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Semimicroanalysis of Saline Soil Solutions

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A system of photometric and volumetric analytical semimicromethods for ions that contribute to soil salinity is described. These methods involve a considerable reduction in the quantity of soil solution required, which is an important consideration in the extraction of such solutions. In addition, they involve a saving of reagents and time. The precision and accuracy of the methods are considered adequate for most soil analyses.

THE detailed analysis of soil solutions is rendered difficult by the large volume of sample required by the standard analytical methods. The method of extraction, size of apparatus, size of soil sample, necessity for repetition of the extraction, and length of time required for extraction have been influenced by the necessity of securing sufficient solution for complete analysis. (In this paper, the term "soil solution" refers to the aqueous solution occurring in the soil at field moisture; the term "soil extract" refers to the solution obtained from a soil that has been mixed with an artificially high quantity of water-e. g., at soil-water weight ratios of 1 to 2 and 1 to 5.) In a discussion of these factors, Eaton and Sokoloff (10) pointed out that "a material reduction in the quantity of solution required in the laboratory would minimize some of the difficulties". Anderson, Keyes', and Cromer (4) recently mentioned the necessity of altering analytical conditions in the direction of microchemistry.

The staff of this laboratory has been engaged in the examination and development of methods for the extraction of soil solutions, particularly of saline and irrigated soils. The pressure-membrane method, described by Richards (28), is an effective means of obtaining solutions from soils covering wide ranges of moisture content, texture, structure, and salt content. This method appears to be especially well adapted to soils at low moisture contents--e. g., near the wilting range (27). The advantages of the pressure-membrane method would be largely lost if it were necessary to apply the conventional analytical methods to the limited volumes of solution obtainable from comparatively dry soils.

Consequently, the development of the pressure-membrane method has emphasized the need for semimicroanalytical methods applicable to small samples of soil solution. In addition to the small amount of sample required, the methods outlined here involve a saving of time and reagent, a consideration which might be of even greater importance to some analysts. There is an expanding interest in the application of microanalytical methods to problems of agricultural chemistry. Peech **(24)** recently published a scheme for the microdetermination of exchangeable bases in soils. Wall (36) has developed a set of microprocedures for the determination of some inorganic constituents of plant ash.

This article presents photometric and volumetric methods for the semimicrodetermination of calcium, niagnesium, sodium, potassium, ammonium, carbonate, bicarbonate, chloride, sulfate, and nitrate ions. These methods generally represent adaptations of other methods previously published for the analysis of soils, waters, plants, and clinical specimens. The methods necessarily vary as to convenience and accuracy. The aim has been to develop simple procedures whose precision would not be seriously less than that of corresponding macromethods.

The methods described apply primarily to saline alkaline soils in which salts of alkali and alkaline earth metals predominate. For use under other conditions, where additional interfering substances might occur, appropriate modifications might be necessary. If the soil solution is not analyzed immediately, the concentration of some ions may be appreciably altered by the activity of microorganisms. Treatments to minimize the direct effect of such processes on the nutrient ions and indirect effects on other ions are usually not reliable. In addition, calcium carbonate and calcium sulfate precipitate from some solutions after extraction. For these reasons, soil solutions should be analyzed as soon as possible.

The centrifuge procedures involve the use of an 8-place centrifuge head rotating at 3000 r. p. m. in a No. 2 International centrifuge. Heavy-duty 12-ml. conical centrifuge tubes are necessary at this high speed in place of the ordinary 15-ml. tubes. A 16-place head rotating at 2000 r. p. m. also provides acceptable results, and may even be more practical for a large number of samples. An angle head rotating at 3000 r. p. m. was tested, but in general the results were inferior with respect to precipitate compaction and over-all accuracy.

A photoelectric photometer is very satisfactory for the colorimetric measurements because of its speed and relative precision. An Aminco Type F double photocell photometer (manufactured by the American Instrument Company, Silver Spring, Md.) was used in this work. It has a permanent mounting of six pairs of matched color filters and permits the use of both optical cells and photometer test tubes. A constant-voltage transformer in the 115-volt power line eliminates fluctuations in light transmission due to voltage variations. Other color-measuring instruments,

TABLE I. DESCRIPTION OF SOILS AND THEIR EXTRACTS

Accession No.	Soil Type	Location	pH	C, %	Extract Characteristics: C, %	PH	Electrical conductivity, K X 10⁸ @ 25° C.	Soil: water ratio	Color
56[a]	Imperial clay	M&land, Cslif.	7.8	0.34	0.000094	7.8	125	1:2	very pale yellow
57	Imperial clay	Imperial, Calif.	7.2	0.33	...	7.0	803	1:5	Colorless
62	Oasis clay loam	Delta. Utah	7.7	1.07	...	7.6	998	1:5	Yellow
63[a]	Oasis clay subsoil	Delta, Utah	8.0	0.58		8.1	557	1:5	Yellow
68[a]	Vale silty clay loam	Vale. Ore.	10.0	0.27	0.0032[b]	10.2	555	1:5	Brown
79	Cajon silty clay loam	Glendale; Ariz.	7.7	1.10	0.0044	7.3	75	1:2	Yellow
84	Gila adobe clay	Las Cruces, N. Mex.	8.0	0.92	0.0034	8.1	85	1:2	Pale yellow
85	Regan clay loam	Roswell. Ii. Mex.	7.8	0.93	0.0032	7.6	353	1:2	Pale yellow
86	Fort Collins loam	Laramie, Wyo.	8.0	0.99	0.0053	7.6	544	1:2	Pale yellow
248[a]	Indio very fine sandy loam	Coachella, Calif.	9.1	0.49	0.0047	9.0	72	1:2	Reddish brown
314	Merced clay loam	Buttonwillow. Calif.	7.8	2.34	0.0047 [b]	7.6	625	1:5	Dark yellow

[a] Soils used only for organic matter investigation.
[b] Values corrected for medium chloride contents and probably less accurate than those for other soils.

such as spectrophotometers, gradation photometers, neutral wedge photometers, and visual color comparators, can also be used. For accurate photometric work it is usually hazardous to rely on permanent photometer calibration curves, because of the variable conditions that affect colorimetric procedures. In this laboratory it is a matter of routine to take a series of standards through the analytical procedure each time a group of samples is analyzed.

The calibration of microburets and small pipets is recommended. A 2-liter beaker covered by a 20-hole perforated brass plate, which holds the centrifuge tubes vertical, makes an adequate water bath.

Ionic concentrations are calculated in terms of milliequivalents per liter (m. e./l.). Attention is called to the increasing use among water chemists of the term "equivalent per million", e. p. m. (3). This unit of concentration is numerically the same as milliequivalents per liter if the specific gravity of the solution is unity.

In addition to the determination of ionic concentrations, the analysis of soil solutions usually includes the pH value and the electrical conductivity as a measure of the total electrolyte concentration. For conductivity measurements on small samples, a micromodification of the common pipet type of conductivity cell, which holds approximately 5 ml., is very convenient. For pH measurements a Beckman "one-drop" glass electrode (manufactured by National Technical Laboratories, South Pasadena, Calif.) is satisfactory. Capillary glass microelectrodes, which require even less sample, are also available.

Description of Soils and Extracts

The systematic investigations of organic matter and precision and accuracy reported here were made on extracts of eleven soil samples of different soil types from various localities. Pertinent characteristics of these soils and extracts are presented in Table I. Two of these soils, 68 and 248, are "black alkali" soils.

The organic carbon contents of the soils and extracts were determined by the chromic acid oxidation method of Schollenberger (SO), involving the modified phosphoric acid reagent of Purvis and Higson (26). The experimental values were multiplied by the factor 1.15, according to Allison (I), which corrects for incomplete oxidation of the organic matter. The carbon contents of the extracts were determined on the evaporation residue of 25-ml. aliquots. Chloride reduces chromic acid, and appropriate corrections are included in the eight extract values reported. The soil carbon contents of the more saline soils also include corrections for chloride, which are very slight compared to those for the corresponding extracts.

The pH values were determined by a glass electrode assembly. The soil pH measurements were made on saturated soil pastes.

Removal of Organic Matter

The possible interference of organic matter in the analysis of soil solutions often raises questions concerning the necessity for its removal. It may interfere in such ways as color masking, reducing action, mechanical contamination of precipitates, and in other direct and indirect ways. The magnitude of these effects is usually unknown. In some systems of analysis, all samples are treated to remove organic matter, regardless of the amount and composition. In other cases the solutions are analyzed without prior separation of the organic fraction.

As the time involved in the preliminary removal of organic matter represents an appreciable fraction of the total time required for analysis, information as to the feasibility of omitting this operation is important, especially in the routine analysis of a large number of soil samples. It is also possible that some or all of the methods for removing organic matter may actually introduce errors into the analytical results. These considerations may affect both macro- and microanalytical methods.

The eleven water extracts of Table I were subjected to four different treatments: ignition, oxidation by hydrogen peroxide, oxidation by bromine, and adsorption by carbon. Other possible treatments were not investigated systematically because they would definitely introduce various kinds of interference. The treated and untreated samples were analyzed for the ions mentioned except nitrate and ammonium. Sodium was determined by a gravimetric uranyl zinc acetate procedure [39, Sect. 70 (b), p. 42] instead of the colorimetric method.

Bromine removed the color from all samples, but the analytical values agreed with those of the untreated solutions. Consequently, there would be no advantage in the use of this oxidant in the scheme of analysis described here.

Carbon not only adsorbed the colored constituents but significantly reduced the concentrations of most of the ions, especially calcium and magnesium, and lowered the bicarbonate-carbonate value of every sample. However, it did not affect the chloride values, which indicates that carbon treatment may be useful in removing color that interferes with the chloride titration.

Hydrogen peroxide treatment at a temperature not exceeding 100" C. caused a general decrease of ions in most samples, particularly of sodium and chloride. The loss of chloride is probably a result of oxidation to chlorine. The cause of the losses of the other ions remains somewhat obscure. These results, coupled with the resistance of some organic matter to oxidation by peroxide and the possibility of the catalytic decomposition of peroxide by soil constituents, make this treatment unsatisfactory.

The ignition treatments were made in porcelain casseroles

at 600° C. The more resistant organic constituents did not decompose completely over extended periods at lower temperatures. This has been observed also on base-exchange residue ignitions. The results of ignition were variable. Sodium and chloride were lost from every sample, to about the same extent. Decreases in magnesium and sulfate occurred in several samples. Calcium showed no significant' change except an increase in one "black alkali" sample, 68. Potassium was extremely high in ten ignited samples, upwards to 590 per cent of the correct value. Fresh samples of four solutions that were extremely erratic in this respect were ignited in platinum dishes, and these yielded the correct values for potassium. The excess potassium evidently originated in the material of the casseroles; the results indicate an exchange of sodium for potassium.

The two black alkali samples were also treated with nitric acid and boiled, to precipitate the colored humates: Analysis of the filtrates showed no appreciable deviation-from the untreated samples.

In this investigation, carbonate determinations were made only on the untreated and the carbon-treated samples, as the' other treatments precluded this determination. Chlorides could not be determined on the bromine-treated sample.

The results of this investigation suggest the following recommendations and possible conclusions. For titration of carbonate species in dark solutions, a potentiometric titration can be substituted for the indicator procedure. Purified decolorizing carbon can safely be used to treat dark solutions prior to the chloride titration. Carbon can evidently be used to remove the color of solutions prior to the determination' of sodium and potassium. Ignition may cause appreciable loss of many ions common to saline soils, especially sodium and chloride.' Ignitions should not be made in porcelain ware. Oxidation of organic matter by bromine and hydrogen peroxide accomplishes no apparent beneficial results. With especial regard to the inorganic composition of black alkali solutions, the inclusions of ions such as calcium and magnesium that may be combined with the humates may not always be desirable.

Precision and Accuracy

To demonstrate the possible ranges of precision and accuracy that can be expected from the various methods, water extracts of seven soils of Table I were systematically analyzed in duplicate by the semimicromethods and by the corresponding macro- or standard methods in use at this laboratory. The results for each particular ion are presented in the section devoted to the discussion of that method.

Organic matter was not removed from these extracts prior to their analysis by either the macro- or microprocedures. As the extracts vary considerably in composition, some reported determinations may involve quantities of ions that do not represent favorable conditions for the evaluation of the accuracy of a method. This applies also to the macromethods.

In addition to the comparisons reported here, many other similar studies have been made on soil solutions and soil extracts, waters, plant nutrient culture solutions, and plant ash extracts. These studies have yielded results as satisfactory as those presented in this paper.

In the succeeding tables, several symbols and terms are used that possibly require brief explanations. The letters A and B indicate duplicate determinations. The mean is the average of the duplicate values, reported to the same decimal point. The per cent deviation represents the average deviation of the duplicates from the mean divided by the mean value, and times 100; this figure is an index of precision or reproducibility. The average per cent deviation is the arithmetical average of the per cent deviation values for the entire group of extracts; comparison of the two values obtained for two methods provides a measure of the relative precision of the two methods. The per cent error represents the algebraic percentile deviation of the mean semimicro value from the mean macro value; this calculation assumes that the macromethod usually provides the more correct result. The average per cent error is the arithmetical average of the percentile errors for the entire group of semimicrodeterminations; this figure provides a general index of the over-all accuracy of the method.

VOLUMETRIC DETERMINATION OF CALCIUM

Calcium is determined by a method involving precipitation as the oxalate, centrifugal washing, and direct titration in perchloric acid solution with ammonium hexanitrato cerate, with nitro-ferroin as indicator. (The ammonium hexanitrato cerate and nitro-ferroin can be obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio.) The precipitation and washing technique represents a combination of modiied Clark and Collip (9) and Blasdale (8) procedures, while the titration technique follows the procedure'of Smith and Gets (32). The use of cerate and nitro-ferroin permits a direct titration at room temperature with a very sharp endpoint change from red to pale blue, and a low blank correction.

REAGENTS. (Keep reagents B, C, D, and E in Pyrex bottles.)

A. Methyl orange, 0.01 per cent in water.

B. 1 to 1 hydrochloric acid.

C. ¼ N oxalic acid.

D. 1 to 1 ammonium hydroxide.

E. 1 to 50 ammonium hydroxide.

F. 4 N perchloric acid. Dilute 340 ml. of 70 per cent perchloric acid or 430 ml. of 60 per cent perchloric acid to 1 liter.

G. **0.01 N ammonium hexanitrato cerate in 1 N perchloric acid. Dissolve 5.76 grams of "standard or reference purity" ammonium hexanitrato cerate in 250 ml. of 4 N perchloric acid and dilute to 1 liter. The reagent should be standardized in the following manner: Pipet 5 or 10 ml. of fresh standard 0.01 N sodium oxalate into a small beaker containing 5 ml. of 4 N perchloric acid, add 0.2 ml. of nitro-ferroin indicator and titrate with the cerate solution to the pale blue endpoint. Determine a blank titration correction on a similar sample minus the oxalate solution. The milliliters of oxalate used divided by the corrected milliliters of cerate and times 0.01 provide the normality of the cerate. Do not attempt to adjust the solution to exactly 0.01 N, and restandardize whenever the reagent is used several days or more apart. Keep in a dark bottle away from light.**

H. Nitro-ferroin indicator (nitro-orthophenanthroline ferrous sulfate). Dilute the stock 0.025 M indicator solution 1 to 20. Use 0.1 ml. in analyses and 0.2 ml. in standardizations.

PROCEDURE. **Pipet an aliquot containing 0.005 to 0.08m. e. of calcium into a clean 12-ml. conical centrifuge tube, dilute or evaporate to 5 ml., and add 1 drop of (A), 2 drops of (B), and 1 ml. of (C). Heat to the boiling point in a water bath. While twirling the tube, add (D) dropwise until the solution just turns yellow. Replace in the bath, and after 30 minutes cool the tube in air or in water. If necessary add more (D) to keep the solution just yellow.**

Centrifuge at 3000 r. p. m. for 10 minutes. Carefully decant the supernatant liquid into a 25-, 50-, or 100-ml. volumetric flask. Stir the precipitate, and rinse the sides of the tube with a stream of 5 ml. of (E) blown from a pipet. Centrifuge at 3000 r. p. m. for 10 minutes. Decant the washings into the same flask. Drain the tube by inversion on filter paper for 10 minutes. Wipe the mouth of the tube with a clean towel or lintless filter paper.

Blow into the tube 3 ml. of (F) from a pipet. When the precipitate is dissolved, add 0.1 ml. of (H). Titrate with (G) from a 10-ml. microburet to the pale blue end point. If more than 5 ml. of (G) is required, transfer the sample to a small beaker and complete the titration. Determine the blank correction in the same manner; it usually is about 0.03 ml. Dilute the supernatant liquids in the volumetric flask to volume and save for the magnesium determination.

CALCULATION. **M. e. of Ca per liter = (corrected ml. of cerate solution X normality of cerate X 1000) ÷ ml. in sample aliquot.**

Precision and Accuracy

The calcium concentrations of the seven soil extracts indicated in Table I were determined by this procedure and by a calcium oxalate-potassium permanganate volumetric macromethod outlined by Wilcox (39, pp. 33-9) and based on the calcium-magnesium separation technique of Blasdale (8).

TABLE II COMPARISON OF MACRO- AND SEMIMICROMETHODS FOR CALCIUM

Soil No.	Macromethod Aliquot	Macromethod Calcium A	Macromethod Calcium B	Macromethod Calcium Mean	Macromethod Deviation	Semimicromethod Aliquot	Semimicromethod Calcium A	Semimicromethod Calcium B	Semimicromethod Calcium Mean	Semimicromethod Deviation	Error
	Ml.	M. e./liter			%	Ml.	M. e./liter			%	%
57	50	39.05	39.09	39.07	0.05	1	39.9	39.9	39.9	0.00	+2.1
62	200	3.53	3.54	3.54	0.14	10	3.57	3.58	3.58	0.14	+1.1
79	200	2.19	2.20	2.20	0.23	10	2.16	2.18	2.17	0.46	−1.4
84	200	2.34	2.34	2.34	0.00	10	2.32	2.34	2.33	0.43	−0.4
85	50	28.35	28.45	28.40	0.18	2	28.6	28.9	28.8	0.52	+1.4
86	50	24.35	24.51	24.43	0.33	2	24.8	24.9	24.9	0.20	+1.9
314	200	7.71	7.80	7.76	0.68	5	7.75	7.80	7.78	0.32	+0.3
				AV.	0.22					0.30	1.2

The results, presented in Table II, indicate highly satisfactory precision and accuracy for the semimicromethod. The reproducibility data demonstrate that it usually is unnecessary to replicate analytical samples.

It has been known that the clinical calcium methods involve a negative error due to loss of calcium oxalate on decanting and a positive error resulting from incomplete washing of the precipitate; Wang (37) indicates that these two errors are very evenly balanced in most analyses. The present results support this view and show that the net resultant error is of slight magnitude.

COLORIMETRIC DETERMINATION OF MAGNESIUM

Magnesium is determined on calcium-free solutions by precipitation as magnesium ammonium phosphate hexahydrate, centrifugal washing, and colorimetric estimation of the phosphate content by the ceruleomolybdate reaction. This standard clinical procedure, recently described for plant ash by Wall (36), has been modified in some details. No method for the precise determination of small amounts of magnesium in soils appears to have been advanced. Although the method described does not involve a high degree of precision, the use of duplicate analytical samples usually provides satisfactory accuracy.

Because of the sensitivity of the colorimetric phosphate measurement, usually only a fraction of the filtrate from the calcium determination is used for the magnesium determination. This practice is also influenced by the inhibition of precipitation of magnesium by high concentrations of oxalate ion (14, 24), which must be reduced to a safe value.

REAGENTS. The concentrations of molybdate and sulfuric acid used in the development of the blue color are those recommended by Truog and Meyer (34), but the strength of the reagent has been modified slightly. The stannous chloride reagent is prepared daily and not acidified, according to Zinzadze (41). Because of the effect of time on the color, especially of darker solutions, photometer readings are made at exactly 10 minutes after addition of the stannous chloride.

The ammoniacal wash liquid is similar to that recommended by Wang (37) for the washing of calcium oxalate precipitates. Reagents A, B, D, E, and F should be kept in Pyrex bottles and replaced if the precipitation blank color becomes too intense.

A. 3 per cent ammonium chloride solution. Dissolve 30 grams of recrystallized ammonium chloride in water and dilute to 100 ml. Filter before use.

B. 5 per cent ammonium dihydrogen phosphate solution. Dissolve 25 grams of ammonium dihydrogen phosphate in water and dilute to 500 ml. Filter before use.

C. Phenolphthalein, 1 per cent in 60 per cent ethanol.

D. Concentrated ammonium hydroxide.

E. Ammoniacal wash liquid. Mix 20 ml. of concentrated ammonium hydroxide with 980 ml. of water, 100 ml. of ethanol, and 100 ml. of ether.

F. Standard 0.001 N magnesium sulfate. This is best prepared by dilution of a more concentrated solution of magnesium sulfate that has been standardized by gravimetric determination of magnesium.

G. 1 N sulfuric acid.

H. Ammonium molybdate reagent. Dissolve 40 grams of ammonium molybdate in 400 ml. of water at 60° C., add 150 ml. of arsenic-free concentrated sulfuric acid to 1000 ml. of water, and cool both solutions. Stir the molybdate solution into the acid solution and dilute to 2000 ml. when cool. The reagent is a 2 per cent solution of ammonium molybdate in 2 N sulfuric acid. It keeps indefinitely in a brown bottle.

I. Stannous chloride reagent. Place 0.300 gram of C. P. stannous chloride dihydrate in a 100-ml volumetric flask. Dissolve rapidly in water, dilute to the mark, and mix. Any turbidity will be removed on mixing with reagent H. Prepare fresh daily.

PROCEDURE. From the volumetric flask containing the calcium-free sample pipet an aliquot containing 0.0005 to 0.003 m. e. of magnesium into a 12-ml. conical centrifuge tube and dilute or evaporate to 5 ml. Add 1 ml. each of (A) and (B) and 1 drop of (C). Heat to 90° C. in a water bath and while twirling the tube add (D) dropwise until pink. Cool, add 2 ml. of (D), and stir with a thin glass rod. Withdraw the rod, stopper the tube, and let stand overnight.

Centrifuge at 3000 r. p. m. for 10 minutes, decant carefully, drain on filter paper for 10 minutes, and wipe the mouth of the tube with a clean towel or lintless filter paper. Wash the precipitate and sides of the tube with a stream of 5 ml. of (E) from a pipet equipped with a rubber aspirator bulb or by a similar arrangement. Centrifuge at 3000 r. p. m. for 5 minutes, decant, drain for 5 minutes, and wipe the mouth of the tube. Repeat this washing procedure once.

Pipet 2 ml. of (G) into the tube and dilute to about 10 ml. After 5 minutes, wash the contents into a 100-ml. volumetric flask to which exactly 5 ml. of (H) have previously been added. Dilute to about 60 ml. and pipet in 1 ml. of (I) while rapidly twirling the flask. Dilute to the mark and mix. At exactly 10 minutes after adding (I) measure the light transmission of the blue solution in a photometer test tube through the 650-millimicron filter versus that of water in a similar tube. Previously, the photometer is balanced at 100 per cent transmission with water in both tubes. The accuracy is increased somewhat by the use of the same test tube for all samples and standards.

Prepare a photometer calibration curve on semilogarithmic graph paper by taking a series of 0, 0.5, 1, 2, and 3 ml. of (F) through the same entire procedure. A typical calibration is shown in Figure 1. The amount of magnesium in the sample is obtained by simple interpolation on the curve.

Because of the effect of oxalate, the magnesium sample should not represent more than one fifth of the calcium sample. If the magnesium concentration is so low that it cannot be accurately

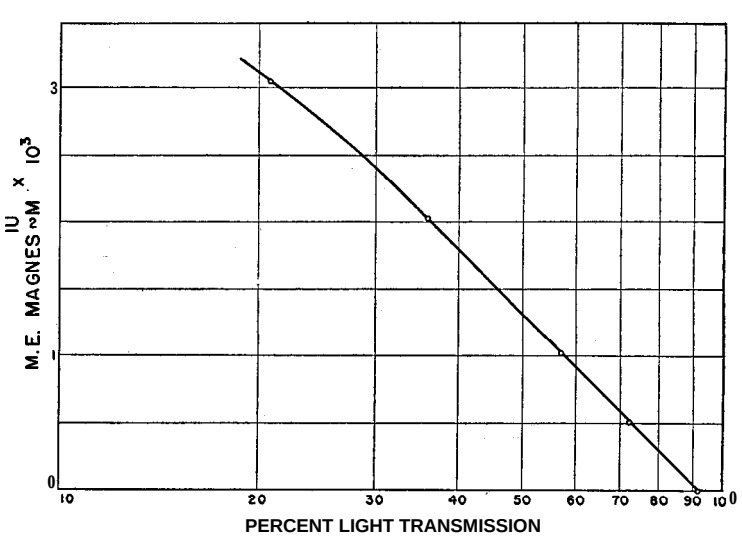

FIGURE 1. PHOTOMETER CALIBRATION CURVE FOR MAGNESIUM

TABLE VIII. COMPARISON OF MACRO- AND SEMIMICROMETHODS FOR SULFATE

Soil No.	Macromethod Aliquot	Sulfate A	Sulfate B	Sulfate Mean	Deviation	Semimicromethod Aliquot	Sulfate A	Sulfate B	Sulfate Mean	Deviation	Error
	Ml.	*M. e./liter*			%	*Ml.*	*M. e./liter*			%	%
57	100	14.85	14.94	14.90	0.30	3	15.0	15.0	15.0	0.00	+0.7
62	100	13.76	13.78	13.77	0.07	3	14.0	14.1	14.1	0.35	+2.4
79	200	2.49	2.49	2.49	0.00	10	2.40	2.45	2.43	1.03	−2.4
84	200	4.38	4.39	4.39	0.11	10	4.50	4.53	4.52	0.33	+3.0
85	50	41.54	41.60	41.57	0.07	1	42.5	42.6	42.6	0.12	+2.5
86	50	71.70	71.80	71.75	0.07	1	72.9	73.0	73.0	0.07	+1.7
314	50	47.58	47.90	47.74	0.34	1	48.5	48.8	48.7	0.31	+2.0
				Av.	0.14					0.32	2.1

The distillation equipment includes microburners, 100-ml. Kjeldahl flasks, and "inverted U" air condensers of 1.9-cm. (0.75-inch) diameter. (These glassware is obtainable from the Hengar Company, Philadelphia, Penna.) A distillation rack of six units is convenient. The Nessler reagent is prepared according to Koch and McMeekin (20). The distilled water should be practically free of nitrogen compounds.

REAGENTS. A. Devarda's alloy. Boil a quantity in 0.2 N sodium hydroxide for a few minutes to reduce the nitrogen content. Wash and dry.

B. 2 N sodium hydroxide. Dissolve 80 grams of nitrogen-free sodium hydroxide in 1 liter of water.

C. 0.01 N sulfuric acid.

D. Standard 0.01 N potassium nitrate. Dissolve 1.011 grams of dry recrystallized potassium nitrate in water and dilute to exactly 1 liter.

E. Standard 0.01 N ammonium sulfate. Dissolve 0.6607 gram of pyridine-free ammonium sulfate in water and dilute to exactly 1 liter.

F. Nessler reagent *(19).* "Dissolve 22.5 grams of iodine in 20 cc. of water containing 30 grams of potassium iodide. After the solution is completed, add 30 grams of pure metallic mercury, and shake the mixture well, keeping it from becoming hot by immersing in tap water from time to time. Continue this until the supernatant liquid has lost all of the yellow color due to iodine. Decant the supernatant aqueous solution and test a portion by adding a few drops thereof to 1 cc. of a 1 per cent soluble starch solution. Unless the starch test for iodine is obtained the solution may contain mercurous compounds. To the remaining solution add a few drops of an iodine solution of the same concentration as employed above, until a faint excess of free iodine can be detected by adding a few drops thereof to 1 cc. of the starch solution. Dilute to 200 cc. and mix well. To 975 cc. or an accurately prepared 10 per cent sodium hydroxide solution now add the entire solution of potassium mercuric iodide prepared above. Mix thoroughly and allow to clear by standing." Keep in a brown bottle.

PROCEDURE. Pipet an aliquot containing 0.002 to 0.015 m. e. of nitrate into a 100-ml. Kjeldahl flask. (If the sample contains carbonate, barely neutralize the aliquot with dilute sulfuric acid.) Add 0.50 gram of (A) and dilute to 30 ml. Immerse the tip of the delivery tube in a mixture of 3 ml. of (C) and 20 ml. of water in a 100-ml. beaker.

Carefully run 2 ml. of (B) down the neck of the flask. Connect the flask to the air condenser by the rubber sleeve and twirl it to mix the contents. Heat the flask with a microburner until 15 ml. have been distilled. Raise the distillation assembly free of the acid and distill one minute longer. Disconnect the delivery tube and rinse it into the beaker. Wash the contents of the beaker into a 100-ml. volumetric flask, add exactly 5 ml. of (F) while whirling the flask, dilute to the mark, and mix. After 15 minutes, measure the light transmission through the 460-millimicron filter in a 1-inch optical cell against that of water in a similar cell. Previously balance the photometer at 100 per cent transmission with water in both cells.

Distill and treat a series of 0, 0.2, 0.5, 1, and 1.5 ml. of (D) in the same manner. From the photometer readings plot a calibration curve on semilogarithmic graph paper. The milliequivalents of nitrate in the aliquot are determined by interpolation on this curve. A typical curve is shown in Figure 4.

If a qualitative nesslerization test indicates a measurable amount of ammonia, both ammonium and nitrate can be determined on the same aliquot by the following modification. Distill, nesslerize, and measure light transmission in the manner described, omitting (A) and substituting a boiling stone. Now immerse the tip of the rinsed delivery tube in a fresh mixture of 3 ml. of (C) and 20 ml. of water in a clean beaker; add to the cooled Kjeldahl flask 0.50 gram of (A) and 15 ml. of water. Immediately connect to the condenser, distill, and treat in the same manner.

To obtain the ammonium calibration curve, distill, without Devarda's alloy, a series of 0, 0.2. 0.5, 1, and 1.5 ml. of (E), nesslerize, and measure the light transmission. Prepare the nitrate calibration curve as previously outlined. Determine the milliequivalents of ammonium and nitrate ions in the aliquot by interpolation on their respective curves.

If it is suspected that hydrolyzable nitrogenous organic compounds are increasing the ammonium value, an additional distillation should be made in which the Kjeldahl flask contents are buffered at pH 7.4, according to Shrikhande (31). A series of ammonium standards can be treated in the same manner.

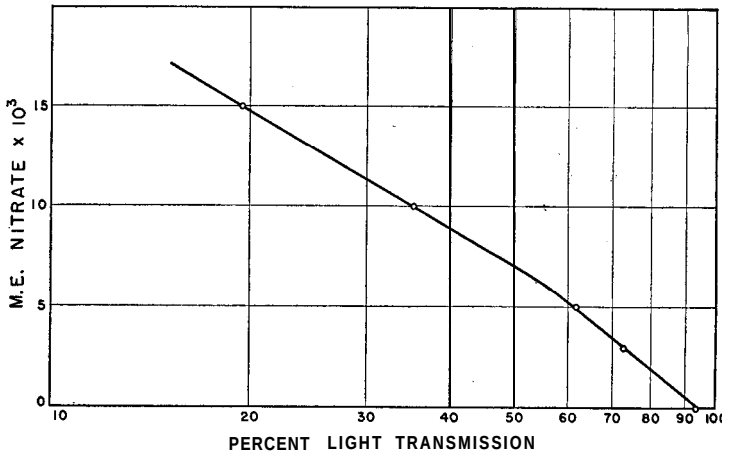

FIGURE 4. PHOTOMETER CALIBRATION CURVE FOR NITRATE

TABLE IX. COMPARISON OF MACRO- AND SEMIMICROMETHODS FOR NITRATE

Soil NO.	Macromethod Aliquot	Nitrate A	Nitrate B	Nitrate Mean	Deviation	Semimicromethod Aliquot	Nitrate A	Nitrate B	Nitrate Mean	Deviation	Error
	MI.	*M. e./liter*			%	*Ml.*	*iu. e./liter*			%	%
57	200	3.72	3.73	3.73	0.13	4	3.63	3.63	3.63	0.00	− 2.7
62	400	0.673	0.680	0.677	0.52	10	0.675	0.690	0.683	1.10	+ 0.9
79	400	0.432	0.439	0.436	0.80	10	0.445	0.450	0.448	0.56	+ 2.8
84	400	0.259	0.259	0.259	0.00	20	0.280	0.285	0.283	0.88	+ 9.3
85	400	2.35	2.36	2.36	0.21	5	2.36	2.36	2.36	0.00	0.0
86	400	0.309	0.309	0.309	0.00	30	0.345	0.345	0.345	0.00	+11.6
314	400	0.877	0.879	0.878	0.11	10	0.875	0.895	0.885	1.13	+ 0.8
				Av.	0.25					0.52	4.0

CALCULATION. M. e. of NO_3 per liter = (m. e. of NO_3 in aliquot as found by interpolation on NO_3 curve X 1000) ÷ ml. in aliquot. M. e. of NH_4 per liter = (m. e. of NH_4 in aliquot as found by interpolation on NH_4 curve X 1000) ÷ ml. in aliquot.

Precision and Accuracy

The seven soil extracts were analyzed for nitrate by this method and by a regular Devarda procedure (39, p. 21) in which the ammonia is collected in boric acid solution and titrated with 0.05 N sulfuric acid. For this purpose, no preliminary separation of ammonia was made, and the values in Table IX include all nitrogen that would be liberated under the analytical conditions.

The extracts of soils 84 and 86 contained so little nitrate that the results by the titration method are probably inaccurate. By excluding these from the accuracy comparisons, the average "error" of the semimicromethod is reduced from 4.0 to 1.5 per cent. As for some other ions, the semimicromethod sometimes may actually provide more accurate results than the comparison method. The colorimetric method shows a satisfactory degree of precision.

Discussion

The volume of sample used in the semimicroanalysis of the seven soil extracts comprised $^1/_{15}$ to $^1/_{36}$ of that used in the comparison methods, with an average of $^1/_{24}$. This represents a considerable reduction in sample requirements. In general, semimicroanalysis requires less time, although no quantitative comparisons have been made. The economy effected in the analytical reagents is often important.

The accuracy obtainable under these conditions is not seriously reduced, and is adequate for most soil analyses. The average predictable error of the methods is about 2 per cent, based on comparisons with methods involving much larger samples. Many of the methods are sufficiently precise that replication of determinations usually is unnecessary. The quantitative reproducibility data presented for the various methods should assist in determining the desirability of replication, based on particular requirements.

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