the quotients are the variables. The extrapolation involved is much less than with the (alignment chart) method given in the body of the paper.

Literature Cited.

1. W. R. Bousfield and T. M. Lowry, "The Purification and Properties of Acetic Acid," *J. Chem. Soc.*, **99**, 1432–1441 (1911).

2. A. L. Crelle, "Rechentafeln," Berlin, 1880.

3. J. N. Currie, "Flavor of Roquefort Cheese," *J. Agr. Res.*, **2**, 1–14, 429–434 (1914).

4. A. W. Dox and R. E. Neidig, "The Volatile Aliphatic Acids in Corn Silage," Iowa Agr. Exp. Sta., *Research Bull.* **7** (1912).

5. E. Duclaux, "De l'origine de l'acide acétique produit pendant la fermentation alcoolique," *Annales (scientifiques) de l'École Normale Supérieure*, **2**, 270–290 (1865).

6. E. Duclaux, "Recherches sur les vins. II. Sur les acides volatils du vin," *Ann. chim. phys.*, [5] **2**, 289–324 (1874).

7. E. Duclaux, "Traité de Microbiologie," **3**, 385 (1900).

8. D. C. Dyer, "A New Method of Steam Distillation for the Determination of the Volatile Fatty Acids, Including a Series of Colorimetric Qualitative Reactions for their Identification," *J. Biol. Chem.*, **28**, 445–473 (1917).

9. A. Harden, "The Chemical Action of *Bacillus coli communis* and Similar Organisms on Carbohydrates and Allied Compounds," *J. Chem. Soc.*, **79**, 610 (1901).

10. E. B. Hart and J. J. Williman, "Volatile Fatty Acids and Alcohols in Corn Silage," THIS JOURNAL, **34**, 1619–1625 (1912).

11. O. Jensen, "Études sur les acides gras volatils du fromage et contributions à la biologie des ferments du fromage," *Ann. agr. Suisse*, **5**, 229 (1904).

12. Friedrich Kohlrausch, "Lehrbuch der praktischen Physik," 11th Ed., 20–22, Leipzig and Berlin (1910).

13. Justus von Leibig, *Ann.*, **71**, 355.

14. I. K. Phelps and H. E. Palmer, "The Separation and Estimation of Butyric Acid in Biological Products," *J. Biol. Chem.*, **29**, 199–205 (1917).

15. H. Droop Richmond, "Studies in Steam Distillation," *Analyst*, **33**, 209–217, 305–313 (1908).

16. G. Seliber, "Détermination des acides dans les produits de fermentation de quelques microbes d'après la méthode de Duclaux," *Compt. rend.*, **150**, 1267–1270 (1910).

17. S. K. Suzuki, E. G. Hastings and E. B. Hart, "The Production of Volatile Fatty Acids and Esters in Cheddar Cheese and their Relation to the Development of Flavor," *J. Biol. Chem.*, **7**, 431 (1909).

18. Fred W. Upson, H. M. Plum and J. E. Schott, "On the Duclaux Method for the Estimation of the Volatile Fatty Acids," THIS JOURNAL, **39**, 731 (1917).

WASHINGTON, D. C.

[CONTRIBUTION FROM THE LABORATORIES OF THE OFFICE OF SOIL FERTILITY, BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.]

A SIMPLIFIED MICRO-COMBUSTION METHOD FOR THE DETERMINATION OF CARBON AND HYDROGEN.

BY LOUIS ELSBERG WISE.

Received June 25, 1917.

The micro-combustion method devised by Pregl[1] and successfully used by various European investigators, has apparently received scant atten-

---

[1] F. Pregl, in "Abderhalden's Handbuch der Biochem. Arbeitsmethoden," [ii] **5**, 1307.

tion in this country, notwithstanding the fact that it permits the use of very small amounts of substance and is capable of giving excellent results. This may perhaps be due to the inherent difficulties attending its use, and to the apparent necessity of using a Kuhlmann micro-balance, which Pregl featured in his original description of the method and which at present is unavailable.

The author has found that if the analyst is content to use 12 to 22 mg. samples for analysis, instead of the 8 to 13 mg. samples recommended by Pregl and the 4 to 7 mg. samples used by Dubsky,[1] the weighings may be carried out on a sensitive analytical balance. Furthermore, some of the difficulties of the micro-combustion, as described by Pregl have been overcome with the aid of certain modifications and simplifications in the apparatus and procedure used, and by the use of some simple but necessary precautions in weighing and in carrying out the combustion proper. In its present form the micro-combustion method may be used and perhaps be of service in the average, well equipped biochemical and organic laboratory.

The Combustion Tube.

The tube[2] in which the combustion is carried out is of hard glass, 1.5 cm. in diameter (with a bore of 1.2 cm.) and 38 cm. in length. About 4 cm. from one end, the tube is drawn to a smooth taper, as shown in Fig. 1, care being taken not to form more than a slight bulge near the extremity of the tube, so that a rubber connection will fit the end snugly. The tip of the tube should be 3 mm. in diameter. The combustion tube is filled with the following materials (introduced into the tube in the order named): (1) a pledget of fine glass wool, loosely packed into the constricted portion of the tube; (2) a 2.5 cm. layer of asbestos treated with cupric oxide,[3] containing a few short lengths of cupric oxide wire; (3) a

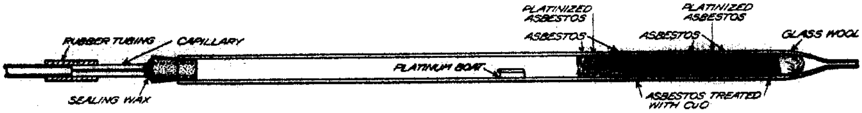

Fig. 1.—Micro-combustion tube.

---

[1] Dubsky, *Chem. Ztg.*, 38, 510 (1914).

[2] The author's combustions were carried out in Jena glass tubes, which seem to be unobtainable at present. There is no reason to believe that other hard glass tubes may not serve equally well. Since the heat required in carrying out micro-combustions is less than that required in the case of macro-combustions, the tubes devitrify very slowly. The Jena tube now used by the author is still serviceable (although slightly devitrified) after 50 or more combustions.

[3] The cupric-oxide-treated asbestos is prepared by impregnating acid-washed asbestos with a concentrated cupric nitrate solution, drying, and carefully igniting. The treated asbestos should be very dark gray in color, and several treatments with cupric nitrate are sometimes necessary. Platinized asbestos is formed by immersing purified asbestos in 10% hydrochloroplatinic acid solution, drying and igniting.

0.5 cm. layer of plain asbestos; (4) a 1.5 cm. layer of platinized asbestos; (5) a 0.5 cm. layer of plain asbestos; (6) a 3.5 cm. layer of asbestos treated with cupric oxide and mixed with cupric oxide wire; (7) a 0.5 cm. layer of plain asbestos; (8) a 1.5 cm. layer of platinized asbestos; and finally (9) a 0.5 cm. layer of plain asbestos.

A single thickness of damp 1/64" asbestos paper is molded around the filled portion of the tube and fastened into place by means of 2 or 3 short pieces of stout asbestos cord. The tube is then dried at 100° (in a hot air oven) and later heated to remove residual water according to the directions given later.

A compact light support for the combustion tube is shown in the photograph. It consists of a brass trough (U), 8 inches long, mounted on an iron support (H) about 10 inches in height. That part of the trough which corresponds to the empty portion of the combustion tube is lined with 1/64" asbestos paper.

The Drying Train.

The photograph shows the form of drying train (A and B) used in the micro-combustion. The train consists of a "side-neck" test tube, the side tube of which is sealed to that of a Schwarz U-tube provided with perforated, ground-in glass stopcocks (*a*) and (*b*). Both the test tube (A) and the U-tube (B) are about 120 mm. long. The inlet tube (*m*), which enters (A) through a one-hole stopper, is made of 4 mm. glass tubing. Its lower end, which should not quite reach the bottom of (A) is drawn to a 1 mm. tip, which serves as a means of observing the rate with which air is drawn through the drying train.

The drying train is filled as follows: A large pledget of glass wool is introduced into (B) so as to completely fill the bend of the tube, thus dividing (B) into two equal compartments. The compartment further from the tube (A) is then nearly filled with anhydrous calcium chloride (12 mesh) which is held in place by another smaller pledget of glass wool. The calcium chloride is then saturated with carbon dioxide.[1] The stream of carbon dioxide, which should enter through (A) and leave by way of (B), should be maintained for about 1 hour. During this treatment the side-arm of (B) should be protected with a calcium chloride guard tube. After saturation of the calcium chloride, the excess of carbon dioxide is removed by aspirating dry air through (A) and (B), precautions being

---

[1] This appears to be essential. All anhydrous calcium chloride, unless specially treated contains appreciable quantities of lime (cf. Morse, "Exercises in Quantitative Chemistry," p. 340). The anhydrous, granular calcium chloride contained in the absorption tube used for the determination of water is invariably freed from lime by treatment with carbon dioxide. Lime is a markedly less efficient drying agent than is calcium chloride, and if *appreciable amounts* of lime are present in the calcium chloride of the drying train, and *none* in calcium chloride of the absorption tube, high results might be expected.

taken to prevent moisture from entering the upper portion of the calcium chloride layer. The other compartment of the U-tube (B) is then nearly filled with a layer of moist soda-lime (containing 10 to 13% of water), which is followed by a pledget of glass wool. The soda lime serves to absorb the carbon dioxide, as well as many other gases which might be present in small amounts in the atmosphere of a laboratory.[1] To the tube (A) are added about 4 cc. of concentrated sulfuric acid which serves to remove ammonia, as well as moisture. In some of the earlier analyses made by the author (see Table I), a 50% solution of potassium hydroxide was used in the tube (A), whereas tube (B) contained calcium chloride only. The drying system described above, however, has been shown by numerous "blank" combustions to be more effective, and has therefore been adopted.

By means of a clean piece of seamless rubber tubing[2] ($^1/_8$″ bore), the free side arm of (B), which is about 6 mm. in diameter, is connected and brought into close contact with a heavy wall capillary tube, 6 mm. in diameter, with a capillary bore of 0.4 mm. The capillary tube, which is about 6 cm. in length, enters the combustion tube through a tight-fitting No. 00 rubber stopper, the end of the tube being cut flush with the end of the stopper, as shown in Fig. 1.

Micro-combustion Boats and Micro-Desiccators.

The combustion boats are of platinum. A small flat boat, similar to that described by Pregl (shown in Fig. 1) is 1.4 cm. long, 0.9 cm. wide and about 0.3 cm. in height. It weighs approximately 0.5 gm. and is very serviceable when readily combustible, non-volatile substances like the carbohydrates, hydroxy-acids, etc., are to be analyzed. If the substance used for analysis is readily volatile, easily fusible, difficult to burn, or if it contains nitrogen, it is better to use a more capacious canoe-shaped boat, which is about 5 cm. long, 0.5 cm. wide and 0.8 cm. high, and which weighs about 4.5 g. The boats are best handled with a small pair of forceps (similar to those shown in the photograph).

The low form of "Stender dish," with its top surface ground into a groove in the plate glass cover, may be readily converted into a micro-desiccator, in which the platinum boats or the substance to be analyzed can be kept prior to the combustion. Such an improvised desiccator (Q) is shown in the photograph.

---

[1] Guareschi, *Atti. accad. sci. Torino*, 51, 4–26, 59–82, 263–78, *et al.* (1916).

[2] The short lengths of red, hand-made rubber tubing used in joining together various pieces of the micro-combustion apparatus, and in preparing "caps" for absorption tubes must be thoroughly cleaned before they are used. A glass rod, coated with glycerol, serves to loosen and remove particles of rubber dust adhering to the inner surface of the rubber tubing, which is subsequently washed with water and carefully dried. Cleansed pieces of tubing should be kept in dust-proof dishes.

Absorption Tubes.

The straight calcium chloride tube, used for the absorption of the water formed during the combustion is represented in Fig. 2. Although larger, it has very nearly the same form as that suggested by Pregl. It is made of thin walled 8 mm. glass tubing, and is about 21 cm. in length. The body of the tube, which is about 14 cm. long, the four capillary constrictions (which are about 1 mm. in diameter), and the blown spherical enlargement near each end are all clearly shown in the figure. The tube terminates at each end in straight tubes about 2 cm. in length and 3 mm. in diameter, having a bore of about 2 mm. The calcium chloride tube is best constructed in two sections, which are shown separated from each other in the diagram by the dotted line. After the introduction into the larger of these sections of a pledget of glass wool, followed by a 8.5 cm. layer of 12-mesh granular calcium chloride and another pledget of glass wool, the two parts are sealed together. The calcium chloride is then saturated with carbon dioxide, care being taken to avoid the absorption of small quantities of water, and to provide for the removal of the last traces of carbon dioxide. A filled calcium chloride tube should weigh roughly 11 g. When not in use, the calcium chloride tubes should be kept securely "capped" with a short length of $^{1}/_{8}''$ red rubber tubing plugged with a short piece of 4 mm. glass rod, *the glass surface of the rod meeting that of the absorption tube.* A calcium chloride tube prepared according to the above method may be used in 10 to 15 combustions without recharging. The tube may then either be broken (at the point indicated on the diagram by the dotted line), cleaned out, dried, refilled, and the glass parts resealed, or else the water may be removed by capping the end of the tube *furthest from the moist calcium chloride layer,* joining the other end with that of a calcium chloride guard tube, and connecting the latter with the laboratory suction. By maintaining a *very moderate* heat (with the aid of a fish-tail burner) under that portion of the absorption tube which contains moist calcium chloride, the water gradually distils from this tube into the cooler guard tube, leaving the calcium chloride in the absorption tube in dry, granular form. After such treatment the calcium chloride in the absorption tube should again be saturated with carbon dioxide.

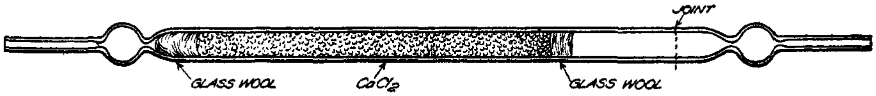

Fig. 2.—Calcium chloride absorption tube.

The carbon dioxide absorption tube originally devised by Pregl (which contained glass wool impregnated with 50% potassium hydroxide solution and anhydrous calcium chloride) was discarded because of the difficulty in preventing an excessive amount of water from distilling from the potash into the calcium chloride. At times the latter became so moist that a stoppage occurred in the absorption tube. This difficulty was also noted by Fisceman,[1] who recently devised a carbon dioxide absorption train consisting of a small gas-washing bottle containing caustic potash coupled with an absorption tube containing soda-lime and calcium chloride.

The tube used by the author for absorbing the carbon dioxide generated during the combustion is shown in Fig. 3. Its dimensions are very similar to those of the calcium chloride tube, but it differs from this tube in having a ground-in glass joint near one end, which permits the repeated filling and cleaning of the tube. The tube is filled in the following manner: A pledget of glass wool is introduced into the tube up to the first capillary constriction. This is followed by an 8.5 cm. layer of moist soda lime (containing 10–13% of water), a 1 cm. layer of dry soda lime, a pledget of glass wool, a 1.25 cm. layer of calcium chloride, and finally another pledget of glass wool. The latter should be sufficiently removed from the joint to prevent loose threads of glass wool from coming into contact with the ground-glass surfaces. The ground glass is lubricated with the minimum amount of vaseline. The ends of the tube, when not in use are kept tightly capped. A filled soda-lime tube weighs approximately 14 grams. It should be cleaned out and recharged after each combustion.

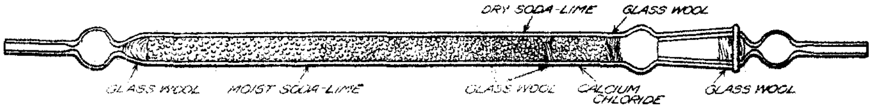

Fig. 3.—Soda-lime absorption tube.

Preliminary Heating of the Combustion Tube.

The combustion tube (see photograph) placed on its support so that its shoulder projects just beyond the end of the trough, is connected with the drying train. The constricted end of the tube is connected with the laboratory suction, and the stopcocks (*a*) and (*b*) of the U-tube (B) are opened. Air is aspirated through the train at such a rate that not more than 3 bubbles per second pass through (A). Alundum tiles (G) made from alundum half tubes $^{3}/_{4}''$ diameter and $^{1}/_{8}''$ thick, and $1^{1}/_{2}$ to 2 inches long are used to cover the entire length of the combustion trough. The combustion tube is heated by means of two burners, a fish-tail burner (W) and a Bunsen burner (R). The preliminary heating should continue until all the water has been removed from the combustion tube F.[2] To facilitate the removal of the last traces of moisture from the constricted part of the combustion tube, the following simple but effective "rider" (de-

---

[1] Fisceman, *Rend. acad. sci. Napoli*, **22**, 31 (1916).

[2] Whether or not all water and carbon dioxide have been removed from a recently charged combustion tube is best determined by carrying out a blank combustion.

vised by Pregl) was used. A 10 cm. length of No. 18 copper wire is bent into the form of a long armed U. The ends of the rider are laid on the trough at each side of the combustion tube and the bend of the rider is laid on the combustion tube at its tapered end, within 0.5 cm. of the rubber tubing which connects the combustion tube with the suction. The heat conducted through the copper is sufficient to vaporize condensed water, without injuring the rubber connection.

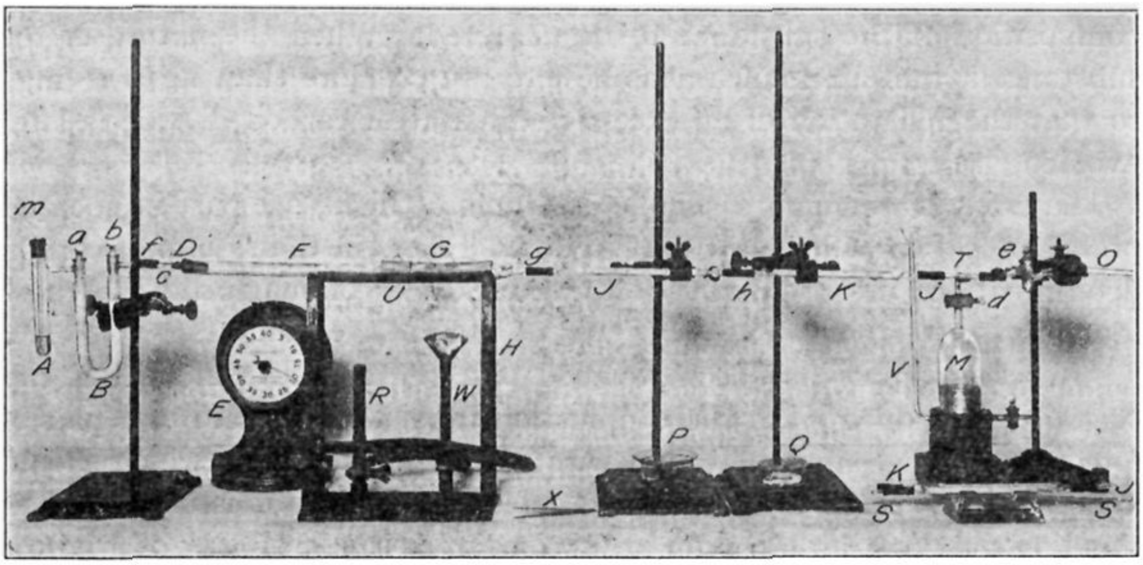

Fig. 4.

Photograph of micro-combustion apparatus.

A, side-neck test tube provided with inlet tube *m*; *B*, side-arm Schwarz U-tube with perforated stopcocks *a* and *b*; *c*, heavy-walled capillary tube entering combustion tube *F* through stopper *D*; *f*, *g*, *h*, and *j*, red rubber connections; *E*, interval timer; *G*, alundum tiles; *J*, calcium chloride absorption tube; *K*, soda-lime absorption tube; *M*, safety manometer provided with side-arm *V* and stopcock *d*; *T*, T-tube leading to *M* and to the stopcock *e* which is connected with tube *O* leading to suction; *U* brass trough resting on support *H*; *W*, wing-top burner; *R*, Bunsen burner; *P*, covered container for "caps;" *Q*, micro-desiccator; *S*, wire used to introduce combustion boat into tube; *X*, forceps for handling combustion boat.

After the preliminary heating is complete, the suction is cut off, and the flames then turned out. The outlet of the combustion tube is capped while the tube is still hot. When the tube has cooled slightly the stopcocks (*a*) and (*b*) of the tube (B) are closed.

Weighings.

All weighings are made on a sensitive analytical balance.[1] Readily combustible non-volatile substances like the carbohydrates, are weighed in the small platinum boat. Difficultly combustible, volatile, or nitrogenous substances are weighed in the canoe-shaped platinum boat, and

---

[1] The balance used by the author is a Becker's Sons' Rotterdam balance, which shows a sensibility of about 5 scale divisions per mg. for a 4 g. load. Using small loads, it was ordinarily found possible to weigh accurately to 0.03 mg.

subsequently mixed with freshly ignited (cool) cupric oxide, and covered with a layer of the oxide so that the entire boat is filled.

Experience has shown that 12 to 22 mg. samples may be used in the micro-analysis, the actual amount chosen depending on the nature and composition of the substance to be analyzed. When the nature of the substance is quite unknown, it is best to use no more than 14 to 16 mg., so that the generation of an excessive amount of carbon dioxide is later avoided.

The error in weighing a sample is seldom as high as 1 part in 200 and probably seldom as low as 1 part in 600. Errors in the results of carbon determinations are ordinarily of the same order of magnitude as those found in the weighings.

After the sample has been weighed out, the combustion boat is *immediately* introduced into the combustion tube, with the aid of a pair of forceps. A long, stout platinum wire with a hooked end (which should invariably be heated to redness and cooled *just* before it is used), serves to guide the boat into position, a few cm. from the last layer of asbestos in the combustion tube. The tube should never be kept open for more than a minute or two at a time, and after the introduction of the boat, should immediately be reconnected with the drying train.

The following technique is always used in weighing the soda lime and calcium chloride absorption tubes: After the removal of the "caps" the surface of the absorption tube is rubbed during one minute with "lens-cloth" (a chemically treated nainsook). The tube is then placed on a counterpoised tube holder on the balance pan, *and weighed at the end of just 4 minutes.* (An interval timer has been found very serviceable in this connection.) The tube is then removed from the balance pan, again wiped during one minute with lens-cloth, and weighed at the end of four minutes. If the difference between the two weighings is greater than 0.05 mg., the entire operation is repeated. Ordinarily however, constant weight is reached after the first two operations.

The Combustion.

The cap is removed from the end of the combustion tube, which is then joined to the calcium-chloride tube (J) by means of a short length of red rubber tubing as shown in the photograph. The calcium-chloride tube is then connected with the end of the soda-lime tube (K) *furthest* from the ground-in joint. The importance of having tight-fitting joints cannot be over-emphasized, adjacent glass surfaces must meet each other at every joint, and must be held securely and rigidly in place. The other end of the soda-lime tube is connected by means of a T tube (T) with the suction (controlled by the stopcock (*e*)) and also with the mercury manometer (M). The latter consists of a reservoir of about 175 cc. capacity, surmounted by the stopcock (*d*) which is bent at right angles, and provided with the side arm (V). The reservoir contains about 1 kilogram of mercury. (M) serves a useful purpose as a manometer, registering the fluctuations in pressure during the combustion. (During the course of a combustion the mercury in (V) ordinarily stands at a level 1 to 2 cm. lower than that in the reservoir.) It acts further as an automatic safety device which permits air to be drawn through the side arm (V) into the reservoir in case an obstruction should occur in the combustion train, which might cause a leak at some joint. It serves a similar purpose when the substance burns too rapidly. In this case the carbon is absorbed with undue rapidity in the soda-lime tube, thus causing a drop in pressure in part of the tube, which, in the absence of (M), might cause a leak inwards at the ground-glass joint. While a leak of this type would probably not be serious, since the position of the joint prevents any gases which might be drawn into the soda-lime tube from coming in contact with the absorbents, the use of (M) prevents this from occurring.

When the proper connections have been made, alundum tiles (G) are placed over the asbestos-covered portion of the combustion tube (F), and the fish-tail burner (W) is lighted. Immediately thereafter, the stopcocks (*a*) and (*b*) of the drying train, and (*d*) of the manometer are opened. Stopcock (*e*) leading to the suction is then very gradually and cautiously opened, so that air is drawn through (A) at a rate of 2 to 3 bubbles per second. After the trough over (W) has been maintained at full red heat for about 5 minutes, one of the hot tiles (G) is held for a moment over that portion of the combustion tube which shelters the combustion boat. If, after a minute, there are no signs of combustion (*i. e.*, no cloud of moisture is visible in the blown enlargement of the calcium-chloride tube), the tile is reheated and replaced over the boat. With volatile or readily combustible substances, the operation of alternately removing and replacing a hot tile should be continued until the first signs of burning are noted. From this point on, the combustion should proceed slowly and steadily. Carbon dioxide should be generated with sufficient speed to cause the soda lime to become quite warm, but never so rapidly that the absorption tube becomes uncomfortably hot. The area in which the heat is generated in the absorption tube should not extend beyond the first portion of the tube, and should never spread to that section of the soda-lime which is immediately adjacent to the calcium chloride layer.

At just what stage of the combustion the second burner (R) should be lighted depends entirely on the ease with which the substance burns.[1] Not infrequently, with readily sublimable substances, the second burner need only be resorted to at the close of the combustion. With difficultly

---

[1] Before carrying out a combustion the analyst should heat, and gradually burn a small sample of the substance on a platinum crucible cover in order to determine the behavior of the substance on heating.

combustible substances, however, the burner must be used at the very outset of the combustion. At the final stage of the combustion, the entire length of the combustion trough is covered with alundum tiles and heated with the full heat of both burners.

To drive the water from the combustion tube into the calcium chloride tube, the same copper wire device is employed, as that previously described under "Preliminary heating." A hot wire is often serviceable in aiding the transfer of water condensed in the blown enlargement of the calcium-chloride tube, past the capillary constriction, into the body of the tube.

When the soda-lime tube *begins* to cool, the burning of the substance may be considered complete, but the combustion is continued for 5 minutes longer to insure the removal of the last traces of carbon dioxide. At the end of this time period, the soda-lime tube should be quite cool, and no visible trace of water should remain in the blown enlargement of the calcium-chloride tube. The average time required for a total combustion (*i. e.*, the time elapsing between the lighting of the first burner, and the actual disconnection of the absorption tubes), is about 20 minutes. An interval timer has been found to be very serviceable in timing the various stages of the combustion. When the combustion is complete, the flames are extinguished. *Immediately thereafter*, the stopcocks (*e*) and (*d*) are closed. The soda-lime tube is then carefully disconnected and the ends are wiped with lens-cloth and capped. The calcium-chloride tube is then disconnected and similarly wiped and capped. The end of the combustion tube is subsequently capped.

TABLE I.—RESULTS OF A SERIES OF MICRO-COMBUSTIONS IN WHICH 50% POTASSIUM HYDROXIDE AND CALCIUM CHLORIDE WERE USED IN THE DRYING TRAIN.

Substance analyzed.	Weight of sample taken for analysis in mg.	Weight of CO_2 found. Mg.	Weight of H_2O found. Mg.	Carbon. Found. %.	Carbon. Calculated. %.	Hydrogen. Found. %.	Hydrogen. Calculated. %.
Toluic acid[1]	21.80	56.35	12.05	70.50	70.56	6.18	5.93
	21.85	56.65	11.70	70.72	70.56	5.99	5.93
Sucrose[2]	22.08	34.00	12.10	42.00	42.08	6.12	6.48
Benzoic acid[1]	20.13	50.8	8.8	68.82	68.82	4.89	4.96
	21.28	54.15	9.30	69.40	68.82	4.89	4.96
Tartaric acid[2]	21.25	25.15	7.4	32.28	31.99	3.89	4.03
	21.32	25.15	7.65	32.17	31.99	4.01	4.03
p-Hydroxybenzoic acid[1]	20.73	46.02	8.5	60.54	60.85	4.58	4.38
	19.65	43.70	8.05	60.65	60.85	4.58	4.38
	20.02	44.80	7.85	61.02	60.85	4.38	4.38
Vanillin[1]	20.30	47.20	10.20	63.41	63.13	5.62	5.30
Hydroquinone[1]	21.35	51.30	10.85	65.54	65.42	5.68	5.50

[1] Burnt in canoe-shaped boat with cupric oxide.

[2] Burnt without cupric oxide in flat platinum micro-combustion boat.

The absorption tubes may be weighed immediately, the same precautions in wiping and weighing being taken as those previously described. The tubes should be taken to constant weight (within 0.05 mg.).

The results of two series of micro-analyses of compounds containing carbon, hydrogen and oxygen, are given in Tables I and II. The significance of the corrections applied in the case of hydrogen values is discussed later.

TABLE II.—RESULTS OF A SERIES OF MICRO-COMBUSTIONS IN WHICH CONCENTRATED SULFURIC ACID, MOIST SODA-LIME, AND CALCIUM CHLORIDE WERE USED IN THE DRYING TRAIN.

Substance analyzed.	Weight of sample taken in mg.	Weight of CO_2 found. Mg.	Weight of H_2O found. Mg.	Weight of H_2O after subtracting average blank (cf Table IV). Mg.	Carbon.		Hydrogen.		
					Found. %.	Calculated. %.	Found. (uncorr.). %.	Found. (corr. for blank). %.	Calculated. %.
Sucrose[2]............	17.72	27.34	9.83	9.64	42.08	42.08	6.20	6.08	6.48
	10.81	16.65	6.47	6.28	42.00	42.08	6.69	6.50	6.48
Tartaric acid[2].......	20.10	23.49	7.25	7.06	31.87	31.99	4.03	3.93	4.03
	13.43	15.70	4.92	4.73	31.88	31.99	4.10	3.94	4.03
Benzoic acid[1]........	12.73	31.95	6.05	5.86	68.45	68.82	5.31	5.14	4.96
	13.42	34.00	6.11	5.92	69.02	68.82	5.09	4.93	4.96
Hydroquinone[1]......	15.38	36.62	7.70	7.51	64.90	65.42	5.60	5.47	5.50
Citric acid[2]..........	13.93	19.10	5.35	5.16	37.38	37.49	4.29	4.14	4.19
p - Hydroxybenzoic acid[1].............	14.11	31.42	5.60	5.41	60.73	60.85	4.44	4.29	4.38
	13.35	29.68	5.47	5.28	60.63	60.85	4.57	4.41	4.38
Succinic acid[2]........	14.20	21.13	6.68	6.49	40.58	40.66	5.26	5.11	5.13

Combustion of Nitrogenous Substances.

In his description of the micro-combustion method, Pregl recommended the use of a special grade of lead peroxide, which when introduced into the combustion tube served to arrest oxides of nitrogen. This grade of lead peroxide is listed by an European manufacturer, who gives a reference to one of Pregl's earlier articles on macro-combustion[3] but this simply refers to the fact that the above manufacturer has prepared the lead peroxide in question and no method for the preparation of the substance is given.

The difficulty in obtaining granular lead peroxide on the American market, coupled with failures resulting from the use of other grades of lead peroxide, led the author to undertake a series of experiments in which the combustion tube described above was used without modification. From a limited number of experiments, it appears probable that if the nitrogenous substance be mixed with cupric oxide, covered with a layer of the oxide, and burnt slowly in a current of air, none or only very small

---

[1] Burnt with cupric oxide in canoe-shaped boat.

[2] Burnt without cupric oxide.

[3] Pregl, *Ber.*, 38, 1434 (1905).

amounts of the oxides of nitrogen of the type which may be absorbed by soda lime will be formed during the course of a combustion. The serviceability and limitations of the micro-combustion as applied to the analysis of nitrogenous compounds may be judged from the results given in the following table.

TABLE III.—RESULTS OF COMBUSTIONS OF NITROGENOUS COMPOUNDS BURNT IN THE PRESENCE OF CUPRIC OXIDE WITHOUT THE USE OF LEAD PEROXIDE.

						Carbon.		Hydrogen.		
Substance analyzed.	Nitrogen % (calc.).	Wt. of sample taken for analysis. Mg.	Wt. of CO_2 found. Mg.	Wt. of H_2O found. Mg.	Wt. of H_2O corrected for blank (cf. Table IV). Mg.	Found. %.	Calculated. %.	Found (uncorr.). %.	Found (corr. for blank). %.	Calculated. %.
Urea	46.7	18.22	13.60	11.27	11.08	20.36	19.99	6.92	6.80	6.71
	46.7	18.13	13.25	11.00	10.81	19.93	19.99	6.78	6.66	6.71
o-Nitrobenzoic acid	8.4	14.03	25.77	4.15	3.96	50.09	50.25	3.31	3.16	3.02
	8.4	19.68	36.28	5.60	5.41	50.28	50.25	3.18	3.07	3.02
Acetanilide	10.4	15.02	39.1	9.13	8.94	71.00	71.07	6.73	6.59	6.71
p-Nitraniline	20.3	16.75	32.33	6.95	6.76	52.64	52.15	4.68	4.55	4.38
Cyanuric acid	32.6	16.57	16.95	3.90	3.71	27.90	27.90	2.63	2.50	2.34

"Blank" Combustions.

It is frequently desirable, although not always necessary, to carry out "blank" combustions in connection with the combustion proper. The blank combustion differs from the actual combustion only in that no boat containing organic material is introduced into the combustion tube prior to the combustion, and that no special precautions are taken in heating the combustion tube. Otherwise the same technique is employed as in the case of the actual combustion. The time required for carrying out a blank combustion is also 20 minutes.

After a drying train has been replenished, the analyst should always carry out a blank combustion to assure himself that the air aspirated through the combustion tube is properly purified. A blank combustion is also advisable after a newly filled combustion tube has been "glowed out" for the first time. It is essential from time to time during a long series of combustions to carry out blank combustions in order to make sure that the drying train has not been exhausted and that the rubber connections are free from minute cracks.

A series of blank combustions was carried out by the author after inaugurating the use of the sulfuric acid—soda-lime—calcium chloride drying train described above, and the results of these blanks are given in Table IV.

These results are stated in terms of mg. of CO_2 or H_2O to be added or deducted, respectively, from the weights of the carbon dioxide and water found in an actual analysis. It will be noted that corrections to be applied in the case of the carbon dioxide are very slight, positive or negative, and that the average correction is only —0.01 mg. of CO_2, which is quite negligible. On the other hand, the corrections to be applied to the weight of water are all negative, and the average correction (—0.19 mg.) although slight, deserves consideration. Due correction in the amounts of water found and in the corresponding percentages of hydrogen were therefore made and the effect of applying such corrections may easily be judged by comparing Cols. 8 and 9 of Table II. The uncorrected values found for hydrogen are frequently 0.10 to 0.15% higher than the corresponding corrected values, which in most cases shows a much better agreement with the calculated values.

TABLE IV.—CORRECTIONS BASED ON "BLANK" COMBUSTIONS IN WHICH H_2SO_4, SODA LIME, AND CALCIUM CHLORIDE WERE USED IN THE DRYING TRAIN.

"Blank" combustion No.	Correction to be applied to the weight of CO_2 in mg.	Correction to be applied to the weight of H_2O in mg.
1	—0.05	—0.14
2	+0.08	—0.27
3	—0.10	—0.20
4	+0.04	—0.13
5	±0.00	—0.10
6	+0.03	—0.22
7	—0.08	—0.28
Average	—0.01 mg.	—0.19 mg.[1]

Discussion.

The accuracy of the micro-combustion method as applied to the determination of carbon appears to be limited largely by the errors in weighing on an analytical balance. The average error of all carbon determinations given in Tables I, II and III, is about 1 part in 250, whereas the estimated precision in weighing 15 mg. of substance on this balance is about 1 part in 300. The error in the hydrogen determination is a far greater one, however. In this case the average error (using the corrected values in Tables II and III) is about 1 part in 40. This may be due in part to errors in weighing the relatively small quantities of water formed, rather than to errors in weighing the sample.

It would appear therefore that the use of a micro-balance would increase the accuracy of the carbon determination, and perhaps to a lesser degree that of the hydrogen determination. On the other hand even when weighings are made on an analytical balance the micro-combustion as herein described is capable of giving results which compare favorably with those obtained with the ordinary macro-combustion methods. Furthermore the applicability of the micro-method is greatly increased when the micro-balance becomes unnecessary.

The micro-combustion method is rapid. Including the weighings and calculations, less than two hours are required to carry out a combustion. No complicated apparatus is required, and, if a large number of combus-

---

[1] Cf. Tables II and III.

tions are carried out, the necessary precautions soon become part of the daily routine. Furthermore, the materials which become exhausted after repeated combustions, are inexpensive and can easily be replaced. The combustion tube, since it is not heated for a long period of time at the high temperature used in the average macro-combustion, can be used repeatedly and devitrifies very slowly. These points may appeal to the organic chemist who carries out control combustions in connection with the manufacture of rare organic chemicals and to the biochemist, whose identification of exceedingly small amounts of an organic compound depends on the use of suitable micro-methods. The method has already proved of value in aiding in the identification of very small amounts of organic substances isolated from soil, and may well find other applications in organic and biological chemistry.

Summary.

1. A modification of the Pregl micro-combustion method for the determination of carbon and hydrogen has been described in detail.

2. The simplified method, in which 11 to 22 mg. samples of substance are taken for analysis, does not require the use of a micro-balance. A sensitive analytical balance serves as a substitute for the micro-balance advocated by Pregl.

3. The usual carbon-dioxide absorption tube has been replaced by a tube with a ground-in joint. This tube may be readily cleaned out and refilled. Soda lime has been substituted for caustic potash as an absorbent for carbon dioxide.

4. The original drying train used in the Pregl method has been modified. Sulfuric acid, soda lime, and calcium chloride saturated with carbon dioxide have been used as absorbents instead of 50% caustic potash and calcium chloride.

5. The technique used in weighing and in carrying out the combustion has been modified and standardized.

6. The value of carrying out "blank" combustions and of making corrections in the case of hydrogen determinations has been demonstrated.

7. The results of two series of micro-analyses of pure substances containing carbon, hydrogen and oxygen, serve to show that the accuracy of the modified micro-combuston method is comparable with that of the ordinary macro-combustion methods.

8. From a limited number of experiments with pure nitrogenous compounds it appears that the method, without further modifications, may be applicable to the micro-analysis of a variety of organic compounds containing nitrogen.

9. The limitations of the micro-method and the possible applications of the method are briefly discussed.

WASHINGTON, D. C.