CSIRO
Division of Fisheries and Oceanography

REPORT 127

Methods for the Determination of Dissolved and Particulate Organic Carbon in Marine Samples

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1980

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION
DIVISION OF FISHERIES AND OCEANOGRAPHY
P.O. BOX 21, CRONULLA, NSW 2230

National Library of Australia Cataloguing-in-Publication Entry

Airey, D.

Method for the determination of dissolved and particulate organic carbon in marine samples.

(Commonwealth Scientific and Industrial Research Organization. Division of Fisheries and Oceanography. Report; 127).
Bibliography

ISBN 0 643 02644 4

1. Biological chemistry. 2. Chemistry, Organic.
3. Marine biology. I. Hogan, M., joint author.
II. Title. (Series).

574.19'24'028

Printed by CSIRO, Melbourne.

80.556-600

METHODS FOR THE DETERMINATION OF DISSOLVED AND PARTICULATE ORGANIC CARBON IN MARINE SAMPLES

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Aust. CSIRO Div. Fish. Oceanogr. Rep. 127 (1980)

Abstract

Detailed technical instructions are given on the use of the Oceanographic International Carbon Analyser to determine concentrations of dissolved and particulate carbon by Menzel and Vaccaro's ampoule technique and of particulate carbon by a furnace technique.

CONTENTS

1. INTRODUCTION

Organic carbon compounds in the marine environment may exist in a dissolved form (DOC) or in a particulate form (POC). POC may be suspended in the water column, associated with living plants and animals, or found in detrital material and sediments.

All reports of organic compound concentrations must be related to the techniques used to collect, pretreat, store and analyse the samples. Only carbon con-centrations determined by identical procedures may be compared. This is because different procedures give different results because organic compounds vary greatly in their molecular weights, their volatilities and their susceptibilities to oxidation. Also, DOC is defined as the fraction that will pass through a 0.45 µm filter, everything greater than 0.45 µm being POC, but the precision of this division depends on the type of filter (glass fibre or silver) used and how the residual carbon is removed from it before use (ignition or sulphuric-chromic acid treatment).

We recommend that new users of this manual should first read Chapter 13 in "Analytical Methods in Oceanography" on Measurement of Organic Carbon in Seawater, by P.J. Wangersky (1974). It gives an excellent insight into the problems associated with the determination of organic carbon in seawater.

The methods to determine carbon described in this manual have been used routinely by CSIRO Division of Fisheries and Oceanography at Cronulla to measure concentrations of biological material, to identify areas of significant runoff and to examine carbon fluxes at sediment-water interfaces. The techniques are relatively simple and, even though they require some technical skill, they can be done routinely with precision if attention is paid to detail. The methods are designed to measure the low levels of organic carbon found in saline environments, but can also be used for carbon analyses of freshwater samples. They are not intended to be standard methods and will be modified if better results can be obtained by different techniques.

2. AMPOULE TECHNIQUES

THE DETERMINATION OF DISSOLVED ORGANIC CARBON BY AN AMPOULE TECHNIQUE

A filtered seawater sample is placed in a borosilicate ampoule. Potassium persulphate is added, followed by phosphoric acid. The mixture is purged with purified oxygen to remove inorganic carbon dioxide and carbonate ions (Appendix II). The ampoule is sealed using a micro-burner, then autoclaved to oxidize the organic carbon to carbon dioxide. The ampoule is broken and the carbon dioxide is determined in a non-dispersive infrared analyser relative to D-glucose standards.

Experimental (Note 1 - Appendix IV)

Special apparatus and equipment

1. An Oceanography International Carbon (OIC) analysing unit.
2. An OIC purging and sealing unit.
3. Bottled gases: oxygen, propane, nitrogen "zerogas", compressed air containing about 350 ppm CO_2 "span gas".
4. An autoclave unit operating at 124°C and 100 kPa (15 lb in^{-2}).
5. A furnace operating at 500-550°C.
6. Aluminium foil.
7. 10 mℓ glass ampoules.

Ampoule preparation

Cover the neck of each new ampoule with a small square of aluminium foil. Place in a rack and put the rack in a furnace set at 550°C for at least 3 hours. (Note 2).

8. Glass fibre (GFC) filter papers.

Filter preparation

Glass fibre filter pads to be used for filtering seawater samples for DOC analysis must be pre-fired at 500°C for one hour. (Notes 1,2,3)

Reagents (Notes 1,4)

1. Potassium persulphate ($K_2S_2O_8$).
   Use analytical reagent grade crystals. (Note 5).
2. Phosphoric acid (H_3PO_4).
   Make a 6% solution by diluting 60 mℓ of A.R. grade H_3PO_4 to 1000 mℓ with carbon-free distilled water. Store in a glass bottle.
3. Magnesium perchlorate.
4. Carbon-free distilled water (CFDW).
   Use water that has been distilled in an all-glass apparatus. (Note 6).

Standards

1. Stock solution
   High purity D-glucose is used as a reference standard. Weigh out accurately 100 mg and make up to 100 mℓ in a volumetric flask using CFDW. One millilitre of this solution contains 400 µg carbon.
2. Working standards
   The stock solution may conveniently be pipetted in volumes of 5, 15, 40 and 75 µℓ (2, 6, 16 and 30 µg carbon).

Methods

Procedure for preparing the standards

1. Take a new pre-fired ampoule and add:
   0.2 g $K_2S_2O_8$ with the scoop provided with the OIC analyser;
   5 mℓ CFDW using a glass pipette or syringe;
   0.25 mℓ 6% H_3PO_4 using a syringe. (Note 7);
   5-75 µℓ of glucose working standard (Note 8).
2. Purge and seal the ampoules as described in Appendix II using the OIC purging and sealing unit. (Notes 21.1 to 21.6).
3. Autoclave the sealed ampoules for at least one hour to oxidize the organic carbon (Note 9).
   Samples or standards can be stored indefinitely in this state.

Procedure for determining the blank (Note 10).

1. A seawater blank must be analysed for each new batch of phosphoric acid or persulphate used. This blank is different from that of the distilled water standards due to a different response by the infrared analyser to an increased pressure in the ampoules caused by the chlorine formed during oxidation. Add to each ampoule (Note 11) only 1-5 mℓ of a filtered seawater with a low DOC concentration (< 0.5 mg C ℓ^{-1}) (collected from deep waters, preferably below 500 m).
2. Add the $K_2S_2O_8$ and H_3PO_4.
3. Purge, seal, autoclave and analyse as before but in duplicate.
4. See page on calculation of the blank.

Procedure for determining DOC in water samples

1. Collect the seawater without contaminating the sample. (Note 12).
2. Filter (Note 13) the sample immediately through a pre-fired GFC filter. Use two aliquots of filtrate to rinse the filtration system.
3. Measure 5 mℓ of the filtrate into a pre-cleaned ampoule (Note 14).
4. If sealing and analysis cannot be started immediately, replace the aluminium foil over the mouth of the ampoule, place in a box to protect the sample from contamination by organic vapours, and store at -20°C (Note 15).
5. Add 0.2 g $K_2S_2O_8$ with the scoop provided.
6. Add 0.25 mℓ of 6% H_3PO_4.
7. Purge and seal as described in Appendix II.
8. Analyse as described in Appendix III.
9. If the concentration measured is greater than 6 mg C ℓ^{-1}, dilute the sample by taking the appropriate volume of sample and making it up to 5 mℓ with CFDW.

THE DETERMINATION OF PARTICULATE ORGANIC CARBON BY AN AMPOULE TECHNIQUE

A section of a filter which retains POC is placed with CFDW in a borosilicate ampoule. The method outlined is then similar to that described on page 2 for DOC.

Experimental

Sample preparation and procedure

1. Prepare the GFC filter pads by igniting for one hour at 500°C or by soaking in hot sulphuric-chromic acid, and rinsing with CFDW.
2. Store the filters in aluminium foil or other container protected from organic carbon contamination, and use only cleaned forceps to handle them.
3. If the weight of particulate matter is of interest, weigh the filters and place in numbered containers.
4. Mix the water sample well and filter it with a vacuum of 30 kPa (250 mm Hg). (Note 13). Record the volume of filtrate.
5. Remove the pad carefully and if the dry weight of material is to be measured freeze dry or dry at < 60°C in an oven, otherwise they should be placed immediately in a perspex or metal pad holder and stored at -70°C. (-20°C freezers can be used as second best alternatives).
6. Place the filter on the filter cutting apparatus as shown in Fig. 1.
7. Cut into eight segments, and place opposite pieces, called arbitrarily 1 and 5, 2 and 6, 3 and 7, 4 and 8, in four cleaned ampoules.
8. Add the $K_2S_2O_8$, CFDW and H_3PO_4, then purge, seal and autoclave.
9. Analyse as described for the DOC determinations.
10. With each batch prepare eight blank ampoules containing the same CFDW and a section of unused ignited GFC filter.

Calculation of dissolved and particulate organic carbon concentrations

From the procedures in sections 1 and 2, you will have the following data:

Standards - Integrated peak areas (IPAs) as a function of standards with up to 6 mg glucose C ℓ^{-1} (30 µg C per ampoule)

1. Plot a graph of IPA against concentration. (See Fig. 2). (Note 16).
2. Extrapolate to zero. The intercepts will give IPA and the carbon content of reagents plus distilled water. This should not exceed 0.5 mg C ℓ^{-1}.
3. Subtract the intercept IPA value (range 20-200 units on a span of 40-60) from the IPAs measured for the standards and redraw the graph through zero to get the standard curve (see Fig. 2).

Seawater blank - Integrated peak areas for increasing volumes of seawater

1. Plot a graph (Fig. 3) of IPA against volume of low carbon seawater.
2. Extrapolate to the y intercept to get the IPA for zero volume of seawater. This IPA represents the carbon in the reagents used to oxidize seawater samples.

Samples - Integrated peak areas from the DOC in seawater or POC samples

1 For seawater DOC samples, subtract the reagent blank IPA from the sample IPA to give the IPA due to carbon in the seawater sample.

2 For POC on filter sections, subtract the average IPA of the CFDW blanks from the sample IPA to give the IPA due to carbon on the filter pad.

3 Determine the carbon in the samples using the corrected sample IPA values and the standard curves.

4 For POC, combine the results of the four pairs of filter sections to give a total weight of carbon on the pad.

Discussion on the ampoule methods for DOC and POC determinations

DOC

The method described is based on the procedure of Menzel and Vaccaro (1964), which is discussed in detail by Strickland and Parsons (1972). It is a precise and sensitive method and can be performed after minimal training. The organic carbon measured is expressed in terms of D-glucose carbon standards. Since 1964 numerous individual organic compounds known to exist in seawater have been used to test the procedure. It is generally believed that no organic matter, except the volatile fraction which is blown off with the inorganic carbon, is escaping detection. However, this is difficult to prove and it is possible that some organic carbon is not detected. Recently there have been suggestions that some labile organic carbon is oxidized at room temperature and lost with the purged inorganic carbon (Sharp 1973; Wangersky 1974; Skopintsev et al. 1977). Techniques which may clarify this point are presently being investigated in this laboratory.

The concentrations of DOC found in most samples lie in the range 0.5-3 mg C ℓ^{-1} (measured as glucose equivalents). Table 1 shows some typical DOC concentrations and the standard deviations from the mean of replicate analyses. Normally the standard deviation (σ) of the mean is ± 6%. However, near the detection limit the standard deviation of the mean can be ± 10%.

Table 1. Some typical dissolved organic carbon concentrations in seawater and percentage standard deviations from the means of replicate analyses.

Mean (mg C ℓ^{-1})	No. of determinations	Percentage standard deviation (± σ%)
0.44	3	1.8
0.60	3	9.8
0.61	3	4.5
0.68	3	9.0
0.68	7	16.2
1.62	4	4.5
1.80	3	3.0
1.73	3	3.0
1.55	5	5.6
8.20	2	4.3

The detection limit as defined by Strickland and Parsons (1972) is expressed as 3 σ of the mean concentrations measured in replicate samples with low but just measurable concentrations. This gives a detection limit of about 0.33 mg C ℓ^{-1} for samples.

Table 2 shows that for glucose standards with concentrations < 2 µg C per ampoule (< 0.4 mg ℓ^{-1}) the standard deviation can be ± 30% of the mean. The limit of detection (3 σ) for standards is 0.35 mg C ℓ^{-1} (1.75 µg C per ampoule), not significantly different from that of the samples.

The 95% confidence range at the 1 mg C ℓ^{-1} (5 µg C per ampoule) level is estimated to be ± 0.06 mg C ℓ^{-1} (0.30 µg C per ampoule).

Table 2. Means, percentage standard deviations of the means and calculated range at a 95% confidence level of replicate glucose standard analyses.

Standard µg C per ampoule	Mean integrated area of n determinations	Range at a 95% confidence level* $\left(\frac{2\sigma}{\sqrt{n}}\right)$	n	Percentage standard deviation of mean (% σ)
0	179	± 32.3	16	36.0
2	220	± 31.4	17	29.0
5	461	17.0	17	7.5
10	686	17.0	17	5.1
15	900	31.6	10	5.5
20	1253	23.5	11	3.1
25	1479	28.9	3	1.5
30	1694	21.0	4	1.2
35	1901	14.0	4	0.7
40	2143	25.0	4	1.2

* See Strickland and Parsons (1972) for definitions

POC

POC concentrations of 0.08-0.9 mg C ℓ^{-1} with standard deviations of 5-50% of the mean have been found. Selected examples are shown in Table 3. Reproduceable standard deviations and confidence levels of these determinations are difficult to calculate because of (i) uneven distribution of the particulate matter on the filter pad due to insufficient vacuum to clear air trapped in the pad or tilting of the filter holder during filtration, (ii) the variability in the carbon content of large and small particles that are distributed unevenly on the pad (Wangersky 1974), and (iii) the experimental and instrumental variability described for DOC determination (normally ± 6%).

For these reasons it is necessary to measure four pairs of the dissected filter pad and total the results, making the assumption that the experimental error is similar to that of the standards and DOC discussed above. However, this is a time consuming technique and if a furnace is available the furnace technique described below is normally used. It has the added advantage that large samples can be processed.

Table 3. Some typical particulate organic carbon concentrations in seawater and percentage standard deviations from the means of replicate analyses.

Mean (mg C ℓ^{-1})	No. of determinations	Percentage standard deviation ($\pm \sigma\%$)
0.094	4	11.9
0.074	3	19.3
0.230	4	20.6
0.340	4	48.8
0.340	4	15.5
0.700	4	7.5

Time requirements for the ampoule method

It takes 5-10 minutes to analyse and record the carbon content of each ampoule. The number of determinations that can be done per hour is dependent on whether a sample is done singly, in duplicate, in triplicate or in quadruplicate. However, it must be remembered that it also takes about 4 hours to prepare (to cap with aluminium foil, bake and store) 300 ampoules; 6 hours to add reagents, purge and autoclave 100 ampoules; and 6 hours to analyse and plot the results from 10 samples in quadruplicate. In order to process more samples at the expense of accuracy, it is suggested that, of the 4 ampoules prepared for each sample, only the first two integrated peak areas measured that are approximately the same should be taken as the correct value, and the remaining ampoules discarded.

3. THE DETERMINATION OF PARTICULATE ORGANIC CARBON BY A FURNACE TECHNIQUE

Particulate organic carbon is decomposed to carbon dioxide by a stream of oxygen in a furnace at 550°C (Fig. 4). Inorganic carbon is not decomposed. The CO_2 is cleaned and measured (i) gravimetrically or (ii) by non-dispersive infrared spectrometry.

Experimental

The two methods described below can be used to determine organic carbon in 12-24 samples of solid material per day.

Reagents

1 Ascarite - soda asbestos which is replaced when the colour changes from light brown to whitish in 3/4 of the column.

2 Magnesium perchlorate (anhydrous). Replace this when it becomes wet as it may block the tube and change the flow rate.

3 Manganese dioxide. Replace about once a month.

4 Antimony powder. Replace about once a month when iodine coats the particles. (Note 17).

5 Potassium iodide (10% solution). Replace when the colour has changed from clear to dark orangy-brown.

6 Standard glucose solution for I.R. CO_2 detection method. dissolve 6.25 g analytical grade D-glucose in 100 mℓ of distilled water (25 mg C $m\ell^{-1}$). Add 2 crystals of lead nitrate as a preservative.

Working standards

Pipette 2, 4, 8, 12 and 16 µl of stock standard (50, 100, 200, 300, 400 µg carbon) into the sample combustion boat.

Apparatus

Figure 4 shows a silica tube (A1-A2) (76 cm long, 1.5 cm I.D., 2 cm O.D.) around which is wound Nichrome wire (B) to make a furnace which in half its length (A1) reaches 900-1000°C, and in the combustion zone (A2) reaches 550-650°C. The wire around A1 is connected directly to the mains and that around A2 to a variable transformer (C). The hot part of the oven (A1) contains silica chips (D) which disperse the volatiles released at 550°C and ensure their complete oxidation. At the cool end of the furnace (A2) there is a removable silicone rubber bung (E) and an oxygen inlet tube (F). The tube furnace is enclosed in a lagged cover (G). A fireclay sample boat (H) contains the sample. All other apparatus should be made of glass. If ground glass joints are not used, sleeves of tygon must be used over the glass to glass joints. The gases that leave the furnace pass through a 10% potassium iodide solution (I) to remove chlorine. The remaining gases then pass through antimony powder (J) to remove iodine carried over from the potassium iodide solution, manganese dioxide (K) to remove oxides of nitrogen, magnesium perchlorate (L) to remove traces of water vapour and for the gravimetric method a preweighed tygon absorption tube (M) which absorbs the "cleaned" CO_2. The tygon absorption tube has a removable glass capillary (N) at each end. It contains magnesium perchlorate (L) (Note 18), glass wool (O) and the self indicating soda asbestos or ascarite (P). If the CO_2 detection is to be done by infrared absorption, then the ascarite tube is replaced by the OIC analysing unit described in Appendix III.

Sample preparation for particulate organic carbon determinations

Samples must be prepared to meet the requirements of the experiment and can fit into the following categories:

POC on pre-ignited filter pads

POC per unit volume: analyse the damp filter immediately or freeze at -20°C in individual containers away from organic fumes.

POC per unit weight of residue: filter onto a preweighed filter pad; then

1 Freeze dry or oven dry, then reweigh the filter and residue;
2 Place the filter in the sample boat and reweigh in the sample boat before ignition (see below);
3 Reweigh after ignition for ash content (if required).

POC in centrifuge residues, sediments, plankton, seagrasses, etc.

1 Freeze dry or oven dry;
2 Homogenize the sample into a fine powder (individual animals, such as zooplankters, need not be homogenized);
3 Weigh out accurately into the sample boat;
4 Ignite in the furnace (see below);
5 Reweigh to determine ash content (if required).

Methods

Gravimetric method (Range: 0.5-40 mg carbon per sample)

1. Switch on the furnace heating coils.
2. Set the variable transformer to 20-30% of full range.
3. Leave for 30-45 minutes to heat up.
4. Insert a thermocouple 18 cm into the furnace inlet and check the temperature is 550-650°C.
5. Set the flow rate (47 ± 3 mℓ min^{-1}). (Note 19).
6. Weigh the ascarite tube to 1×10^{-5} g and the sample or glucose standard to 1×10^{-4} g.
7. Attach the ascarite tube to the end of the CO_2 "scrubber tubes".
8. Quickly remove the silicone rubber bung, insert the sample boat and replace the bung. (Note 20).
9. Combust for 20 minutes.
10. Reweigh the ascarite tube to determine the CO_2 absorbed.

Calculation

Equation

$$C_6H_{12}O_6 + 6\ O_2 \xrightarrow[550^{\circ}C]{\text{Heat}} 6\ CO_2 + 6\ H_2O$$

glucose — carbon dioxide absorbed on ascarite

let "a" be the weight of glucose
"b" be the weight of sample
"y" be the weight gain of ascarite tube with CO_2.

1. If experimental conditions are correct (i.e. 100% recovery of CO_2)

$$y = \frac{264}{180}\,a = 1.47\,a.$$

2. The percentage carbon of a sample is

$$\left(\frac{y}{b}\right) \cdot \left(\frac{12}{44}\right) \cdot (100) = \frac{27.3_y}{b}\ \%$$

Infrared absorption method (Range: 0.05-0.4 mg carbon per sample)

1. Span the non-dispersive infrared detector supplied with the OIC analyser as described in the manufacturers' manual (e.g. MSA Lira Models 300, 303 and Horiba PIR-2000).
2. Set up the furnace and the potassium iodide, antimony power and magnesium perchlorate "scrubber tubes" as described for the gravimetric method.
3. Zero the instrument using nitrogen "zero gas" as described in the manufacturers' manual.
4. Pipette aliquots of standard glucose into the combustion boat, ignite in the furnace and plot a standard curve. This will be nonlinear (see Fig. 5).
5. Ignite the samples and determine the carbon content from the standard curve.

4. REFERENCES AND FURTHER READING

Instruction Manual Preliminary MSA Model 303 Infrared Analyzer. (Mine Safety Appliances Co.: Pittsburgh, Pa).

Instruction and Procedures Manual: The Total Carbon System Operating Procedures Model 0524B. (Oceangraphy International Corporation: P.O. Box 2980, College Station, Texas 77840, U.S.A.).

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APPENDIX I

Preparation of carbon-free distilled water

Select one of the methods listed below:

1 If distilled water yields high blanks (> 0.2 mg C ℓ^{-1}) redistil the distilled water (not (not de-ionized) in all-glass apparatus.

2 Reflux the distillate (1 ℓ at a time) with 10 $K_2S_2O_8$ and 1-2 mℓ of 85% phosphoric acid for 4 hours.

3 Distil into a clean receiver, reject the first 100 mℓ and last 200 mℓ.

4 Store in a clean glass stoppered bottle which should never be left open

or

Irradiate the distilled water in covered silica tubes with UV light after adding one drop of H_2O_2 to each 100 mℓ.

APPENDIX II

Operation of the ampoule purging and sealing unit

General description

The ampoule sealing unit purges glass ampoules of inorganic and volatile organic carbon components with purified oxygen flowing at approximately 70 mℓ min^{-1}. The oxygen is purified by passing through a 200 mm cupric oxide catalyst tube at 450°C. An oxygen-propane microburner is used to seal the ampoule, the neck of which is held by a clamp. The lower portion of the ampoule is twisted manually after the neck becomes molten. "Purging cones" prevent introduction of carbon dioxide contamination from the microburner during the sealing operation. (See Notes 21.1 to 21.5).

Operation (see Fig. 6)

1 Connect the sealing unit to a cylinder of commercial oxygen fitted with a pressure reducing regulator using a length of thick-walled 6 mm O.D. polypropylene tubing for high pressure and swagelok fittings.

2 Adjust the regulator to deliver at a pressure of 200 kPa (about 50 mℓ min^{-1}).

3 Turn catalyst heater on. Adjust voltage if necessary to give a constant temperature of 450-500°C as indicated on the pyrometer (Fig. 7).
CAUTION: Maximum temperature for the heating element is 525°C.

4 Allow at least 15 minutes for unit to purge before placing ampoules in purging position. (On initial start-up allow one hour to condition catalyst and to purge lines).

5 Place purging cone on each ampoule. For best precision, cones must be cleaned with distilled water before each set of samples.

6 Place ampoule into purging position (Fig. 7) and insert a glass purge tube through purge cone to the bottom of the ampoule. Purge for 20 minutes (see Fig. 8). Glass purge tubes must be cleaned initially by drawing hot distilled water through them with suction. Store purge tubes and purge cones in container provided on unit.

7 Ignite the microburner. See Lighting sequence following. The microburner is adjusted to give the correct heat for sealing, but minor heat adjustments may be necessary for different operators. The valves to the right marked "microburner adjust" are for fine adjustment; the toggle valves marked "propane" and "oxygen" serve as ON-OFF valves. Once the proper flame has been attained the microburner adjust valves should not be turned. Turn the flame on or off with the toggle valves on the left only.

Lighting sequence

1 Open main valve on propane cylinder.
2 Open propane toggle valve.
3 Ignite propane gas at microburner.
4 Wait 10 to 15 seconds for the flame to reach maximum intensity. (This waiting period is necessary due to the very small gas flow through the regulator. Should the oxygen be turned on before this, the mixture of gases will be explosive).
5 Open oxygen toggle valve.
6 Flame should be approximately 1.5 mm long and slightly oxidizing (intense blue).
7 Place a purged ampoule in the clamping assembly (Fig. 9). The glass purge tube should be placed in the tube holder during this operation.
8 Press ampoule holding lever with the thumb and place the base of the ampoule in position.
9 Raise purge tube and press into purge tube holding assembly. (Bottom of purge tube should be level with the underside of the clamp, but lower than apex of purge cone).
10 Swing the microburner into place and seal the ampoule (Fig. 10, 11). (Only 3-4 seconds are necessary for the thin glass neck of the ampoule to melt. When the molten state has been reached the ampoule is pulled down approximately 10 mm. This movement decreases the diameter of the neck. The ampoule is then twisted rapidly for several turns without raising or lowering the ampoule. The twisting motion is necessary to seal off any capillary that might otherwise be formed. Practise this procedure with ampoules filled with water until a good seal can be formed each time. Care must be taken not to allow the partly sealed ampoule to be raised into the flame as gas expansion within the ampoule will cause a thin glass bubble to form at the tip. If the ampoule is pulled down too far the seal will be long, sharp and easily broken. On the other hand, if the ampoule top is too thick, it will be difficult to break during analysis. Several practice ampoules should be placed in the ampoule analysing unit and checked for ease of breaking.
11 Remove the microburner from the sealing position as soon as the ampoule has been sealed. This prevents overheating of the clamp.
12 Place the glass purge tube and purge cone in another ampoule, and place in purging position.
13 Remove the sealed ampoule from the sealing holder.
14 Open the clamp and permit the hot ampoule tip to drop in a glass beaker.

Turning off the sealing unit

1 Close oxygen toggle valve.
2 Wait until a pure propane flame appears.
3 Close propane toggle valve.
4 Close main valve of propane cylinder.
5 Close main valve of oxygen cylinder.
6 Turn off catalyst heater.

APPENDIX III

Operation of the ampoule analysing unit and integrator

General description (see Notes 34.1-34.10)

This unit (Fig. 12) contains a non-dispersive IRA and is used to determine the CO_2 concentration resulting from the wet oxidation of organic matter in the ampoule. Operation of the IRA is described in its instruction manual and initial and final adjustment steps below (see also Fig. 13). The unit allows ampoules to be analysed easily without introducing atmospheric carbon dioxide and removes water vapour from the carrier gas stream prior to reaching the IRA.

Prior to use, fill the drying tubes with anhydrous magnesium perchlorate except the first tube on the left which is a trap to remove gross amounts of water vapour. The second tube is the primary drying tube filled with magnesium perchlorate. Gas flows through this tube from bottom to top. The third tube on the front and the long drying tube inside the cabinet ensure that no water vapour is transmitted in the carrier gas stream to the IRA. Glass wool plugs are placed at the ends of all tubes except the water removal tube. The tubes should be tapped only gently while filling, in order not to restrict the gas flow any more than necessary.

A glass wool filter at the outlet of the Ampoule Breaking Assembly prevents small particles of glass clogging the gas line to the first water removal tube. Replace the glass wool plug when necessary.

The modified Ampoule Breaking Assembly (Note 22) (Fig. 14) should be dismantled and all particles of glass purged out with water after breaking about 30 ampoules (Note 34.1). Small particles of glass tend to pass into the stainless steel purge tube, restricting the flow. This restriction is cleared by removing the plastic gas line and pushing a length of spring steel wire through the purging tube. A build-up of glass particles within the ampoule breaking assembly tends to prevent the free movement of the stainless steel purge tube. Usually several up-and-down movements of the purge tube will remedy this situation. The silicone rubber seal which seals the purge tube within the ampoule cutting plunger should be replaced after several hundred ampoules have been analysed or before if the centre hole in the seal has become enlarged. Silicone grease should be used to lubricate the purge tube whenever necessary.

The primary drying tube will need replacing often. Usually the lower 2 cm of magnesium perchlorate becomes exhausted, indicated by a change from dry granules to a semi-liquid state. Whenever a reduction in the carrier gas flow rate is noted, this tube should be checked first as the probable cause (Note 34.6). Also check the stainless steel purge tube for restriction due to particles of glass within the tube.

Connecting ampoule analysing unit to gas supplies

1 Connect the analyser (right side, back) to the commercial nitrogen ("zerogas") with 6 mm O.D. polyethylene tubing via a cylinder regulator. To the connection nearest the front attach the line for "span gas" (compressed air containing about 350 ppm carbon dioxide) (Note 23).
2 Check that "zero" and "span" gas toggle valves point to the "off" position.
3 Adjust inlet pressures to 80 kPa (12 lb in^{-2}) at the cylinder regulators.
4 If required connect a strip chart recorder (0-10 mV).

Initial zero and span adjustments

1 Turn on and warm up IRA. (Four hours warm-up for maximum thermal equilibrium, leave on overnight if the instrument is used daily.) Before any analysis, purge about six prepared practice ampoules through the instrument (Note 24).
2 Place a standardization vial in the Ampoule Breaking Assembly or place an ampoule in the assembly. (Use plastic adaptor and gum rubber gas seal).
3 Position the two-way valve to "out-to-analyse" position so that the carrier gas flows through the Ampoule Breaking Assembly.
4 Open the "zero" gas toggle valve.
5 Open "flow" toggle valve.
6 Adjust "IR" rate valve to give a flow rate of 200 mℓ min^{-1} (reading of 12 on the rotometer).
7 Allow system to purge, then zero the IRA and the strip chart recorder if one is used (Note 25).
8 Close the "zero" gas toggle valve.
9 Open "span" gas toggle valve.
10 Check "span gas" flow rate and if necessary, adjust the "span" gas regulator for a flow of 200 mℓ min^{-1}.
11 Purge the system with "span gas" until "IR" meter reaches a maximum.
12 Adjust the "span" control on the IRA to give a reading of 100 on the "IR" meter (Note 26).
13 With the recorder range in the most sensitive position (0-10 mV), adjust the "Recorder Adjust" so that the recorder reads the same as the "IR" meter (Note 27).
14 If a strip chart recorder is not used with the OIC analysing unit, the IRA will require re-spanning if the analyser meter goes past a reading of 100. To respan refer to the infrared detector instruction manual supplied with your carbon analyser.

Operation of the unit with sample ampoules

Prepare a standard curve similar to that in Fig. 2 daily (Note 29).

1 Zero and Span adjustments have been made. Electronic integrator has been adjusted to give zero integration.
2 Push 2-way valve to "IR By-Pass" to "in" position.
3 Place sample ampoule in unit (plastic adaptor and gum rubber seal on ampoule (Note 30). (See Fig. 15).
4 Push the stainless steel purge tube down close to the top of the ampoule.
5 Open "purge rate" valve to give a purging rate of approximately 200 mℓ min^{-1} (13 on rotometer).

6 Purge for 10 seconds.
7 Pull 2-way valve to "out" position so that IRA carrier gas flows through the Ampoule Breaking Assembly.
8 Check that flow rate is 200 mℓ min^{-1} (Note 34.1).
9 Stop IR carrier gas by turning "flow" toggle valve to "off".
10 Wait for flow rate to fall to zero.
11 Raise purge tube clear of plunger cutters.
12 Break ampoule top and push purge tube to within 3mm of ampoule bottom.
13 Open the "flow" valve when the rotometer returns to zero flow rate. Check for a flow rate of 12.5 to 13 on the rotometer (Note 31, 34.6).
14 Prepare another ampoule with plastic adaptor and gum rubber seal for placing in the Ampoule Breaking Assembly.
15 Allow gaseous contents of ampoule to pass into IRA.
16 Turn the 2-way valve to "in" after the CO_2 peak has been formed on the recorder chart and the recorder pen is exactly at the 5% point. If only the Electronic Integrator is being used (no strip chart recorder), the 2-way valve may be turned to the "in" position when the IRA meter reads 3%. (Note 32).
17 Place the next sample in the Ampoule Breaking Assembly.
18 Record the electronic integrator reading and zero the integrator after zero integration is noted.
19 Repeat the procedure starting with Step 7. If the 2-way valve was changed at the above given points, adequate purging will have taken place.

Electronic integrator

The integrator converts the D.C. output signal of the IRA to a 5-digit LED read out directly proportional to the total amount of carbon dioxide passed through the IRA.

Four controls are used to operate the integrator. The on-off switch supplies power to the integrator assembly. The "lamp test" push switch causes all LED segments to illuminate. The "area clear" switch, when momentarily pressed in the "manual" position, resets the integrator. Final adjustments with "zero baseline" control give zero integration. All other controls on the integrator panel are for the electronic printer and are not used for normal integrator operation.

Turn the power switch to "on". All digits should be illuminated and show some number. Press the "lamp test" switch to check illumination of all segments of each digit. The IRA should be warmed up (4 hours) with zero gas flowing. Zero the IRA with the "IR zero" control (coarse adjustment) until no integration is occurring. Adjust the integrator "zero baseline" control (fine adjustment, 2% of full-scale) clockwise until integration slowly occurs. If there is not sufficient adjustment with the integrator "zero baseline" control, then slightly readjust the "IRA Zero" control (Note 33). Readjust the integrator "zero baseline" control so that there is no integration. When zero integration has been achieved, clear the integrator by pressing Area Clear switch to the "manual" position. The integrator is now ready for use.

APPENDIX IV

Notes

1. All equipment, samples and reagents for carbon determinations should be kept away from contaminants such as grease and organic solvents, and areas where people smoke.
2. It is more efficient to fire a large number at one time. Cleaned ampoules or filters must be stored in an atmosphere free of organic carbon.
3. Use clean metal forceps to handle. Aluminium foil pre-ignited at 500°C is useful to prevent contamination.
4. If commercially available reagents give a blank value that is too high, refer to Strickland and Parsons (1972) for purification procedures.
5. As the level of impurities varies in different batches of $K_2S_2O_8$, always treat standards and samples from the same bottle.
6. Do not use water that has been deionized through a resin column or the DOC blank will be high. Test the distilled water for low blank before use. If the blank is high, clean,the still and storage container. As a last resort, attempt to remove the DOC using one of the methods listed in Appendix 1.
7. Wet the inside neck of the ampoule with the H_3PO_4 to prevent atmospheric CO_2 contaminating the sample during purging and sealing.
8. Prepare 50 ampoules for each standard concentration. This procedure takes several days as only 100 ampoules can be purged and sealed each day. Purge and seal ampoules as soon as standards are pipetted into them.
9. Raise the temperature in the autoclave to 95-100°C before placing the sealed ampoules inside, then raise it to 124°C and hold at that temperature for one hour.
10. To reduce or eliminate erratic values caused by contamination, all glassware, filters, reagent bottles, pipettes, etc., must be cleaned with hot chromic-sulphuric acid and rinsed with distilled water then CFDW. (See also Notes 1 to 6).
11. For convenience prepare these in batches of 50.
12. Be cautious of grease from winch-cable, boat exhaust, submersible pumps and sample bottles. Thoroughly clean new or dirty water samplers by scrubbing with detergent, rinsing with distilled water and finally with CFDW. Use chromic-sulphuric acid, if necessary. Wrap open ends in pre-ignited aluminium foil to prevent further contamination.
13. Use a vacuum of less than 30 kPa (250 mm Hg) to prevent phytoplankton cell rupture.
14. Depending on the accuracy required, one to four aliquots should be measured.
15. Small, prefired or chromic-sulphuric acid cleaned, glass-stoppered, glass bottles can also be used if immediate processing is impossible. It is advisable to keep some sample in this state in case the sample has to be diluted because of high carbon levels.
16. If a CFDW blank (0 mg Glucose C ℓ^{-1}) is measured, remember that the blank is at the detection limit of the method and there will be a standard deviation of ± 30% compared to approximately ± < 6% at concentrations of 1.5 and 6 mg C ℓ^{-1}.
17. To renovate, rinse quickly in concentrated HCl then in distilled water and then dry in an oven.
18. This is not necessary once the operator is familiar with the procedure and is sure he is not weighing water that should have been removed in the long drying tube prior to the ascarite tube.
19. It is important that it should be neither too fast nor too slow or CO_2 will be lost instead of absorbed onto the ascarite. With experience the operator is able to assess the correct flow rate by examining the bubble pattern in the KI solution. However, initially and periodically for even a routine operator the following procedure must be followed:

Weigh out accurately 2-10 mg D-glucose into a sample boat.
Measure the CO_2 evolved using the procedure for gravimetric determination with an ascarite adsorption tube. The recovery must be 100 ± 5%. If it does not lie in this range, check the flow rate, the temperature in the combustion zone, gas leaks after combustion and quality of the magnesium perchlorate and ascarite.

20. CO_2 will get into the system if this is done slowly.

21.1. Purge and seal first those ampoules with the lowest carbon concentration.

21.2. Always cover ampoules with a sheet of aluminium foil during preparation and prior to sealing to prevent contamination by dust particles.

21.3. Use glass-stoppered bottles for storage of sample water, H_3PO_4 and $K_2S_2O_8$.

21.4. Always use clean purge tubes and purge cones.

21.5. Clean glass purge tubes and purge cones by drawing boiling distilled water through them with suction. Purge cones fit into 5 mm I.D. Tygon tubing attached to the vacuum source. Place a rubber seal (similar to the one used to seal the stainless steel flushing tube in the ampoule crushing assembly) in the 5 mm Tygon tubing to reduce the opening to fit the glass purge tube. Wipe the outside of the tube with a clean damp tissue first and then draw hot water through the tube. Handle only the end of the tube that was placed into the seal. Store in the screw-cap vial until used. Do not attempt to completely dry the tube. The ampoule sealing unit should be placed so that the operator is looking up and under the microburner when sealing an ampoule. This position, and the use of glass-blower's glasses, makes it much easier to determine when the molten state has been reached.

22. The commercial cutter tips were made of stainless steel which tended to crush rather than cut the ampoule, and a considerable amount of downward force was required, often resulting in the ampoule breaking below the neck and causing the pressurized contents to scatter glass and ampoule contents over a wide area. Chlorine generated from the sample corroded the stainless steel cutters to such an extent that they became weakened and splayed outward. Figure 14 shows the modified cutter, in which the four stainless steel jaws are replaced by three tungsten carbide ones. These new cutters do not corrode and only slight pressure is required to cut the ampoule cleanly at the top of the neck, which increases the speed of analysis and reduces the hazard.

23. Compressed air containing 350 ppm carbon dioxide should be used as a span gas. Laboratory compressed air can be used as a second choice. However, the carbon dioxide content of this gas may vary from day to day.

24. When a sample is presented in quadruplicate and two of the first three readings give similar results, the fourth ampoule can be saved for this purpose.

25. First zero the IR analyser with the "zero" control, then zero the Strip Chart Recorder with the displacement control.

26. The "span" control of the IR analyser is shown in Figure 12.

27. This setting will allow analysis of ampoules containing 16-20 µg carbon. If more carbon is expected, the IR analyser will require re-spanning. For best accuracy, the CO_2 peak should be in the middle one-third of the recorder. All carbon dioxide peaks should be on-scale in order to prepare a CO_2 standard curve which encompasses the sample peaks.

28. The re-spanning of the IRA is necessary if analysing the contents of ampoules with high CO_2 concentrations. Re-spanning is an electrical "compression" of the signal output of the IRA amplifier. At about 25% above the 100 meter reading the amplifier output becomes "flat" and extremely nonlinear. Re-spanning to a lower meter reading results in an increased CO_2 range of the analyser.

29. There is some instability in the instrument and daily variations in the blanks. The former is thought to be due to the equilibration of the system with the chlorine liberated from samples. To keep this variability to a tolerable level, the IR cell must be cleaned quarterly, and the corroded lining replaced every 1-2 years (if the instrument is used every day). We keep a spare infrared cell so that, during the period the cell is being relined, analyses can continue.

30. DOC ampoules may be analysed at room temperature. Ampoules can be warmed in an oven to 55°C prior to analysis for faster analysis and less peak tailing. Use caution when placing ampoule in the Ampoule Breaking Assembly. Pull the plunger and the purging tube up so that the top of the ampoule will not be broken when it is tightened into place.

31. Waiting for a zero flow rate in Steps 10 and 13 (Operation of the unit with sample ampoules) in Appendix III has been found to give better precision.

32. The 2-way valve could be changed when the recorder pen reaches the baseline or the electronic integrator shows zero integration, but there is no loss of accuracy or precision when changing the valve as given above if a standardization curve is made using the same technique.

33. In some infrared detectors time is required for equilibrium to be reached after this zero adjustment, but on others there is a quick response. (Refer to IR detector instruction manual).

34.1. Step 8 (Appendix III - Operation of the unit with sample ampoules) is a check for leaks in the system, or obstructions in the gas flow system.

34.2. Use several trial ampoules at the beginning of a batch. This allows a water vapour equilibrium to be established. If trial ampoules are not used, the first few CO_2 peaks may be high.

34.3. Peak area should be used in lieu of peak height for best precision. A disc type integrator accessory attached to the recorder or the electronic integrator will indicate peak area.

34.4. Analyse all ampoules at the same rate i.e. two or three minute intervals for best precision.

34.5. Peak height and peak area change with different flow rates. Flow rate must be kept constant.

34.6. This is a low pressure system. The slightest obstruction in any gas line will change the flow rate. Changes in flow rate are usually due to: primary drying tube exhausted and settled; leaky ampoule seal, crack in ampoule below seal. For this reason the flow is checked Step 13 (Operation of the unit with sample ampoules) Appendix III; small particles of glass lodged in the purge tube.

34.7. Check analyser zero before each sample and span settings before each batch.

34.8. Lubricate purge tube with a light coating of silicone grease when necessary.

34.9. A reduced peak height and a broadened CO_2 peak may be caused by a pin hole in the stainless steel purge tube. When this happens, the flow rate of bubbling in the ampoule will decrease.

34.10. Change number 3 drying tube daily. The gases from ampoules containing seawater are extremely corrosive to the analyser's sample cell when accompanied by even the slightest amount of water vapour.

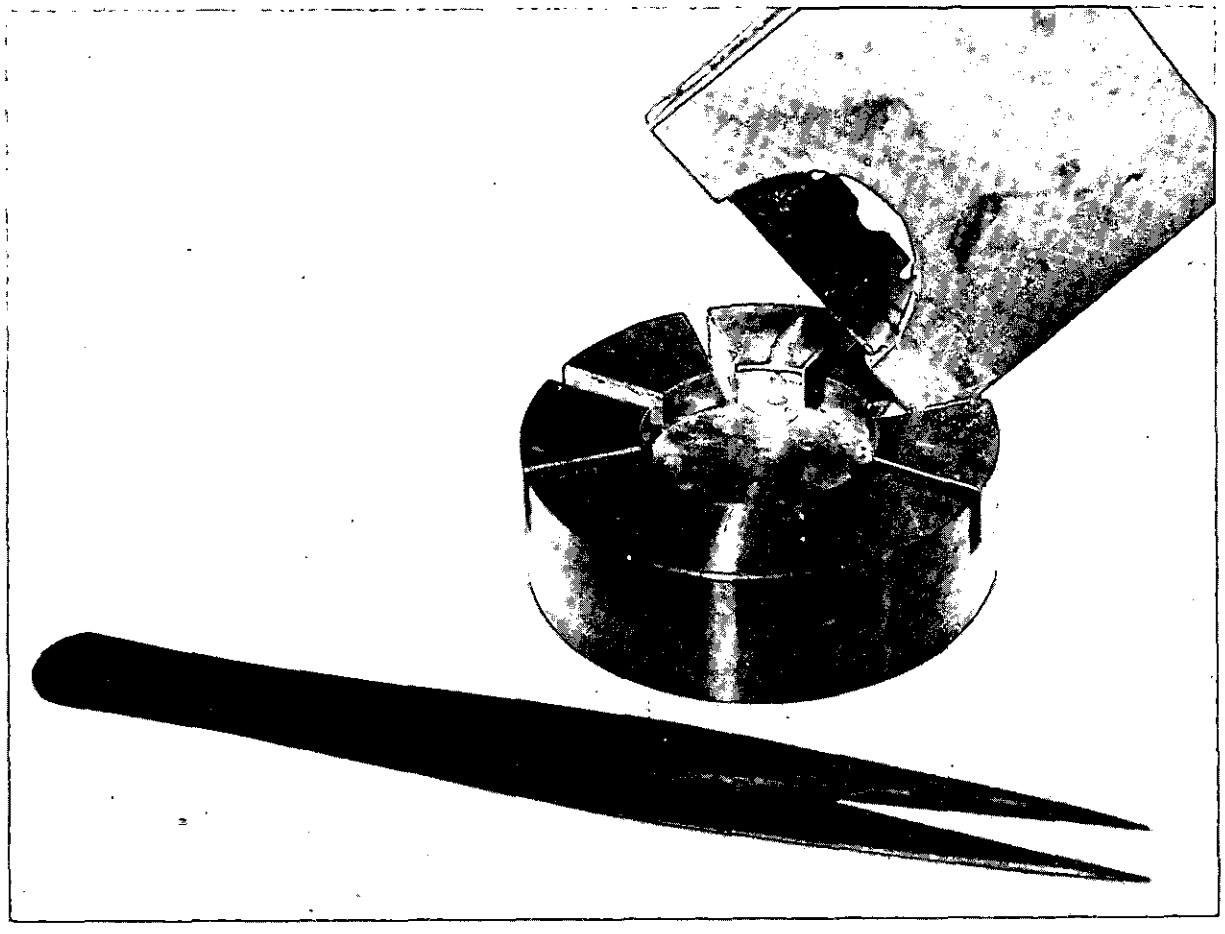

Fig. 1 The apparatus used to segment a filter.

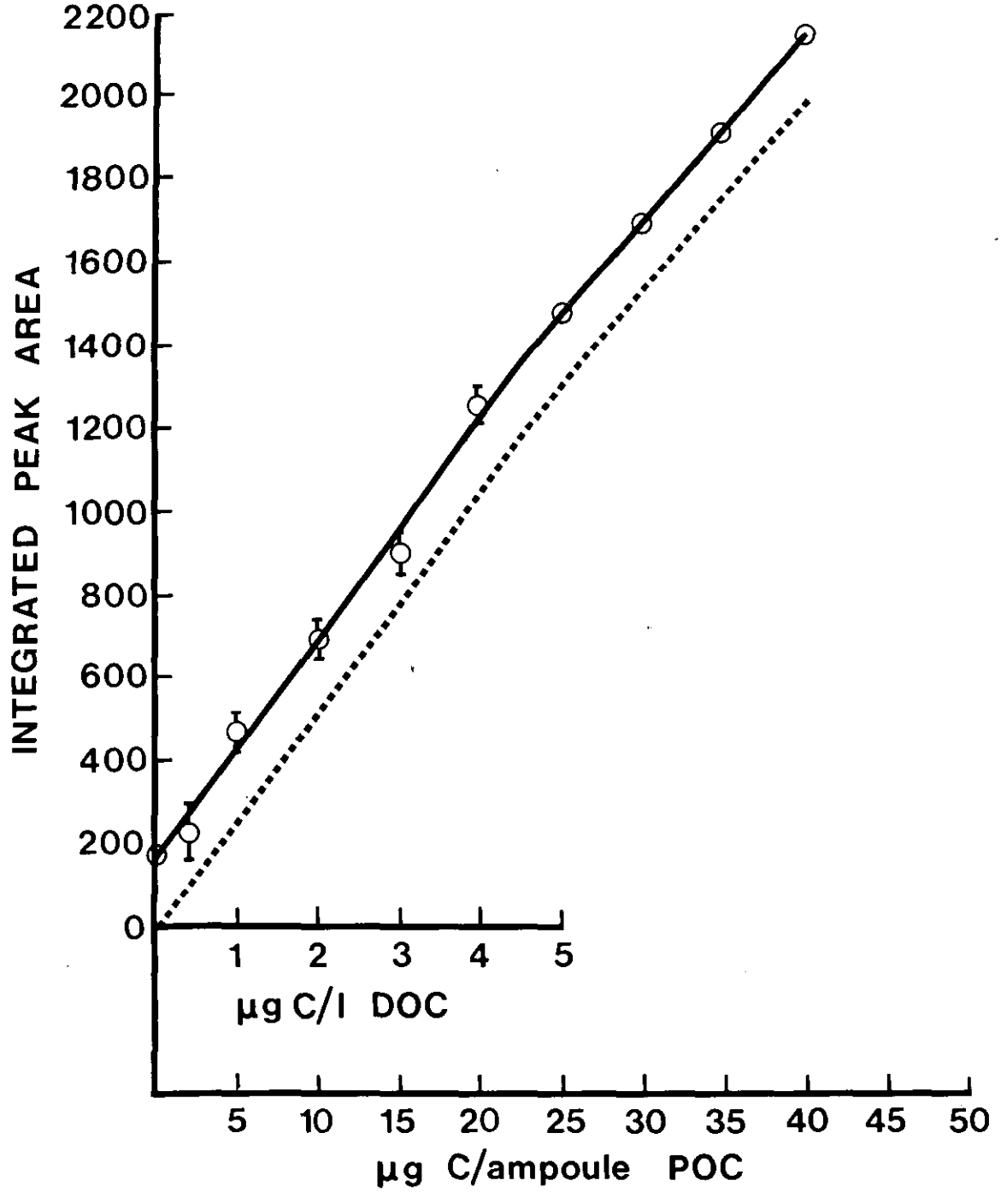

Fig. 2 Integrated peak areas as a function of glucose carbon concentrations (µg C ℓ^{-1} for DOC; µg C per ampoule for POC); including reagents and distilled water blank (the corrected standard curve is dotted).

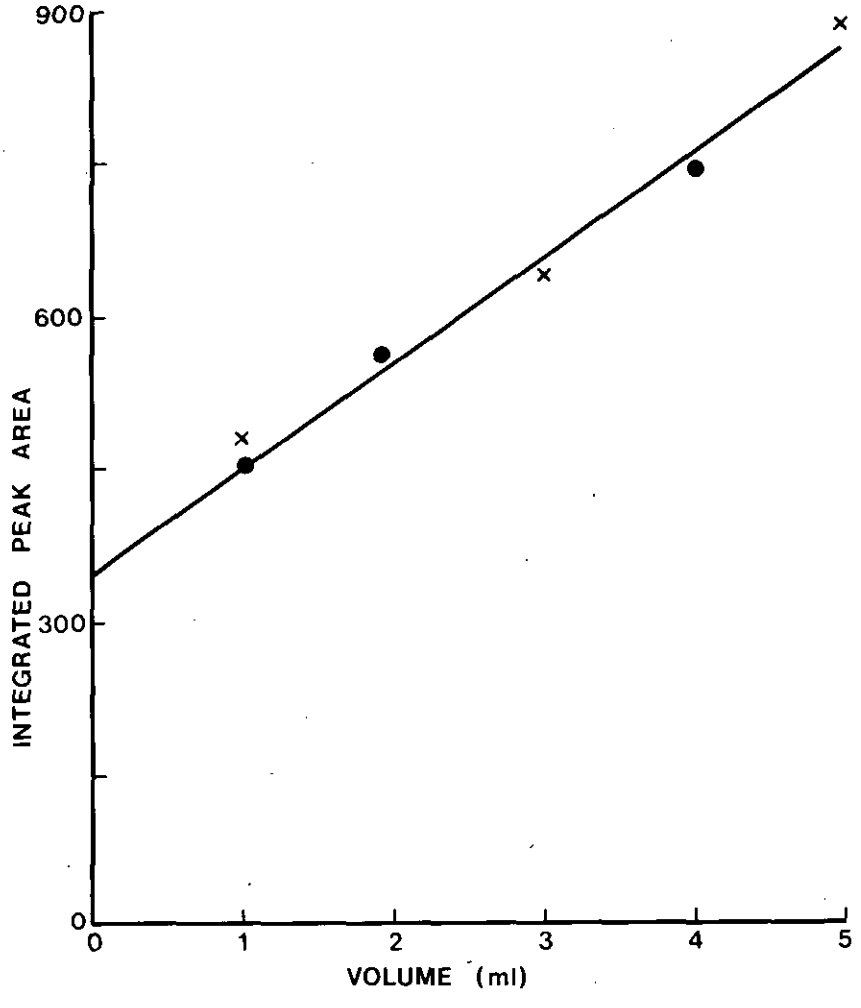

Fig. 3 A graph of integrated peak areas as a function of increasing volume (mℓ) of low carbon seawater for the determination of the reagent blank.

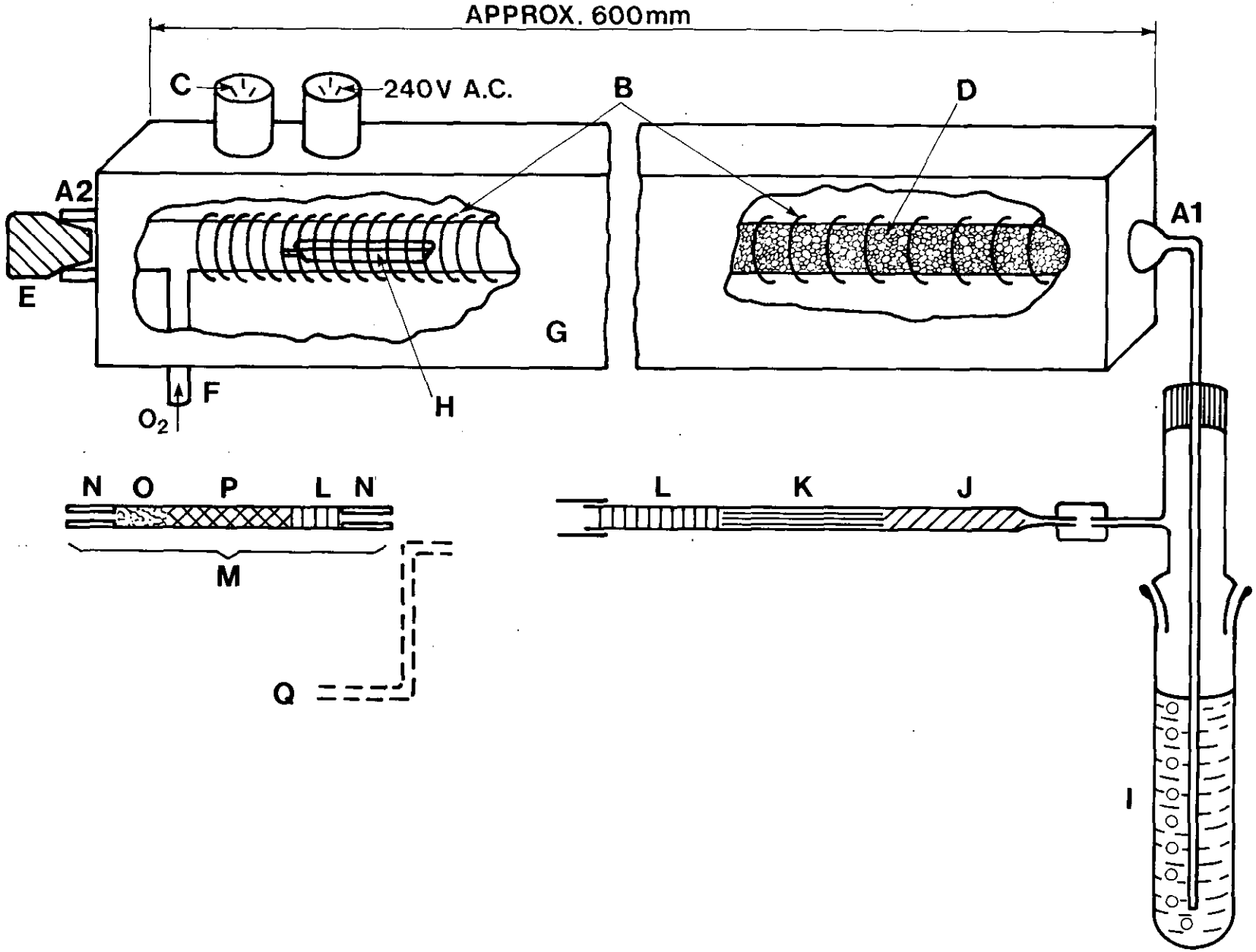

Fig. 4 The combustion furnace for particulate organic carbon analyses by gravimetric or infrared absorbtion methods. See text for details.

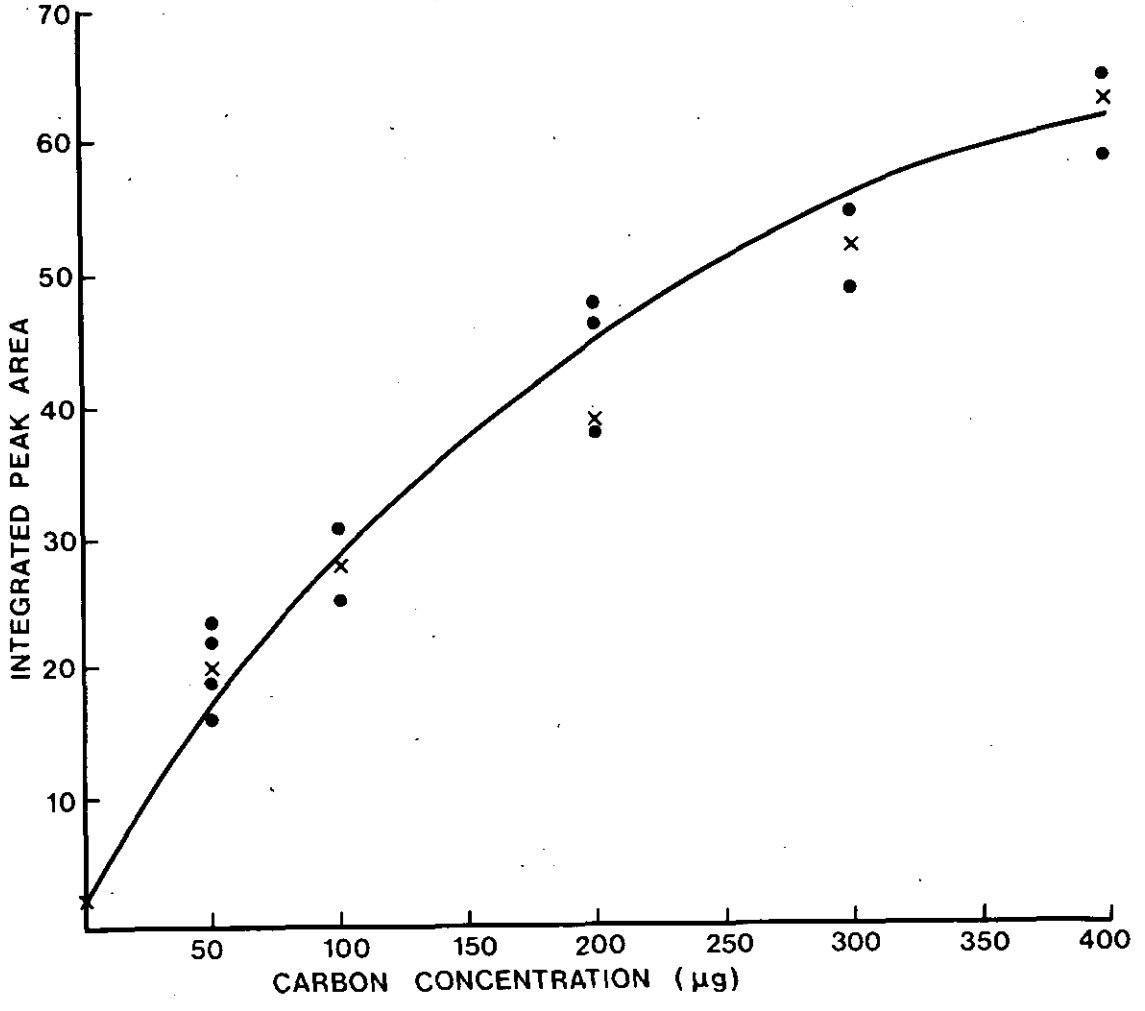

Fig. 5 Standard curve for the POC determination by the furnace technique.

AMPULE SEALING AND PURGING FLOW DIAGRAM

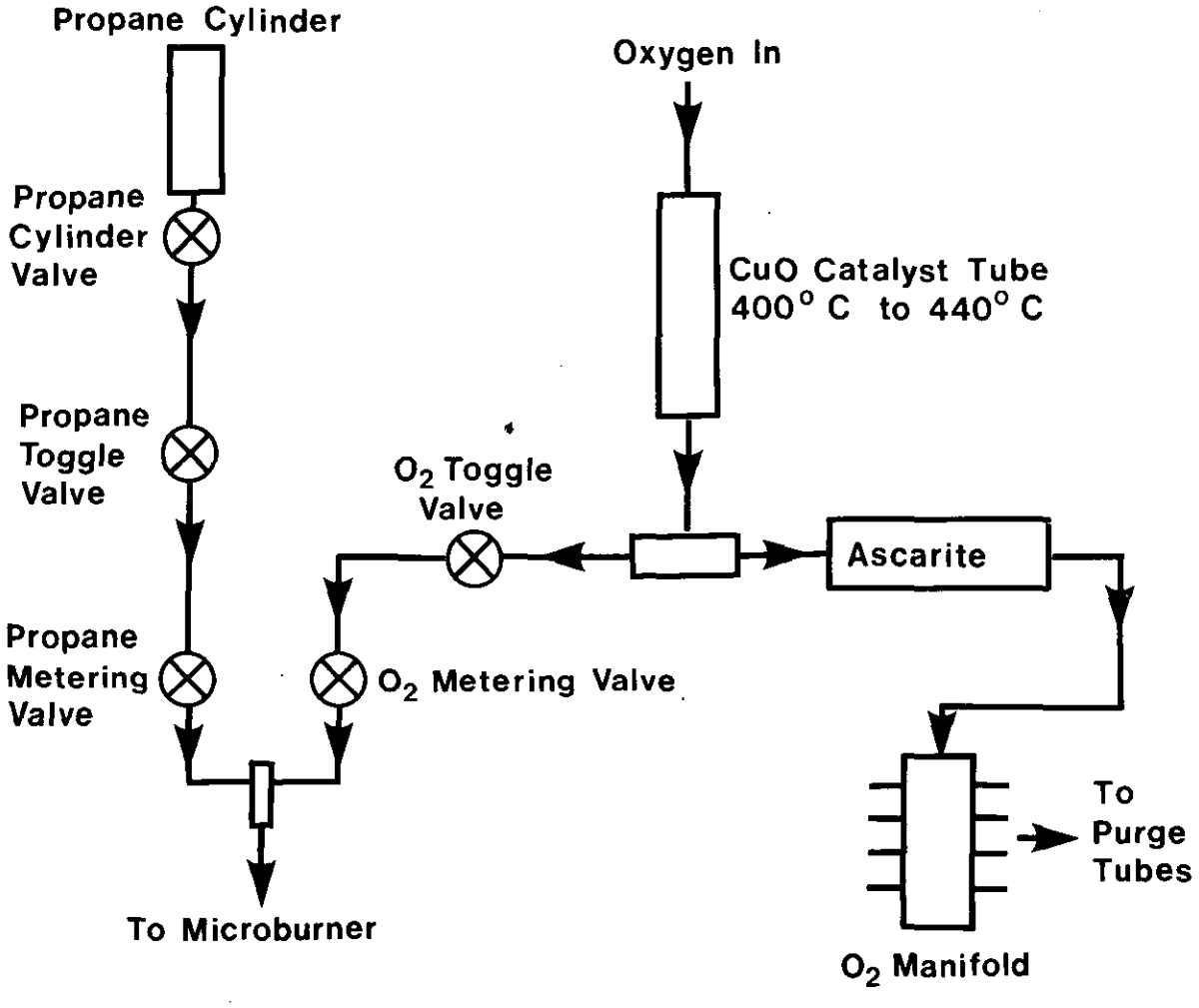

Fig. 6 Diagram of gas flow when ampoules are purged and sealed.

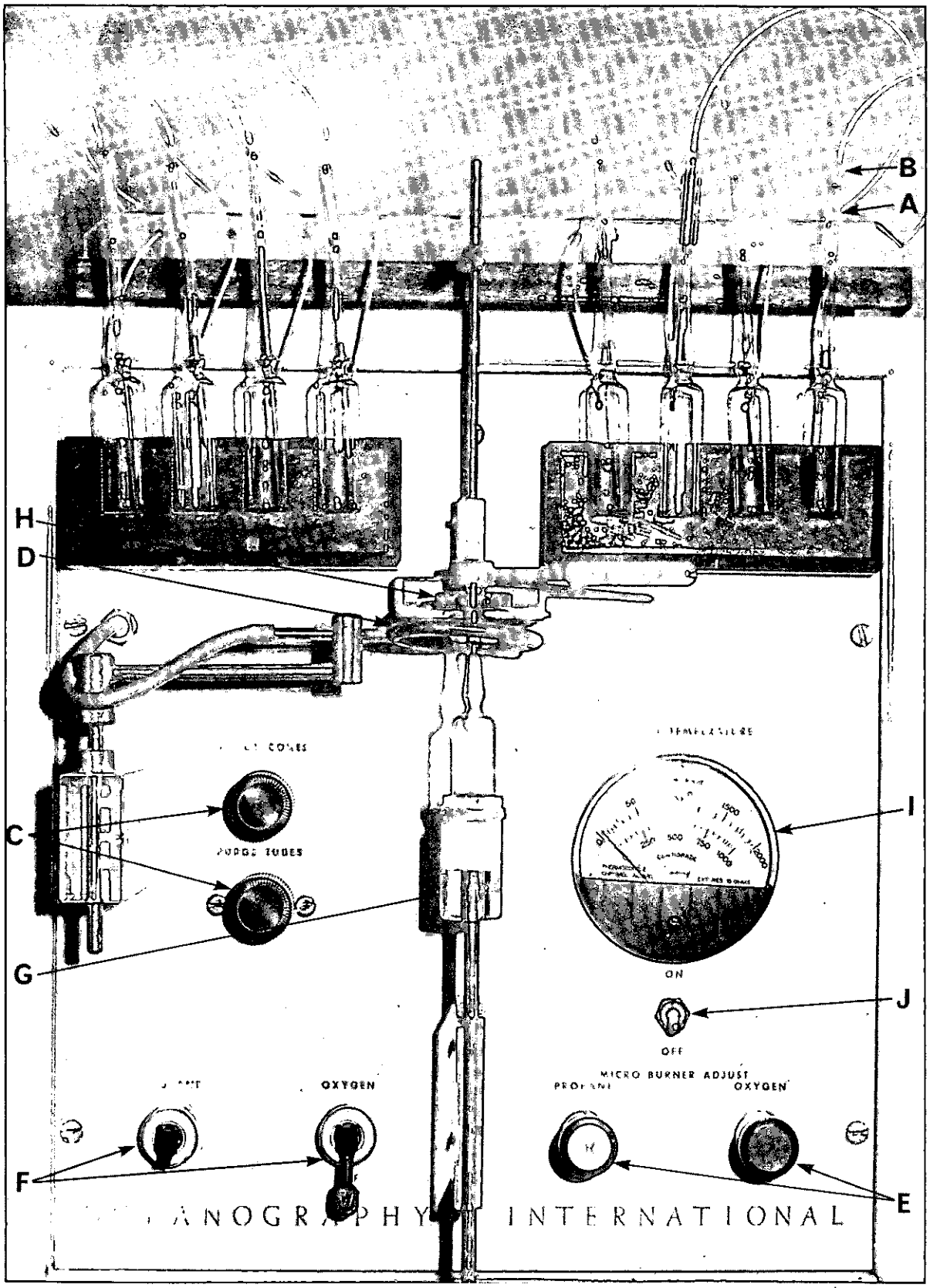

Fig. 7 Front view of the ampoule purging and sealing unit with the ampoules in purging position. A: Purge cone resting on top of ampoule; B: Purge tube in position; C: Storage for purge tube and cones; D: Microburner; E: Fine adjust valves for propane and oxygen for microburner; F: Off-on valves for propane and oxygen for microburner; G. Ampoule receiver; H: Clamping assembly; I: Pyrometer (indicates catalyst temperature); J: Off-on switch for catalyst heater.

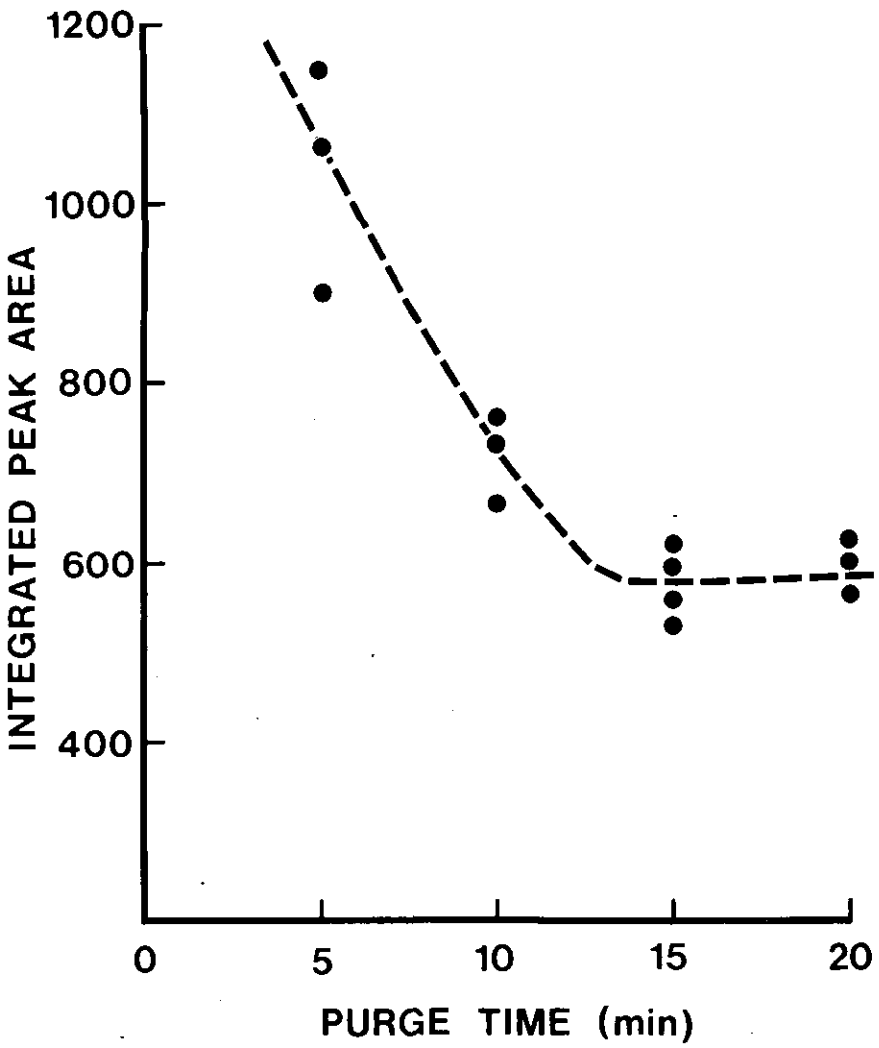

Fig. 8 Integrated peak areas showing the time required to purge inorganic CO_2 from samples.

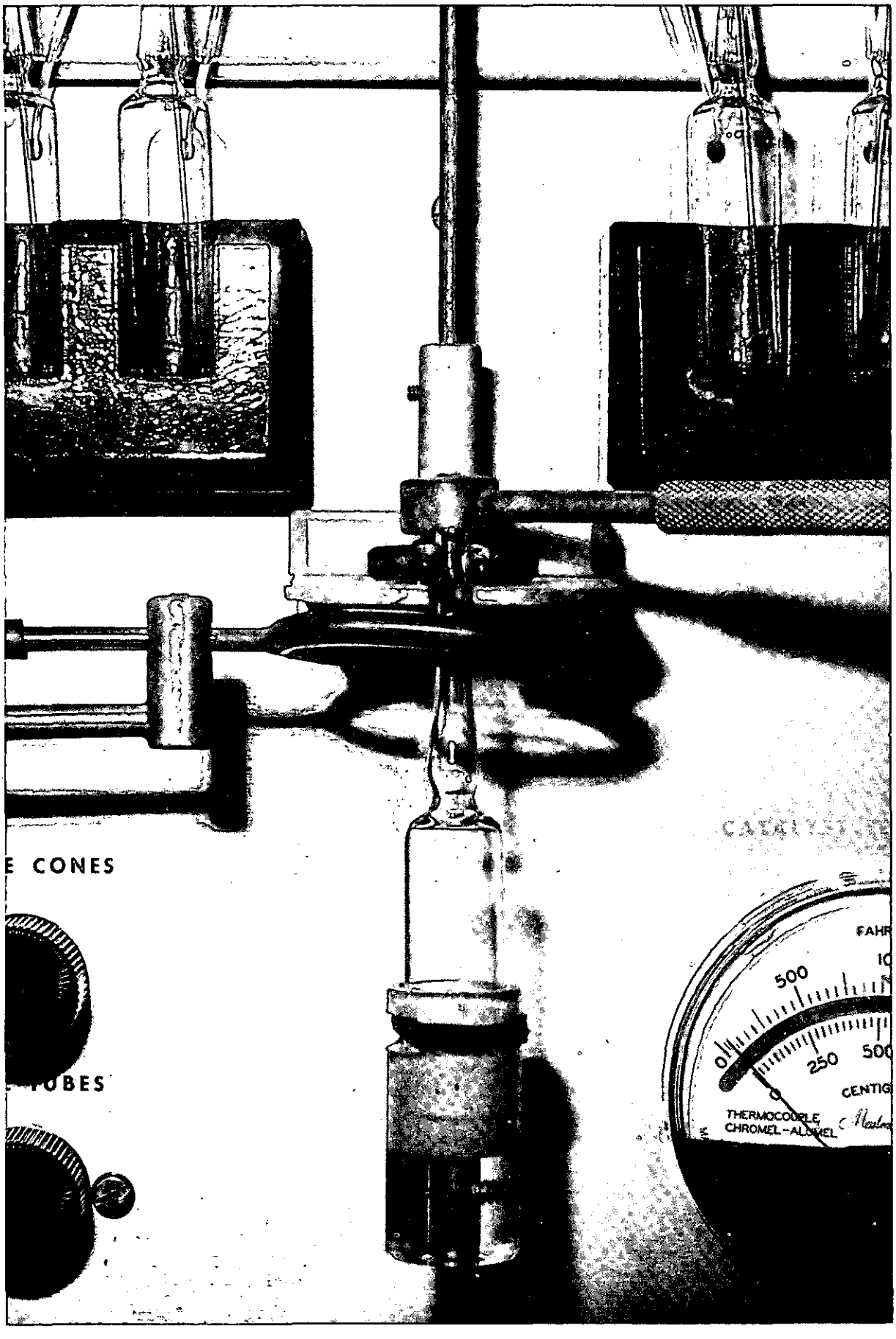

Fig. 9 The ampoule in the clamping assembly.

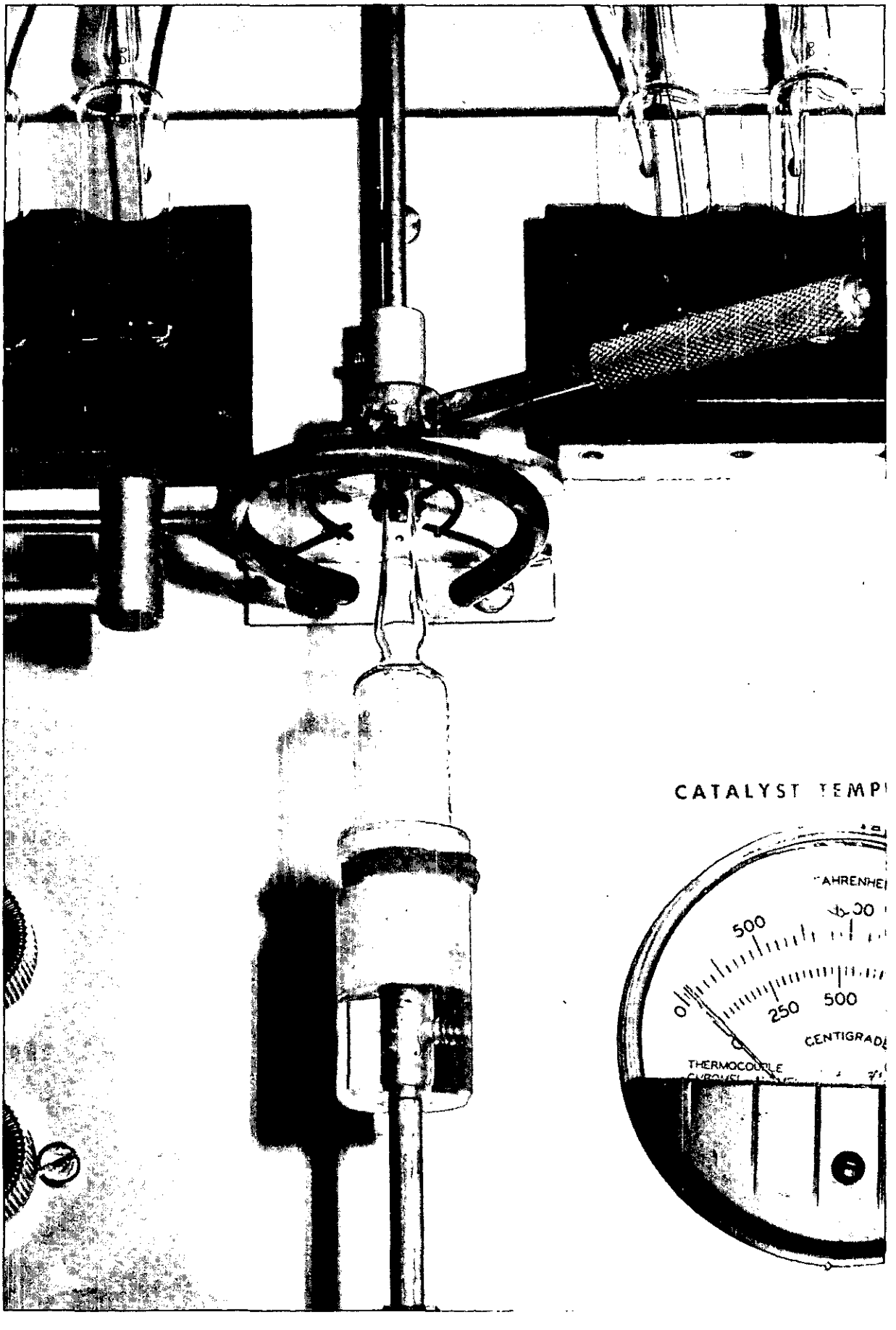

Fig. 10 The ampoule in the sealing position.

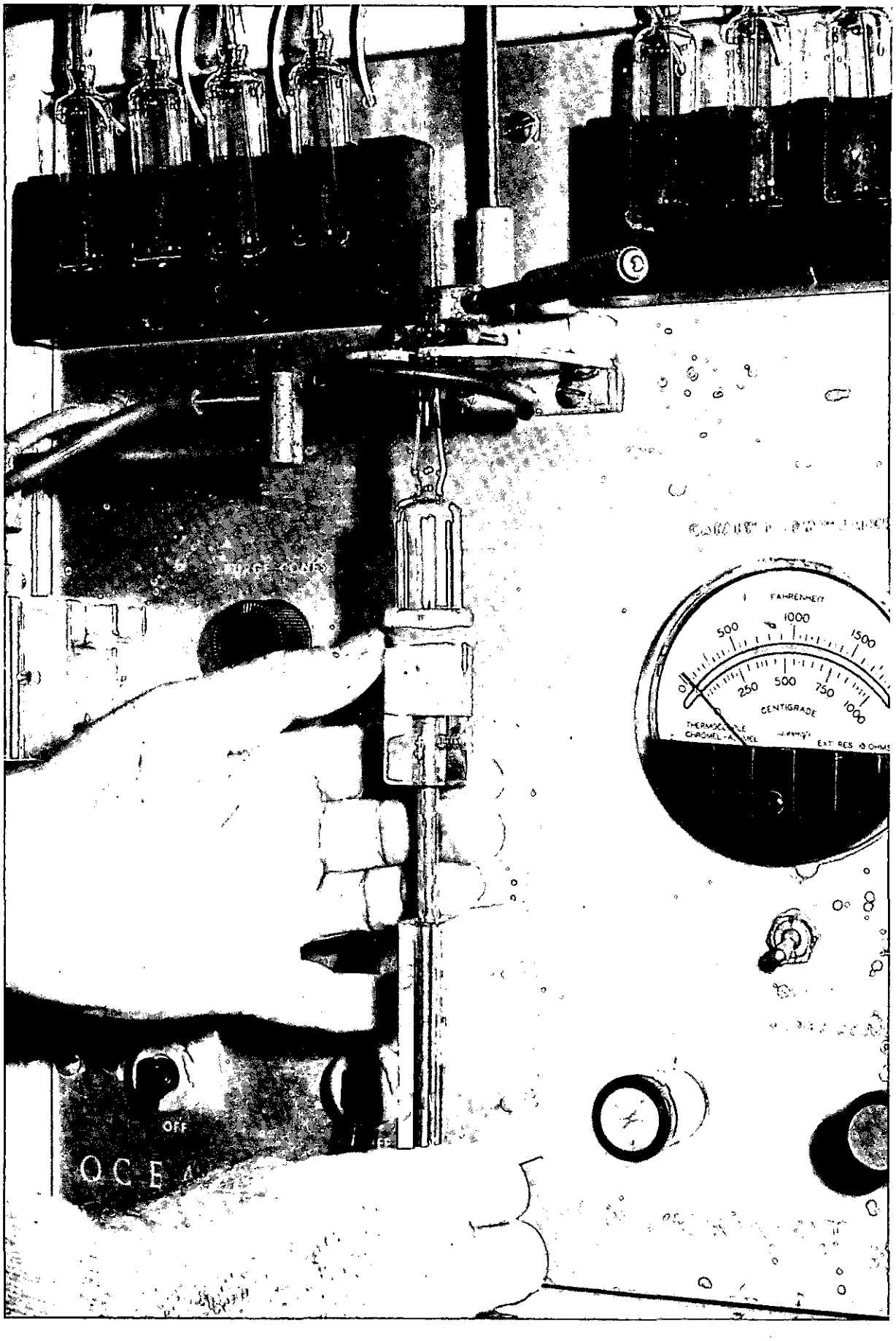

Fig. 11 Position of the hands during sealing operation.

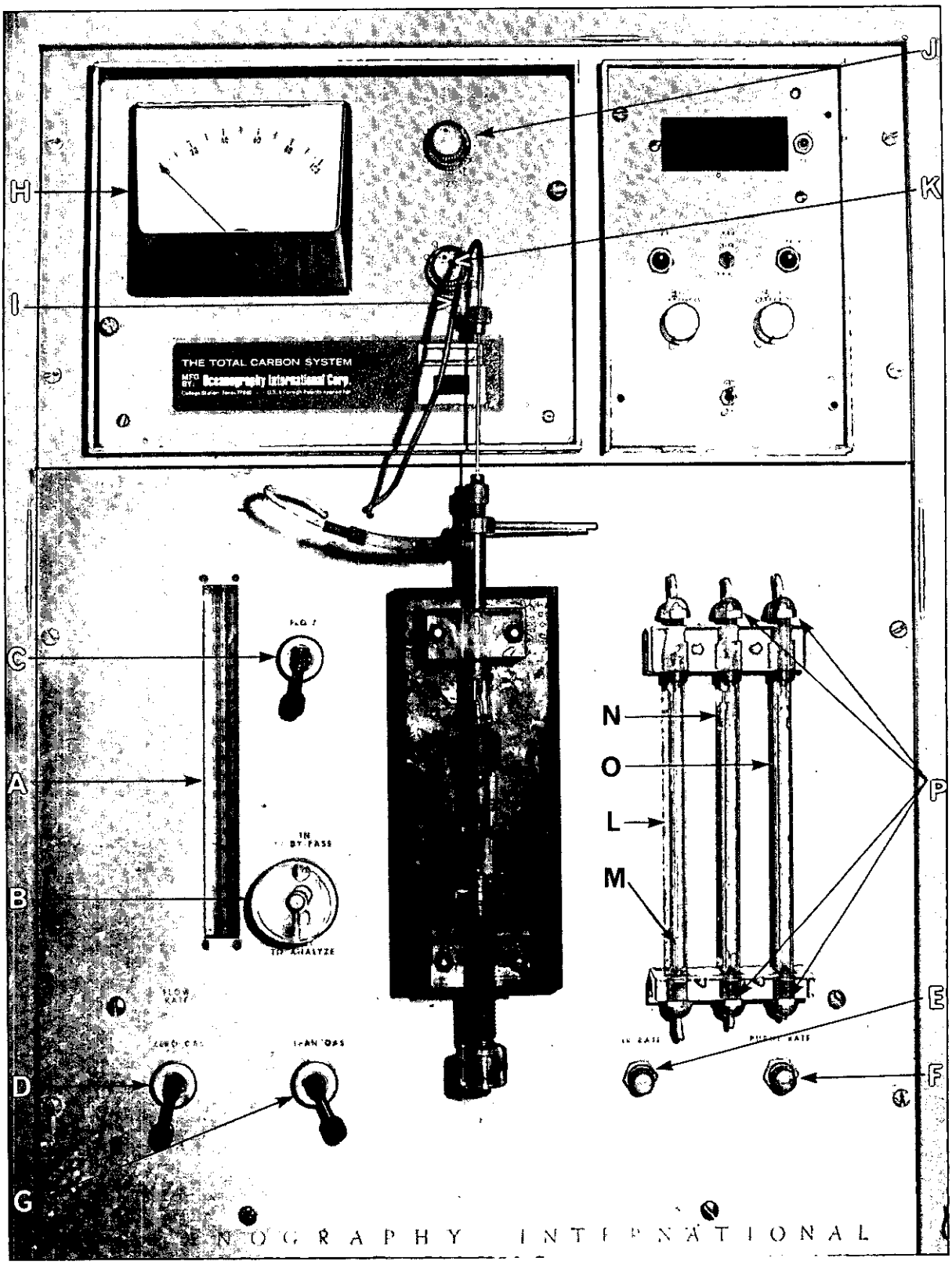

Fig. 12 The ampoule analyser unit. A: Rotometer; B: Two-way valve; C: Flow valve; D: Zero gas valve (on-off); E: IR rate valve; F: Purge rate valve; G: Span gas (on-off); H: IR meter; I: Purging tube shown inserted down into ampoule; J: IR span; K: IR zero; L: Water vapor trap; M: position of inlet plastic tubing; N: Primary drying tube; O: Third drying tube; P: Position of glass wool plugs.

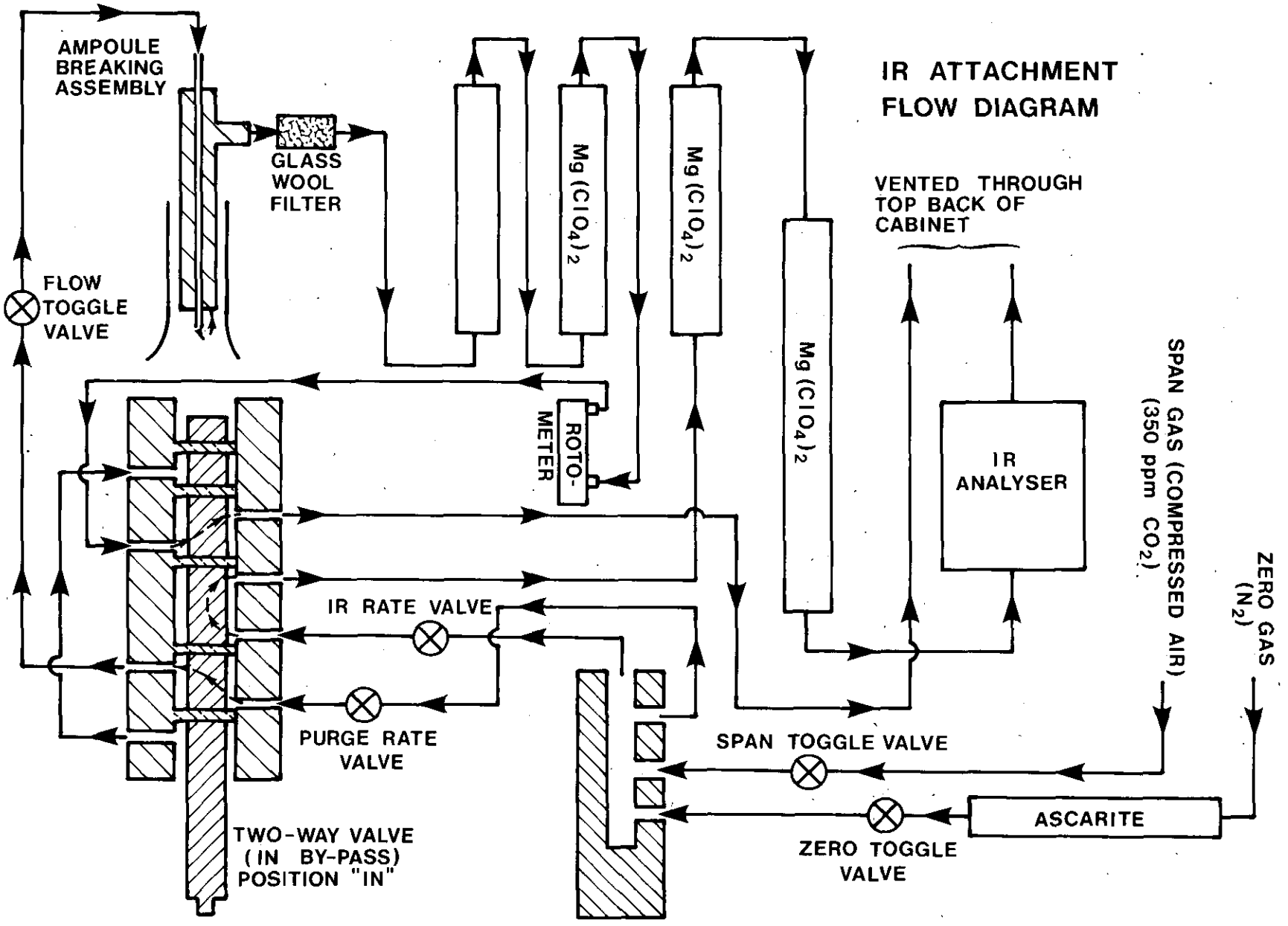

Fig. 13 Gas flow during infrared analysis.

AMPOULE BREAKING ASSEMBLY

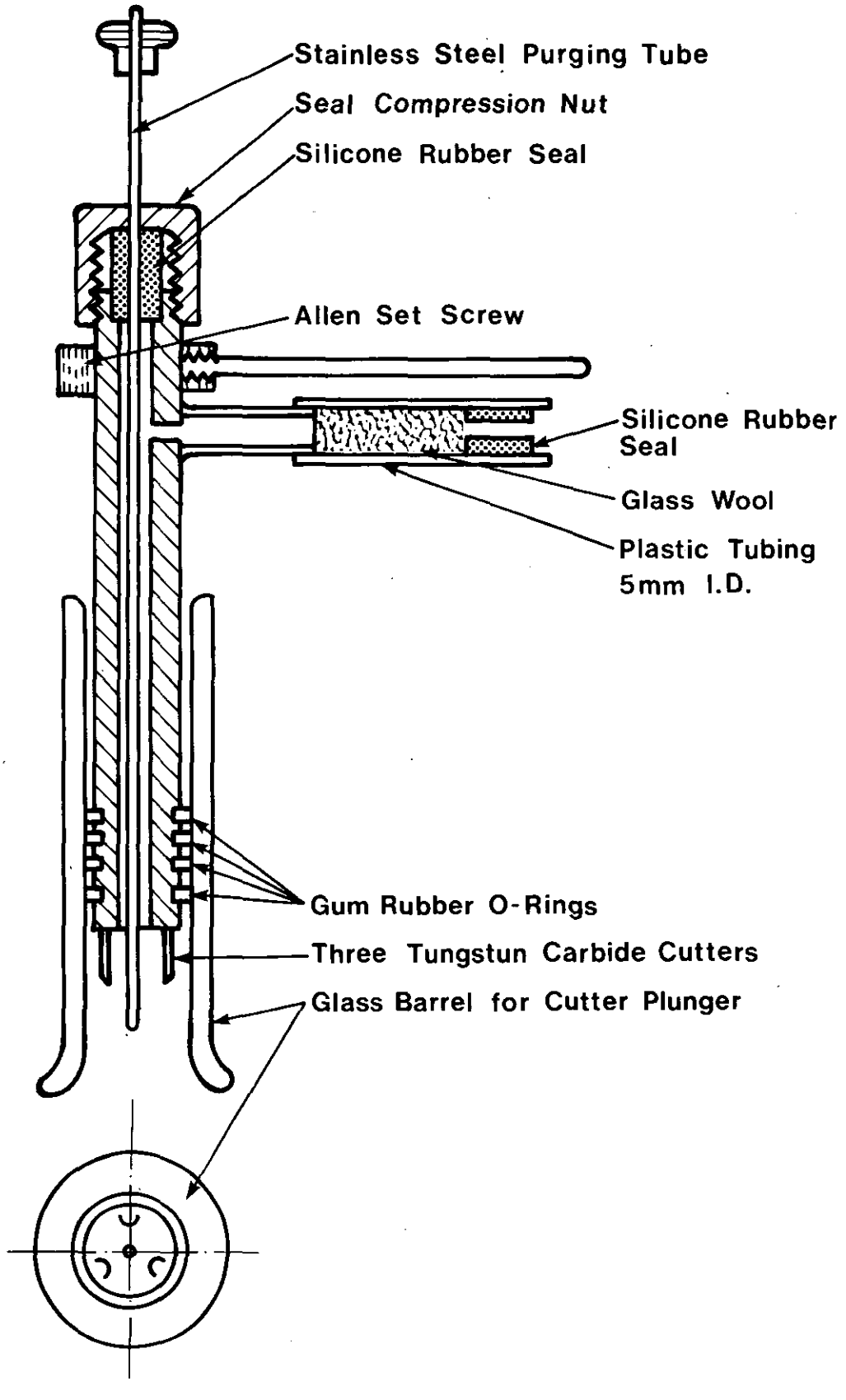

Fig. 14 A cross section of the tungsten carbide ampoule cutter.

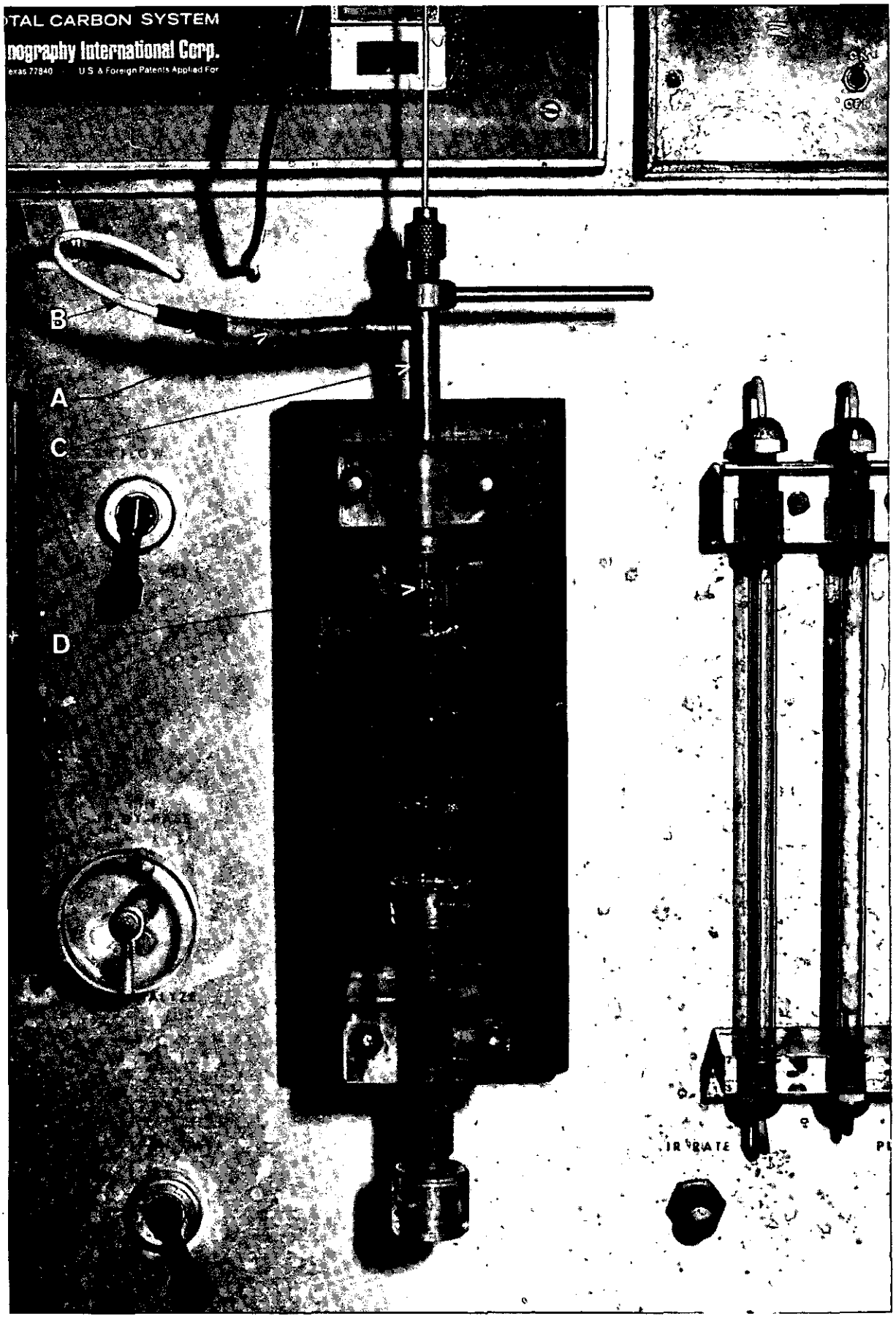

Fig. 15 The ampoule breaking assembly. A: Glass wool filter; B: To water removal tube; C: Ampoule breaking plunger; D: "O" ring seals.

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