ORGANIC MICROANALYSIS IN JAPAN—
PAST AND FUTURE

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INTRODUCTION

In comparison with the United States and European countries, the use of organic microanalysis in Japan is quite new. The first Japanese textbook on this subject appeared in 1927. The author, Dr. Kunihiko Suminokura, was taught by Professor F. Pregl at the University of Graz in 1924. Dr. Suminokura took a deep interest in organic microanalysis and taught the skills he had acquired at Graz to his students at the College of Agriculture of Tottori in Japan. In 1937, the first edition of a book entitled Micro- and Semimicro-Quantitative Determination of Organic Compounds was published by Dr. Eiji Ochiai and Kyosuke Tsuda. Dr. Ochiai had studied microchemical techniques with Professor H. Lieb at Graz in 1931. The book emphasized that the technique of microanalysis is not a difficult one to acquire and thus played an important role in spreading the micro-technique in Japan. In 1956 a fifth, revised edition was published by E. Ochiai, K. Tsuda and S. Sakamoto. Before the Second World War, microanalysts in Japan were mainly concerned with introducing the original Pregl method. In 1932 microanalysis was introduced into the department of Pharmaceutical Sciences, Tokyo University, by Professor E. Ochiai and in 1934 Professor S. Takei initiated it in the department of Agricultural Chemistry, Kyoto University. Takeda Chemical Industries, one of the largest chemical companies in Japan, also began analysis in 1934.

After the Second World War, microanalytical research developed at an accelerated pace and this led to the desire for mutual discussion whereby investigators could exchange experiences and discuss the results of their studies. In 1953, the first symposium on organic microanalysis was held at Kyoto University. The symposium is now held at least twice a year at various places in Japan with 100–150 participants. In 1964, the 27th symposium was held at Osaka in June, and the 28th symposium at Sendai in September. The symposium is conducted by The Association of Organic Microanalysts, which is one of the divisions of The Analytical Chemical Society of Japan. At present, about 230 microanalysts are members of the Association. One of the tasks of the Association is the standardization of microchemical equipment designed by Japanese analysts. In 1963, the first issue on recommended specifications for microchemical apparatus was published by the governing body of the Association, which also recommends reference substances for use in microanalysis. At present, 34 selected substances are being distributed at a reasonable price with a certification of purity from the Association.
MICROCHEMICAL BALANCES

With the introduction of the microtechniques to elementary organic analysis, a number of Kuhlmann's microchemical balances were imported to Japan. When I was involved in graduate study at Kyoto University, two of Kuhlmann's balances were provided in our laboratory, and I used them in my research. At that time there were no such balances manufactured in Japan. In 1955, I decided to construct a new balance. I first attempted to determine why the rest-point of a balance shifts with temperature changes.\(^1,2^a\) The degree of this shifting, which is known as the temperature coefficient of the balance, was measured in several kinds of microbalance including those of Shimadzu, Kuhlmann, and Mettler. *Figure 1* shows typical shifting of the zero-point caused by temperature changes.

![Figure 1: Rest-point shifting of a balance](image)

Various causes of the shifting can be found and the coefficient appears as the sum of the causes. It was surmised that the difference between the linear expansion coefficient for agate and that for the metallic beam material played an important part in the shifting of the beam fulcrum. To confirm this assumption, the temperature coefficients were measured for various reconstructions of the fixing mechanism of the agate knife-edge, and it was found that the shifting of the rest-point can be decreased markedly when the central knife-edge is carefully held in a strain-free state. When the central knife-edge is held in a state of stress with metal fittings, the edge
undergoes some elastic deformation under the temperature gradient, because of the difference in thermal expansion coefficient.

Another cause of shifting is heterogeneity of the knife-edge. A thermal expansion coefficient was measured with agate and it was found that the coefficient in a direction parallel to the stripes in the material was $12.40 \times 10^{-6}$ cm/°C and that in a perpendicular direction was $16.47 \times 10^{-6}$ cm/°C. Because of its heterogeneity agate is an unsuitable material for a knife-edge. The following conclusions were reached concerning the reduction of the shifting.

(i) The material with which a beam is constructed should have a thermal expansion coefficient which is approximately the same as the coefficient of the knife-edge material.

(ii) Agate is not a suitable material for a knife-edge.

(iii) The knife-edge should be held to the beam in a strain-free state.

To find a suitable material for the knife-edge, the plastic deformations of edges made of sapphire, ruby, spinel, agate, fused quartz and a special steel alloy were measured. The results obtained are shown in Figure 2. This diagram shows the radii of edges which underwent a plastic deformation after an arresting-releasing operation had been performed 20 times under the various loadings. These results show that ruby or sapphire are more suitable because they can maintain a smaller radius and there is less possibility of rounding the contact edges. Table 1 illustrates the weighing precision of a microbalance having a central knife-edge radius, $r$; $s$ is the standard deviation of the observed values.
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Because the thermal expansion coefficient of ruby or sapphire is small compared with that of agate, a metallic material such as brass or aluminium, which is usually used for a beam system, is not suitable for the beam material. Metallic titanium was found to be best because its coefficient is small and close to that of ruby or sapphire. Moreover titanium (density = 4.5) is lighter than brass (density = 8.6) and is resistant to corrosion.

Precision in weighing with a microbalance not only depends upon the sharpness of the knife-edges but also upon the flatness and smoothness of the plates in contact with the knife-edges which act as bearing systems. The polished surfaces of plates made of fused quartz, agate, sapphire, ruby and diamond, respectively, were photographed with an electron microscope and are shown in Figure 3. As the photographs show, the surfaces of the plates are covered with scratches. The width and depth of the scratches depend upon the fineness of the polishing powder used. Various attempts have been made to get smoother surfaces but, as yet, none have been successful.

One of the important tasks of the arresting mechanism is to restore the beam and stirrups precisely. It is well-known that the reproducibility of the balance is largely affected by this mechanism. When the balance beam has repeatedly been arrested and released, the location of the contact point of the knife-edge with the flat bearing varies to some extent. The parallel-bracket arrestment (Figure 4a) and the hinge-joint arrestment (Figure 4b) are two typical mechanisms of balances. An investigation was made to determine which of the two arresting mechanisms would be more suitable for the microbalance. Two newly constructed microbalances were used. A flat bearing, F, was fixed in the beam instead of the original knife-edge and the original flat bearing was changed to a knife-edge, K (Figure 5). After careful adjustment the test balance was operated. The change in the contact point could be calculated from the change of the rest-point and the sensitivity of the balance. The following values were obtained: $s$ (standard deviation) = ± 0.33 µ for the parallel-bracket; $s$ = ± 0.051 µ for the hinge-joint arrestment. Thus, the hinge-joint type of mechanism appeared to be better than the parallel-bracket. Based on these observations, I designed a microbalance which was manufactured by Shimadzu Seisakusho Ltd. Figure 6 shows the beam. It is constructed with a beam skeleton of titanium,

<table>
<thead>
<tr>
<th>Material</th>
<th>Radius of the central knife-edge, $r$ (µ)</th>
<th>Estimated standard deviation of the weighings, $s$ (µg) ($n = 20$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agate</td>
<td>12.96</td>
<td>3.65</td>
</tr>
<tr>
<td>Agate</td>
<td>5.00</td>
<td>1.34</td>
</tr>
<tr>
<td>Sapphire</td>
<td>0.71</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Figure 3. Polished surfaces of plates photographed with an electron microscope.

(a) Fused quartz ($\times$ 2000);
(b) agate ($\times$ 2600);
(c) sapphire ($\times$ 3000);
(d) ruby ($\times$ 3000);
(e) diamond ($\times$ 3000)
Figure 6. Beam of a microchemical balance

Figure 7. Shimadzu microchemical balance with retractable fork
three knife-edges of ruby and with a mirror below the central knife-edge. An image of the reticle is first projected to the mirror and is reflected three times between it and the facing mirror fixed to the column. This device serves to magnify the swing angle of the beam remarkably. *Figure 7* shows the complete balance. One of the auxiliary devices is a retractable fork with

*Figure 4.* Two typical arresting mechanisms of balances (a) Parallel-bracket arrestment; (b) hinge-joint arrestment

*Figure 5.* Bearing system of a balance, tested for reproducibility of contact point. F: Flat bearing; K: knife edge

which two absorption tubes can be alternately loaded onto the pan-hook by operating a knob from the outside of the balance. The temperature coefficient of the balance is certified to be $3 \mu g/\degree C$ and the precision (standard deviation) is $\pm 2 \mu g$. 

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Microdetermination of carbon and hydrogen

In 1959 an assembled microapparatus for determining carbon and hydrogen with an electrically heated furnace was first manufactured on a commercial scale by Shimadzu Seisakusho Ltd. At the time I was investigating an automatic combustion system by which, with a combustion furnace of a stationary type, the sample was heated to 300°C in 10 min. The temperature was then raised to 800°C during another 10 min. An automatic apparatus having this device was commercially manufactured in 1953.

It is well-known that absorption tubes used in the determination of carbon and hydrogen are often difficult to weigh exactly, errors in weighing being attributed to the change of the temperature, humidity and the electrostatic charge on the wall surface of the tube. I designed a small, light absorption tube constructed with a thin-wall metal chamber and a pair of needle-tube stoppers. As the tubes are connected to the combustion train by means of a Daiflon tube, wiping of the tubes is not required. Hozumi and Imaeda have discussed the theoretical relationship between diffusion rate, pressure drop and the dimensions of the capillary of the absorption tube and have recommended capillaries of simpler and wider bores and greater lengths. In Japan at the present time almost all microanalysts use either Mitsui's metal tube or Hozumi's glass absorption tube. One of the troubles encountered in Pregl's original method is the use of lead peroxide with which oxides of nitrogen are removed from the combustion products. Since 1960, the following reduction method has been used in our laboratory. A sample is burned in a stream of nitrogen by adding a controlled amount of electrolytically generated oxygen. The nitrogen oxides resulting from a sample containing nitrogen are reduced to nitrogen; thus, the carbon dioxide tube does not represent erroneous weight gain. This method is becoming widely used in Japan, and is especially advantageous when a gas-chromatographic finish is applied to elementary organic analysis.

Microdetermination of nitrogen

In 1953 several investigations of the thermochemical equilibrium between carbon dioxide and cupric oxide or reduced copper were reported. When pure cupric oxide was heated in the combustion tube at a working temperature of 750–800°C under a current of carbon dioxide, a remarkable amount of oxygen evolved forming cuprous oxide. In Pregl's method, no observable oxygen was found in the nitrometer in spite of the fact that one end of the final cupric oxide layer near the reduced copper is heated at a temperature sufficiently high to liberate oxygen. This inconsistency was understood when it was confirmed by quantitative determination that Merck's wire-form cupric oxide contains 75 per cent cuprous oxide, 22 per cent cupric oxide and 2 per cent copper which will absorb oxygen at the working temperature. Consequently, the success of Pregl's filling seems to be attributed to the fact that cuprous oxide instead of cupric oxide was the main component of the so-called wire-form cupric oxide. I proposed a tube followed by a layer of cuprous oxide at 750–800°C. With regard to the temperature at which copper is maintained, Hozumi and Amako observed
that the incomplete reduction of oxides of nitrogen occurred below 500°C. They concluded that the proper temperature for the reduced copper should be in the range of 550–600°C.

I designed a modified Stehr's nitrometer having a flexible polyethylene bottle and a stop valve of Daiflone. Recently a nitrometer having a large capacity for potash solution was reported. It is operated without a levelling bulb, and has a small wad of nickel gauze in the stem to automatically stop the nitrogen bubbles at zero position. Hozumi and Amako designed a nitrometer having a "blank scale", and Mizukami and Miyahara proposed a nitrometer having a vinyl stopper. In the original Dumas procedure, the sample has to be well mixed with copper oxide. This procedure is inconvenient and several devices have been proposed to ensure the combustion in the current of carbon dioxide. In the method proposed by Hozumi and Kinoshita, a boat containing cobaltic oxide is introduced into the tube with the sample and both are heated at the same time. Oxygen released from the cobaltic oxide at 850°C serves to complete the combustion of the sample. Several attempts using gaseous oxygen in order to obtain a complete combustion have been reported. In our laboratory, electrolytically generated oxygen is being used.

**Microdetermination of halogens and sulfur**

Numerous methods for the microdetermination of halogens and sulfur have been reported. One of especial interest is that proposed by Safford and Stragand. The sample is burned in a stream of oxygen, and the liberated halogen is retained by silver gauze maintained at 400–425°C. In my experience the results obtained are not precise enough for this to be a recommended method. The most outstanding trouble seems to arise from the handling of the roll of silver gauze without any container. To overcome this problem an absorption-funnel was designed in which the electrolytically prepared silver sponge could be packed. The funnel is inserted from the tail-end of the combustion tube and is maintained at 425°C as shown in *Figure 8*.

![Figure 8. Tail-end of combustion tube with absorption funnel](image-url)
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The sample is burned in a stream of oxygen and suction from a Mariotte bottle is applied at the tail-end of the combustion tube to draw air through the absorption funnel so that all the halogen resulting from the combustion is introduced into the funnel. The weight gain of the funnel represents the quantity of halogen. Sulfur is also determinable by this method using an absorption funnel with a somewhat longer barrel. The silver sponge absorbs sulfur trioxide quantitatively but it does not absorb sulfur dioxide. At a temperature above 700°C a small amount of sulfur dioxide is produced in the reaction gas by the thermal equilibrium. A longer layer of the silver sponge ensures the formation of sulfur trioxide and the sulfur is thus quantitatively retained in the funnel.

Several modifications of this absorption funnel method have been reported. Imaeda\textsuperscript{22} and Kan and Tsukamoto\textsuperscript{23} have reported a method of simultaneous separate microdetermination of halogen and sulfur with a dual absorption funnel as shown in Figure 9. Kan and Tsukamoto used granulated cobaltic oxide for the absorption of sulfur oxides packed in chamber A and silver sponge for the halogen packed in B. The jointed funnel was maintained at 450–500°C \textit{in situ}. As cobaltic oxide does not absorb halogen, sulfur and halogen in the sample were determined from the weight increase of A and B separately. Hozumi\textsuperscript{24, 25} proposed a method of separate microdetermination of different kinds of halogens with the dual absorption funnel. With this method silver sponge was packed in B and granules of silver iodide in A and the funnel was maintained at 250°C in an auxiliary furnace. Chlorine or bromine given off during the combustion reacted with the silver iodide releasing iodine. The iodine was absorbed by the silver sponge packed in B. As iodine in the combustion products passed through the layer of silver iodide without reaction, the halogens could be identified and determined by weighing chambers A and B separately after they had been disjointed. With the organic sulfur compounds containing metal, the usual combustion method is insufficient to give accurate results. Kan, Tsukamoto and Suzuki\textsuperscript{26} recommended a method in which a sample was heated together with a mixture of vanadium pentoxide and wolfram trioxide under a current of oxygen; the resulting sulfur trioxide was retained in an absorption funnel containing silver sponge. To determine organic fluorine, Oono\textsuperscript{27} used a modified absorption funnel containing ferric oxide. The hydrogen fluoride formed by the combustion of the sample was quanti-
tatively retained in a funnel maintained at 180°C. In the simultaneous
determination of halogens and sulfur, Hozumi and Mizuno\textsuperscript{28} used a modified
absorption funnel filled with glass beads coated with potash solution.
After washing the absorbed halogens and sulfate ions from the funnel into
a beaker, they were determined separately by potentiometric and colorimetric
methods. Simultaneous determination of carbon, hydrogen and
halogens (or sulfur) was proposed by Fujimoto, Utsui and Ose\textsuperscript{29} and Onoe,
Furakawa and Otsuka\textsuperscript{30} using an absorption funnel containing silver wire
or silver sponge to retain the halogens (or sulfur trioxide). The remaining
combustion products were then introduced into absorption tubes to deter-
mine carbon and hydrogen.

Application of the absorption-funnel method for the determination
of elements other than sulfur or halogens

A method of determining mercury in organic compounds was reported
(Mitsui, Yoshikawa and Sakai\textsuperscript{31}) in which the mercury in the combustion
products was retained in an absorption funnel containing silver sponge at a
temperature of 50–100°C. Onoe\textsuperscript{32} recommended an absorption funnel with a
wad of fine gold wire as a mercury absorbent. Kunimine, Ugajin and
Nakamaru\textsuperscript{33} used one containing fine quartz wool for the determination of
selenium in organo-seleno compounds. Onoe\textsuperscript{34} determined arsenic by
burning arsenic compounds in a combustion tube under a stream of oxy-
gen and retaining the resulting arsenic trioxide in an absorption funnel
filled with fine quartz wool. As this method is quite simple, it can easily
be used for routine analysis. Hozumi and Mizuno\textsuperscript{28} used the funnel in the
determination of alkoxyl groups.

The funnel containing silver sponge sometimes has an unexpected gain in
weight caused by an electrostatic charge\textsuperscript{35}. To dissipate the charge an insula-
ting tube of aluminium, into which charged material could be inserted, was
proposed (Mitsui and Yoshikawa\textsuperscript{36}). Ono, Saito and Masuda\textsuperscript{37} recommended
a coating method in which the surface of the funnel was coated with a trans-
parent conductive substance consisting mainly of tin oxide.

Microdetermination of oxygen

In 1948 Kasagi\textsuperscript{38} tried to introduce the Schütze-Unterzaucher micro-
method into Japan with an apparatus of his own construction. Kono
and colleagues\textsuperscript{39, 40} applied his automatic combustion system to oxygen
determination. In this method, the reaction rate was controlled by a
manometer responding to the internal pressure of the reaction tube and the
travelling rate of the sample heater by a relay connected with the mano-
meter. He used Safford–Stragand’s silver gauze method, the oxygen in the
sample being determined as iodine resulting from the reaction of carbon
monoxide with anhydro-iodic acid. Imaeda\textsuperscript{41, 42} suggested a method using an
absorption tube at the final step and in which the oxygen in the sample
was determined as carbon dioxide. For removing oxygen in the carrier gas,
Imaeda\textsuperscript{43} recommended porous copper granules of his own preparation.
 Mizukami\textsuperscript{44} used Raney nickel at room temperature for this purpose and
found that nickel has a much greater capacity than reduced copper. Mitsui,
Yamamoto and Yoshikawa\textsuperscript{31} suggested a method using an absorption funnel
containing silver sponge. The technique is similar to the method for determining halogen mentioned above.

**Group analysis**

A method for the simultaneous microdetermination of alkoxyl compounds including methoxyl, ethoxyl, propoxyl and butoxyl groups has been reported by our laboratory\(^{45, 46}\). The alkyl iodides formed by the Zeisel reaction are absorbed in silica gel filling an absorption tube of stainless steel. After the tube has been weighed, it is heated under a stream of nitrogen at 150°C, and the released alkyl iodides are introduced into the column of the gas chromatograph. The chromatogram obtained illustrates the kinds of alkyl iodides and their molar ratios. The quantitative and the qualitative determinations are thus carried out simultaneously. *Figure 10* shows the alkoxyl apparatus designed by our laboratory.

Fukuda\(^{47-51}\) described a combustion method, in which the alkyl iodide formed by the Zeisel reaction is driven with air into the usual combustion tube which has platinum contacts. Iodine and carbon dioxide so formed are retained by a silver gauze roll and an absorption tube containing soda asbestos. The alkoxyl group content is calculated from the weight of the iodine and the kinds of groups are classified according to the molecular ratio between the carbon dioxide and the iodine. Hozumi\(^{52}\) also reported a combustion method for determining alkoxyl groups in which the liberated alkyl iodides are passed through an absorption funnel containing silver sponge maintained at a temperature of 400–500°C; the iodine so formed is determined from the weight increase of the funnel.

A method of volumetric microdetermination of the formyl group in the presence of the acetyl group and of the acetyl group in the presence of the formyl group was described by Kan, Suzuki and Kashiwagi\(^{63}\).

**RECENT MODELS OF APPARATUS FOR ELEMENTARY ANALYSIS**

At present, several Japanese models of microapparatus are being manufactured commercially. The most widely used is one designed by our laboratory and manufactured by Shimadzu Seisakusho Ltd. It is the so-called “Universal Model” and is shown together with the schematic flow diagram in *Figures 11* and 12. It is used for the microdetermination of carbon–hydrogen, nitrogen, halogens, sulfur, oxygen and mercury. The carbon–hydrogen determination uses the reduction method with nitrogen as the carrier gas, the metal absorption tubes being easily removed from the combustion train with a lever. Nitrogen determination is done under a current of carbon dioxide containing a suitable amount of electrolytic oxygen. Sulfur, halogens and mercury are determined by the absorption-funnel method. Oxygen is also determined with the absorption funnel as the final step.

In an apparatus designed by Oda, Tsuchihashi and Ono\(^{54}\) and manufactured by Tada Scientific Company a sample is dropped, together with a boat, into a combustion tube of the double-walled type installed at an angle of about 45° and is burnt instantaneously. For carbon–hydrogen determination, oxygen is used as the carrier gas and manganese dioxide as the external
absorbent. The total time required for a single analysis is normally 20 min. To determine oxygen, platinized carbon is used as the catalyst and the resulting carbon monoxide is oxidized to carbon dioxide with anhydro-iodic acid and determined with an absorption tube containing soda-asbestos. The apparatus and the flow diagram are shown in Figures 13 and 14.

A rapid combustion apparatus designed by Kimura\textsuperscript{55–57} is offered by Yazawa Manufacturing Company. In this apparatus the sample is introduced with a magnet into a combustion tube maintained at 800°C and is burnt instantaneously. The combustion products, carbon dioxide and water, are swept into the absorption tubes which have two looped capillaries at both ends. The tubes are automatically disconnected from the apparatus when the combustion has ended. The time required for a single analysis is about 5 min. The apparatus and the flow diagram are shown in Figures 15 and 16.

A classical carbon–hydrogen automatic apparatus (Figure 17), in which lead dioxide is still used as the absorbent of oxides of nitrogen, was designed by Kono\textsuperscript{40} and manufactured by Mitamura Riken Kogyo Company.

**SUGGESTIONS FOR THE FUTURE**

In Japan, as in other countries, research has developed along two lines. One is concerned with the instrumentation of the apparatus. A number of methods for the determination of carbon, hydrogen and nitrogen which do not involve the tedious gravimetric procedures at the final step are now being perfected. Recently Hozumi has designed a new apparatus that is offered commercially by the Yanagimoto Manufacturing Company. A view and schematic flow diagram of this apparatus are shown in Figures 18 and 19. The combustion products, water, carbon dioxide and nitrogen, with a carrier gas of helium, are drawn into a cylinder by a piston pump. After the gas mixture has been stored for 3 min to become homogenized, it is pushed slowly out from the cylinder into a series of detectors using three pairs of katharometers and three bridge circuits. The three output signals representing hydrogen, carbon and nitrogen are picked up and recorded successively with short time intervals during which the pump is operated. The total time required for an analysis is 18 min. A further approach to a fully automatized instrument involving an automatic sample introducer and a digital printer is being considered.

Some apparatus using thermal conductivity detection with or without gas-chromatographic separation will be offered in the near future.

The other line of present investigation is toward an ultra-micromethod. Our laboratory has recently conducted experiments on a sub-micromethod for oxygen determination using a sealing tube\textsuperscript{58}, and Hozumi\textsuperscript{59} is attempting to develop his sealing tube method for nitrogen sub-microdetermination.

**References**

elementary Analysis, Japan (1964).
Figure 10. Alkoxyl apparatus
Figure 11. Shimadzu combustion apparatus

Figure 12. Schematic flow diagram for Shimadzu apparatus
Figure 13. Tada combustion apparatus

Figure 14. Schematic flow diagram for Tada apparatus
Figure 15. Yasawa combustion apparatus

Figure 16. Schematic flow diagram for Yasawa apparatus
Figure 17. Mitamura combustion apparatus
Figure 18. Yangimoto CHN recorder

Figure 19. Schematic flow diagram for Yangimoto CHN recorder