

1006

REVIEW OF
FUNDAMENTAL
DEVELOPMENTS
IN ANALYSIS

Organic Microchemistry

C. L. OGG

Eastern Regional Research Laboratory, Philadelphia 18, Pa.

IN THIS biannual review the author has selected those papers which to him seemed to indicate current interest and trends in the field of organic microchemistry, and has not tried to include all the publications on the subject. Another reviewer by stressing different aspects of the subject undoubtedly would have cited a number of papers not included in this review. As it was impossible to test in the laboratory even a small fraction of the methods described, selection of the publications to be stressed was a most difficult task. This was particularly true of those papers which described modifications of existing methods, because frequently only a small change in procedure or apparatus results in a greatly improved method.

CARBON AND HYDROGEN

Much of the work reported during the past 2 years has dealt with the two primary sources of trouble in the carbon and hydrogen determination—namely, the removal of the oxides of nitrogen and the elimination of errors that occur when some of the

less common elements (in organic compounds) are present. This indicates that the methods in use today generally produce satisfactory results for the usual organic materials. It does not mean that there is no room for improvements nor that better methods will not be devised, but that the more pressing problems are the two mentioned above.

Numerous absorbents for nitrogen oxides have been proposed to date and a critical study of their efficiency and capacity seems to be desirable. Of equal importance would be a readily acceptable method for testing those qualities of an absorbent, so that not only the known absorbents could be compared but new materials could be evaluated.

Cropper (13) reported the results of a study of the efficiency of different preparations of lead dioxide. The efficiency was found to depend on the particle size of the lead dioxide; large particles had a low capacity and small particles a high capacity but led to erratic values. Consequently, a medium-capacity material was required for uniform performance. Fortunately, this type of

material can be made by recommended methods. A new absorbent for the oxides of nitrogen was proposed by Cross and Wright (14). This was trishydroxylamine phosphate which, when used in an absorption tube between the water and carbon dioxide absorbers, removed the nitrogen oxides efficiently, increased the accuracy of the carbon values for nitrogen-containing compounds, and improved the precision of the results for nitrogen-free materials. Hussey, Sorensen, and DeFord (23) also claimed increase in precision with ammonium sulfamate dispersed on silica gel in an absorption tube instead of lead dioxide in the combustion tube. Abramson and Brochet (1) recommended diphenylamine and sulfuric acid on alumina as absorbent for the oxides of nitrogen.

The determination of carbon and hydrogen in organosilicon compounds was studied by Klimova, Korshun, and Bereznitskaya (25), and the method they proposed not only eliminated the errors caused by the formation of silicon hydride and prevented carbide formation, but also permitted the simultaneous determination of the silicon. To do this, chromic oxide on asbestos was placed in a special sample holder and volatile silicon compounds were made to react with the chromic oxide to form silica, which was retained in the tube and weighed. Klimova and coworkers (26) used the same technique when analyzing organophosphorus compounds, with equally good results. Even at 900°C. the phosphorus pentoxide was retained on the asbestos and the phosphorus could usually be determined with fairly good accuracy.

Another element which causes erroneous values if present in very large amounts is fluorine. Bodenheimer and Goldstein (8) developed an equation for correcting the hydrogen values obtained for fluorine compounds. They found that the hydrogen values should be diminished by $1/57$ of the percentage of fluorine in the compound. A practical approach to the problem of fluorine in the carbon and hydrogen determination was the interesting method for the simultaneous determination of carbon and fluorine described by Freier, Nippoldt, Olson, and Weiblen (16). Combustion was by moist oxygen in a quartz tube, the hydrofluoric acid formed was determined by acid-base titration, and the carbon dioxide was absorbed by Ascarite. Good results were obtained for a number of different compounds.

Kuck, Altieri, and Towne (28), using the Garner balance, made a critical study of diffusion rates through the capillaries of absorption tubes. Diffusion rates were related to capillary size, and, as expected, the larger the capillary the faster the rate. Standard tubes with 0.25-mm. capillaries filled with oxygen lost weight for about 2 hours, then gained at the rate of 0.201 γ per minute. The standard error of estimate for the Ascarite tube was $\pm 1.65 \gamma$ and for the Drierite tube $\pm 2.65 \gamma$. Mitsui (36) has been actively designing and modifying microchemical apparatus, including many of the more modern and desirable features, and also critically examining such points as design of absorption tubes and preparation of reagents. For determining very small amounts of carbon dioxide produced by combustion, Holt (21) has designed a capillary trap which produces a 4-mm. pressure reading per microgram of carbon.

The Fisher Award Lecture of Van Slyke (58) gives an excellent review of the status of the wet-combustion method for carbon in various materials, for radioactive carbon, and for other elements precipitated with organic reagents.

NITROGEN

McKenzie and Wallace (33) have probably come closer to the optimum conditions for the Kjeldahl digestion than any workers reporting to date. They determined the optimum temperature to be between 380° and 390° C. and obtained that temperature by using 1 gram of potassium sulfate per milliliter of sulfuric acid. Mercuric oxide in sulfuric acid was the catalyst, addition of hydrogen peroxide was unnecessary, and digestion for 15 minutes

after clearing was sufficient. A fixed time of boil would be preferable to 15 minutes after clearing, because some materials do not char. The ammonia was absorbed in 2% rather than the 4% boric acid preferred by most analysts. Scandrett (43) used as his steam source the superheated steam supplied by the laboratory steam line rather than the customary steam generator. This readily available source, although seldom used, was easy to control, safe, and satisfactory despite the introduction of rust particles into the distillation apparatus.

The sealed-tube method of White and Long had given low results for certain nitro compounds, but Baker (4) reported that good results could be obtained if 50 mg. of thiosalicylic acid or glucose were added to the digest. Grunbaum, Kirk, Green, and Koch (19) reported a critical study of the sealed-tube technique, determining the temperature effect on the stability of the ammonia and the effect of the amount of sulfuric acid and the addition of water on ammonia stability. Their study covered both the milligram and microgram ranges.

A new "wrinkle" in indicators proposed by Sher (48) was a two-step or signaling indicator consisting of bromocresol green, new cocine, and *p*-nitrophenol dissolved in the boric acid absorbing solution. He stressed the point, which is sometimes overlooked, that the solution before titration should always be adjusted to a fixed volume.

A simple colorimetric method for 1 to 20 γ of nitrogen was proposed by Boissonnas and Haselbach (9). After digestion with sulfuric acid the digest was neutralized with a lithium hydroxide-acetate solution in 20% Cellosolve, ninhydrin hydrindantin reagent was added, the solution was heated, and the color was measured.

The Dumas method frequently gives low results for certain types of heterocyclic compounds because of retention of nitrogen in the refractory chars formed during analysis. Two groups of analysts, Swift and Morton (55) and Parks and others (42), described methods for recovering this nitrogen by burning the char with oxygen after the usual combustion in carbon dioxide. Swift and Morton used cylinder oxygen and the other group used electrolytically generated oxygen and the nickel-nickel oxide tube filling of Kirsten. In addition to the emphasis on method for refractory materials, the trend is toward more rapid Dumas-type methods. Swift (54) described another such method which required less than 30 minutes per determination and used a carbon dioxide flow rate of 15 to 16 ml. per minute. In a collaborative study reported by this reviewer (37) it was noted that more analysts in this country were shifting to the Shelberg and Zimmermann methods and that these procedures gave results as good as or better than those obtained by the more conventional procedures. Kuck and Altieri (27) extended the Dumas method to permit analysis of samples liberating less than 0.2 ml. of nitrogen. To do this a nitrometer with a capacity of 0.2 ml. was designed and calibrated in microliters and samples were weighed on a Garner quartz fiber balance. With 0.4-mg. samples the standard error of estimate was $\pm 0.28\%$ nitrogen.

SULFUR

A new direct titrimetric method for sulfate applicable on the semimicro scale was proposed by Fritz and Freeland (17). Titration was with standard barium solution using alizarin red S as indicator and was carried out in 30 to 40% alcohol. Equilibrium was quickly attained, permitting a more rapid titration than with sodium rhodizonate indicator, but the coprecipitation errors were greater than with the gravimetric method. When interfering anions were absent, removal with an ion exchange column so improved the results that they were as good as those obtained by the gravimetric procedure. An indirect titrimetric method, described by Wilson, Pearson, and Fitzgerald (60), determined the excess barium ion after barium sulfate precipitation by titration

with 0.02*N* (ethylenedinitrilo)tetraacetic acid (ethylenediamine-tetraacetic acid, EDTA). These workers used ceria or alumina as catalyst in the combustion and found it to be unaffected by chlorine. Of the indirect methods, this one seems promising because of its simplicity and the probability that coprecipitation will be less because precipitation and titration take place in a very dilute solution.

The silver absorbent method was investigated further by Kuck and Grim (30) and Vecera (59). Vecera recommended the use of a tube filled with silver shavings because this prevents mechanical loss of the silver sulfate and permits easy extraction, should halogens be present in the material being analyzed. Kuck and Grim again extended the method to decimilligram samples using the Garner balance and found that as little as about 50 γ of sulfur could be determined satisfactorily. For materials containing alkali and alkaline earth metals, Sirotenko (49) recommended fusion of the sulfate residue in the combustion boat with boron trioxide, thereby liberating the sulfur trioxide.

Electron microscope studies by Schulek, Pungor, and Guba (47) showed that the addition of ammonium chloride at the time the barium sulfate was precipitated changed the morphology of the precipitate and facilitated its filtration. A collaborative study (39) showed that the barium sulfate precipitate obtained in the AOAC Carius and catalytic combustion methods need not be subjected to a second washing and ignition procedure.

Anderson (2) described a spectrophotometric method for determining microgram quantities of sulfur, in which the sulfur was converted to sulfate, benzidine sulfate was precipitated, and the precipitate was dissolved in hydrochloric acid, made to volume, and measured spectrophotometrically. Ignition of organic substances with magnesium powder was proposed by Schöniger (44), who then determined the sulfur iodometrically after distillation of the sulfide by Zimmermann's procedure. The same ignition procedure was also used for chlorine and bromine compounds with the argentometric measurement of Kainz and Resch.

HALOGENS

The Pregl combustion technique followed by a Northrop potentiometric titration was used by Kuck, Daugherty, and Batdorf (29) to determine chlorine in small samples. Use of the silver-silver oxalate reference cell with a silver electrode eliminated the need for graphic location of the equivalence point, since the presence of chloride ion displaced the electrochemical equilibrium. The titration merely involved the addition of standard silver nitrate solution until the equilibrium was restored. Thomas (57) recommended the simple titration with mercuric nitrate using diphenylcarbazone-bromophenol blue mixed indicator for determining chlorides in aqueous solution.

Chateau (10) described an interesting procedure for determining both bromine and iodine even when present with chlorine. The bromates and iodates were formed by oxidation with sodium hypochlorite at pH 5.5 and the excess oxidizer was reduced by potassium formate. At pH 4.1, potassium iodide reduced only the iodate and the liberated iodine was titrated with standard thiosulfate solution. Addition of sulfuric acid to the bromate-iodide solution liberated six atoms of iodine per initial bromine and this was again titrated with thiosulfate. If the ratios of the two halogens were not greater than 200 to 1, reasonably good results were obtained. The method can be applied to silver halides if the halides are put in solution with hydroxylamine in basic solution. The spectrophotometric method of Hunter and Goldspink (22) permitted determination of less than microgram quantities of bromine. To do this two quantitative conversions were necessary: first, that of bromide to bromate by hypochlorite oxidation and, second, formation of tetrabromosaniine by the bromine liberated through the reaction of the bromate with added bromide.

Belcher and coworkers (5, 6) reported methods for fluorine and

for the other three halogens in fluorine-containing compounds using the nickel bomb method with sodium or potassium. Each halogen was determined separately by accepted titrimetric procedures. Clark and Rees (11) also proposed a method for determining all four halogens based on combustion in oxygen. Fluorine was separated from the other halogens by making the latter react with silver. A mathematical calculation was necessary to obtain complete differentiation.

The silver-amalgamated silver electrode system of Clark was adapted to the microdetermination of chlorine, bromine, and iodine by Cogbill and Kirkland (12). They stressed the ease of construction of small electrodes and their sensitivity in very dilute solutions. A novel combustion procedure for relatively nonvolatile materials was described by Schöniger (45). The sample was burned in a piece of ash-free filter paper in an oxygen atmosphere in an Erlenmeyer flask and the combustion products were absorbed and titrated.

OXYGEN

Oita and Conway (40) described a modification of the Schutze-Unterzaucher method for oxygen, which eliminated the need for an 1120° C. furnace. They replaced the carbon filling with one composed of 50% platinized carbon and found that a temperature of 900° C. was sufficient to convert all oxygen to carbon monoxide. They also introduced a copper roll in the heated portion of the tube to remove sulfur and found that with this combination the blanks were usually negligible. Oliver (41) confirmed the findings of Oita and Conway and also described methods for the preparation of the carbon-platinum catalyst, copper gauze, and iodine pentoxide. Four different modifications of the Unterzaucher method were described by Jones (24). Two procedures for determining carbon dioxide, one gravimetric and one volumetric, were used, iodine was absorbed on sodium thiosulfate crystals, and in the fourth pyrolytic hydrogen was removed by diffusion through palladium, followed by the usual liberation and thiosulfate titration of the iodine.

MISCELLANEOUS ELEMENTS

Bernhart and Wreath (7) described a modified spectrophotometric method for determining orthophosphate which is not affected by moderate amounts of pyro-, meta-, or polyphosphates. Color is developed in a solution containing sulfuric acid and at least 25% acetone, and the method is applicable to solutions obtained after either acid digestion or alkaline fusion of organic materials. Di Bacco (15) proposed a molybdenum blue procedure in which sulfuric acid was used but not acetone, and applied a similar method to the determination of arsenic. Lévy (31, 32) also described methods for both phosphorus and arsenic, but used a potentiometric titration procedure in which silver nitrate was added in excess of that needed to precipitate the anion and the excess silver was titrated with a standard chloride solution. In both procedures an empirical correction was necessary. The iodometric determination of arsenic in organic materials on a semimicro scale, proposed by Bähr, Bieling, and Thiele (3), required sulfuric acid digestion in a Kjeldahl flask followed by permanganate and perhydrol oxidation. The iodine liberated by the arsenate was determined by thiosulfate titration. Martin (34) increased the specificity of the colorimetric boron method using chromotrope 2B by replacing the concentrated sulfuric acid with a mixture of sulfuric and acetic acids plus acetic anhydride.

FUNCTIONAL GROUPS

Heron and coworkers (20) made a critical study of the methoxyl and ethoxyl group determinations and found the main source of error to be the thiosulfate in the wash solution. This was replaced by 25% sodium acetate with a considerable improvement

in results, especially when ethoxyl groups were determined. In two collaborative studies reported by Steyermark (50, 53), analysts using the Clark method obtained better results than those using the Elek procedure. Methoxyl and ethoxyl ethers and esters were determined. However, when all analysts used a modified Clark procedure the results were not sufficiently accurate or precise to recommend the adoption of the method as an official AOAC procedure. A further modification of the method following the suggestion of Heron and others (20) did lead to good results in collaborative work concluded this year but not yet published. To determine methoxyl in the presence of ethoxyl, Gran (18) absorbed the iodides in trimethylamine dissolved in isopropyl alcohol. Tetramethylammonium iodide precipitated quantitatively on standing overnight. Mathers and Pro (35) used an entirely different approach. Methoxyl was cleaved to methanol and oxidized to formaldehyde, and the latter was determined spectrophotometrically by the chromotropic acid method. The method gave good results and is specific for the methoxyl group.

Schöniger, Lieb, and El Din Ibrahim (46) used the Parnas-Wagner Kjeldahl distillation apparatus to steam-distill the acetic acid formed either by oxidation of C-methylated substances or by saponification of acetyl compounds. Only 10 minutes were required for a distillation, titration was with standard alkali, and the results were good. In the collaborative study reported by Steyermark (53), neither the Roth-Kuhn nor the Elek and Harte method consistently gave satisfactory results. Tani and Nara (56) avoided distillation of the acetic acid by passing the solution after saponification through a column of cation exchange resin in the hydrogen cycle and titrating the effluent with standard base.

STANDARDIZATION

Official methods for the determination of carbon and hydrogen, nitrogen by the Kjeldahl method, sulfur, chlorine, and bromine have been adopted by the Association of Official Agricultural Chemists (38). A method for methoxyl and ethoxyl determination has been recommended for adoption and a preliminary study of methods for phosphorus has been completed, but neither has been published.

The Committee on Microchemical Apparatus of the Division of Analytical Chemistry, AMERICAN CHEMICAL SOCIETY, has published recommended specifications for numerous pieces of weighing and drying apparatus (51). The committee has also drawn up specifications for a modified Clark alkoxy apparatus (52).

LITERATURE CITED

- (1) Abramson, E., Brochet, A., *Bull. soc. chim. France* 1954, 367-9.
- (2) Anderson, L., *Acta Chem. Scand.* 7, 689-92 (1953).
- (3) Bähr, G., Bieling, H., Thiele, K. H., *Z. anal. Chem.* 143, 103-11 (1954).
- (4) Baker, P. R. W., *Analyst* 80, 481-2 (1955).
- (5) Belcher, R., Fernandez-Caldas, E., Clark, S. J., Macdonald, A., *Mikrochim. Acta* 1953, 283-9.
- (6) Belcher, R., Macdonald, A. M. G., Nutten, A. J., *Ibid.*, 1954, 104-16.
- (7) Bernhart, D. N., Wreath, A. R., *ANAL. CHEM.* 27, 440-1 (1955).

- (8) Bodenheimer, W., Goldstein, M., *Bull. Research Council Israel* 3, No. 1/2, 53-5 (1953).
- (9) Boissonnas, R. A., Haselbach, C. H., *Helv. Chim. Acta* 36, 576-81 (1953).
- (10) Chateau, H., *Science et inds. phot.* 26, 41-6 (1955).
- (11) Clark, H. S., Rees, O. W., Illinois State Geol. Survey, Rept. Invest. 169 (1954).
- (12) Cogbill, E. C., Kirkland, J. J., *ANAL. CHEM.* 27, 1611-13 (1955).
- (13) Cropper, F. R., *Mikrochim. Acta* 1954, 25-48.
- (14) Cross, C. K., Wright, G. F., *ANAL. CHEM.* 26, 886-90 (1954).
- (15) Di Bacco, G., *Boll. chim. farm.* 93, 43-7, 88-90 (1954).
- (16) Freier, H. E., Nippoldt, B. W., Olson, P. B., Weiblen, D. G., *ANAL. CHEM.* 27, 146-9 (1955).
- (17) Fritz, J. S., Freeland, M. Q., *Ibid.*, 26, 1593-5 (1954).
- (18) Gran, Gunnar, *Svensk Papperstidn.* 57, 702-8 (1954).
- (19) Grunbaum, B. W., Kirk, P. L., Green, L. G., Koch, C. W., *ANAL. CHEM.* 27, 384-8 (1955).
- (20) Heron, A. E., Reed, R. H., Stagg, H. E., Watson, H., *Analyst* 79, 671-80 (1954).
- (21) Holt, B. D., *ANAL. CHEM.* 27, 1500-1 (1955).
- (22) Hunter, G., Goldspink, A. A., *Analyst* 79, 467-75 (1954).
- (23) Hussey, A. S., Sorensen, J. H., DeFord, D. D., *ANAL. CHEM.* 27, 280-1 (1955).
- (24) Jones, W. H., *Ibid.*, 25, 1449-52 (1953).
- (25) Klimova, V. A., Korshun, M. O., Bereznitskaya, E. G., *Doklady Akad. Nauk S.S.S.R.* 96, 81-3 (1954).
- (26) *Ibid.*, pp. 287-8.
- (27) Kuck, J. A., Altieri, P. L., *Mikrochim. Acta* 1954, 17-24.
- (28) Kuck, J. A., Altieri, P. L., Towne, K., *Ibid.*, 1954, 1-16.
- (29) Kuck, J. A., Daugherty, Martha, Batdorf, D. K., *Ibid.*, 1954, 297-307.
- (30) Kuck, J. A., Grim, E. C., *Ibid.*, 1954, 201-10.
- (31) Lévy, Roger, *Compt. rend.* 236, 1781-3 (1953).
- (32) *Ibid.*, 238, 2320-1 (1954).
- (33) McKenzie, H. A., Wallace, H. S., *Australian J. Chem.* 7, 55 (1954).
- (34) Martin, Georges, *Bull. soc. chim. biol.* 36, 719-29 (1954).
- (35) Mathers, A. P., Pro, M. J., *ANAL. CHEM.* 27, 1662-4 (1955).
- (36) Mitsui, Tetsuo, *Kagaku no Ryoiki (J. Japan. Chem.)* 5, 687-97 (1951).
- (37) Ogg, C. L., *J. Assoc. Offic. Agr. Chemists* 37, 450-8 (1954).
- (38) *Ibid.*, 38, 365-7 (1955).
- (39) *Ibid.*, pp. 377-80.
- (40) Oita, I. J., Conway, H. S., *ANAL. CHEM.* 26, 600-2 (1954).
- (41) Oliver, F. H., *Analyst* 80, 593-4 (1955).
- (42) Parks, T. D., Bastin, E. L., Agazzi, E. J., Brooks, F. R., *ANAL. CHEM.* 26, 229-32 (1954).
- (43) Scandrett, F. J., *Analyst* 78, 734-7 (1953).
- (44) Schöniger, W., *Mikrochim. Acta* 1954, 74-80.
- (45) *Ibid.*, 1955, 123-9.
- (46) Schöniger, W., Lieb, H., El Din Ibrahim, M. G., *Ibid.*, 1954, 96-103.
- (47) Schulek, E., Pungor, E., Guba, F., *Anal. Chim. Acta* 10, 506-12 (1954).
- (48) Sher, I. H., *ANAL. CHEM.* 27, 831-2 (1955).
- (49) Sirotenko, A. A., *Mikrochim. Acta* 1955, 153-4.
- (50) Steyermark, Al., *J. Assoc. Offic. Agr. Chemists* 38, 367-77 (1955).
- (51) Steyermark, Al., Alber, H. K., Aluise, V. A., Huffman, E. W. D., Jolley, E. L., Kuck, J. A., Moran, J. J., Ogg, C. L., Willits, C. O., *ANAL. CHEM.* 26, 1186-90 (1954).
- (52) *Ibid.*, 28, 112 (1956).
- (53) Steyermark, A., Loeschauer, E. E., *J. Assoc. Offic. Agr. Chemists* 37, 433-49 (1954).
- (54) Swift, H., *Analyst* 79, 718-21 (1954).
- (55) Swift, H., Morton, E. S., *Ibid.*, 77, 392-6 (1952).
- (56) Tani, Hideo, Nara, Akio, *J. Pharm. Soc. Japan* 74, 1399-400 (1954).
- (57) Thomas, J. F., *J. Am. Water Works Assoc.* 46, 257-62 (1954).
- (58) Van Slyke, D. D., *ANAL. CHEM.* 26, 1706-12 (1954).
- (59) Vecera, M., *Chem. Listy* 48, 613-6 (1954).
- (60) Wilson, H. N., Pearson, R. M., Fitzgerald, D. M., *J. Appl. Chem. (London)* 4, 488-96 (1954).