalate, the best results are found in the range 1.2 to 2.2 mg. of calcium.

In Table III results are summarized. The determination of calcium by this procedure must be done with care in the presence of ions which may coprecipitate with calcium or may form a precipitate or some complex with the oxalate ion. Oxidizing substances should be absent.

Calcium in the presence of magnesium may also be determined by this procedure. Several tests have been done using 6×10^{-5} mole of oxalate (Table IV). When the magnesium content is high, the precipitation of calcium is very slow, owing to the interaction of the magnesium and calcium ions which increases the solubility of the calcium oxalate. In the presence of magnesium, the amount of oxalate is increased, using a standard oxalate solution containing 4×10^{-5} mole of oxalate per ml. Three milliliters is poured into a centrifuge tube, from 2 to 6 ml. of the solution of calcium containing magnesium is added, and then distilled water to make up to 10 ml., after addition of some drops of ammonium hydroxide.

Two milliliters of the centrifuged solution is taken and the method previously described is applied. Magnesium does not interfere appreciably in the stability of the ferric-salicylate complex in the absence of oxalate.

The colorimetric method has been applied to the determination of calcium in plant materials; 50 ml. of extract has been obtained per gram of dry material, and 6 ml. of the extract was taken, using 3 ml. of oxalate solution containing 4×10^{-5} mole of oxalate per ml. In Table V the results obtained by the colorimetric method are compared with those obtained by the volumetric method (permanganimetric) from 3 grams of dry material.

Table V. Comparison of Results Obtained by Colorimetric and Volumetric Methods

Sample	Ca in Dry Material, %		Ca in Extract (Colorimetry), P.P.M.
	Volumetry	Colorimetry	
1	0.56	0.54	108
2	0.45	0.46	92
3	0.61	0.64	128
4	0.39	0.38	76

All the tests described in this paper have been carried out in subdued light to avoid photo-decomposition of the ferric-salicylate complex and reduction of the ferric ions in the presence of oxalate (?).

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