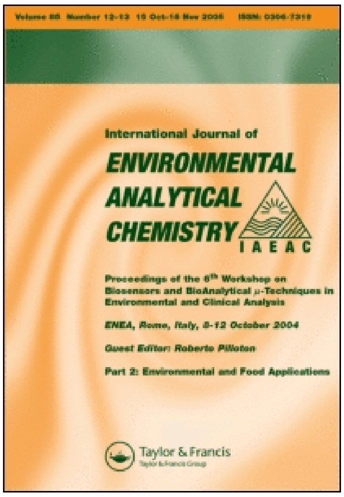

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APPLICATION OF AN AUTOMATED CARBON-NITROGEN ANALYZER FOR ELEMENTAL AND ISOTOPIC ANALYSIS OF SAMPLES RETAINED ON GLASS-FIBER FILTERS

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A method is described for application of an automated carbon-nitrogen elemental analyzer for organic matter collected on glass fiber filters. The method allows a large size glass-fiber filter (47-mm diameter), which is commonly used in aquatic environmental researches, being processed for the automated elemental and isotopic analysis. A hydraulic press was used to destroy the fiber structure of the filter and to form a tablet covered with tin foil with bulk volume of about 0.2 cm^3. The tablet can be introduced in an autosampler that drops a sample into the combustion tube of the analyzer. We also modified the combustion furnace tube by inserting a ceramic inner tube. The ceramic tube protects the quartz combustion tube from invasion by melted glass residue and facilitates post-combustion removal. While the procedure proposed here eliminated much of the operational problems associated with handling of larger glass-fiber filters, we found that analysis of large amount of organic matter (approximately more than 1000 μgC and 200 μg N) by this procedure may result in apparent lower output of carbon and nitrogen signals. Restriction of the amount of organic matter and addition of cobalt oxide as catalytic agent within the tablet is suggested as precautionary measure for the method. Careful examination of combustion yield depending on nature of the sample will also be necessary for actual application of the method.

Keywords: Organic carbon; organic nitrogen; elemental analyzer; isotope mass spectrometry; particulate organic matter; glass-fiber filter

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INTRODUCTION

Commercially available automated elemental analyzers based on Dumas combustion are widely used for determination of carbon and nitrogen content of environmental organic samples. Such analyzers can also be coupled with isotope mass spectrometry which then provides simultaneous determination of carbon and nitrogen content and ^{13}C and ^{15}N isotope enrichment[1–4]. Common design of recent automated analyzers includes an upright-positioned combustion furnace combined with an automatic sampler. The advantage of such design is clearly in simple and reliable operation of the automatic sampler that drops samples into the furnace by gravity. Samples are applied in a tin or an aluminum capsule which yields spontaneous high temperature around the sample in the presence of oxygen gas (flash combustion). Inorganic ash will accumulate in the furnace and require regular removal. Such residual material is of small volume in most organic samples and thus it rarely becomes an operational problem.

In research of the aquatic environment, determination of carbon and nitrogen content for suspended organic particles in water column has been conducted routinely. The ^{13}C and ^{15}N isotope enrichment in suspended particles are also analyzed in biogeochemical studies based on natural abundance variation of these isotopes[3, 4] and in experimental determination of primary productivity and other biogeochemical rate processes following artificial addition of isotope tracers[1, 2]. In these studies, however, suspended organic matter is generally collected on glass-fiber filters. Quartz-fiber, silver-fiber or aluminum oxide porous filters may be used instead, but for the reasons of economy, availability of several different levels of particle retention efficiency, and consistency with previous observations, glass-fiber filters are still of general preference. With the operational temperature for the flash combustion, glass fiber melts and piles up in the furnace tube. The melted glass is quite difficult to remove. It also invades quartz furnace tube, and frequently yields cracking in the tube. Therefore, some modification of combustion system is necessary for analysis of glass-fiber filters. For example, Bechemin et al.[5] reported successful use of an alundam crucible for the purpose protection of the quarts furnace tube in elemental analysis of glass-fiber filter samples.

Glass-fiber filters of both 25- and 47-mm in diameter are commonly used for aquatic particle collection. However, 47-mm filters were difficult to process in the automated elemental analysis systems. The excessive amount of melted glass from the large filters aggravates the damage of quartz tube. Moreover, the large size filter does not fit in the holders of normal automatic sampler. The fabric structure of the filter retains air that may affect nitrogen analysis. Thus published works generally use either a 25-mm filter [e.g. 6] or a small portion of a 47-mm filter[7]. In some research purpose, however, the use of larger size filters is desirable. Filtration can be completed in much shorter time with the large size filter. The filtering time is critical in some experimental determination of metabolic rates with stable isotope tracer. The application of excessive amount of organic particles onto small filters may cause clogging of fiber space. This will further delay the filtering time, as well as enhance retention efficiency of smaller particles and thus affect the analytical reproducibility.

In this study, we developed and examined a conventional procedure for sample preparation with larger size glass fiber filters in an automated analytical system for elemental content of carbon and nitrogen and isotope enrichment of ^{13}C and ^{15}N.

EXPERIMENTAL

An automated Dumas combustion system (Roboprep, Europa Scientific Ltd., UK) installed at the Ocean Research Institute, University of Tokyo was used. The basic design of the system is similar to the Carlo-Erba elemental analyzers; it has an upright combustion (oxidation) column in a furnace followed by a reduced copper column and a gas chromatographic column. The pyrolyzed organic material is then converted to carbon dioxide (CO_2) and nitrogen gas (N_2) by passing through catalytic agents followed by cupric oxide and reduced copper columns with helium carrier gas. The CO_2 and N_2 are separated by gas chromatography and then determined by thermal conductivity or mass spectrometry. Carbon and nitrogen content of the sample were calculated from the integration of chromatographic peak area of CO_2 and N_2. The manufacturer provided a specially designed "large" automatic sampler which has larger sample holders (10 mm in diameter and 10 mm height). The combustion system was coupled with an isotope mass spectrometer (Tracermass, Europa Scientific) and the coupled system was run under the "C-N simultaneous" mode. The duration of oxygen introduction was set to 60 seconds. The carbon and nitrogen content and $^{13}C/^{12}C$ and $^{15}N/^{15}N$ isotope ratio were simultaneously determined by mass spectrometry.

A 47-mm glass-fiber filter (GF/F, Whatman International Ltd., UK) with organic sample was folded with a tin sheet. Either a commercially available square tin foil (37 mm × 37 mm, 0.025 mm thick; Elemental Microanalysis Ltd., UK) or a round tin foil prepared by unfolding a pressed tin sample capsule (20 × 8 mm; either Europa Scientific or Elemental Microanalysis) was used. Care should be taken so that all the outer surface of the folded filter was covered with tin. The folded filter was inserted in the orifice (7-mm diameter) of a custom-made tablet press (Figure 1). The folded filter was then compressed by a hydraulic pressure at about 5 × 10^7 Pa. The compressed filter was in the form of cylindrical tablet coated with tin (7-mm diameter × ca. 5-mm height; approximate volume 0.2 cm^3). The fiber structure of the glass-fiber filter was completely destroyed and was left with practically no air space. The tablet form was stable. These tablets were easily processed with the "large" automatic sampler.

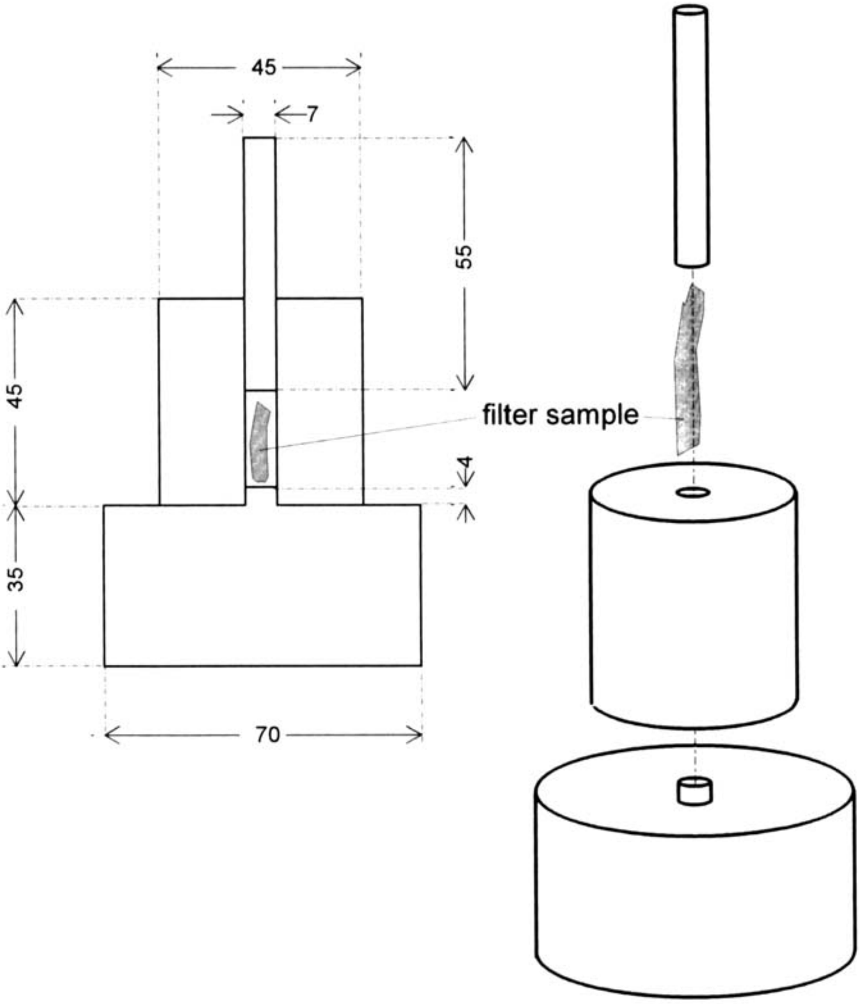

FIGURE 1 A custom made press for tablet formation of 47-mm glass-fiber filters

Test samples were prepared by weighing several reagent standards and lyophilized powder of spinach leaves. The material was placed on the filter surface and then was folded and compressed into the tablet form as described above. The reagent standards used were sodium glutamate, antipyrin, glucose, sulfathiazole, caffeine, cholesterol, acetanilide and m-dinitrobenzene. Sodium glutamate and glucose were of analytical reagent grade obtained from Wako Pure Chemical Industries Ltd., Japan. Other reagents were of organic micro-analytical standard grade obtained from Kishida Chemical Co. Ltd., Japan. When necessary, cobalt oxide (black powder, organic micro-analytical reagent grade, Kishida) was placed on the filter and was compressed together with the reagent.

A standard quarts combustion tube for the Roboprep system (475 mm high, 19 mm outer diameter) was set in the furnace, but the tube packing of catalytic reagents was slightly modified from the original formulae suggested by the manufacturer. The amount of catalytic reagents was reduced in order to make room for a ceramic inner tube. A ceramic tube of outer and inner diameters of 15 mm and 11 mm (Pythagoras Type 610, Haldenwanger, Germany) was used as an inner tube. We cut the tube into 150-mm long and scraped the inner wall of one end of the cut tube. The tapered inner wall prevents the sample tablet from clogging on the tube top. The other end of the tube was plugged with quartz wool. The overall configuration of the oxidation column with the ceramic inner tube was shown in Figure 2. Chromium oxide (granular, high temperature oxidation catalyst) was obtained either from Europa Scientific or Elemental Microanalysis. Copper oxide (wire) and silver (25–30 mesh granule) were of organic elemental analysis grade obtained from Wako Pure Chemical Industries.

RESULTS AND DISCUSSION

Approximately 20 samples on 47-mm glass-fiber filters can be processed in series without replacing the ceramic tube. After analyzing this number of samples, the ceramic tube was still filled with residues to less than half of its height. However, applying more samples sometimes yielded a change of chromatographic peak shape and poor combustion yields. The ceramic tube was replaced after analyzing 20 filter samples and performed excellently in protecting the quarts oxidation tube. Practically no corrosion of quarts by melted glass was found and the average lifetime of a tube has been extended substantially. The oxidation tube can be used for months without replacement, unless the catalytic reagents in the column became inferior. The ceramic tube can be replaced easily

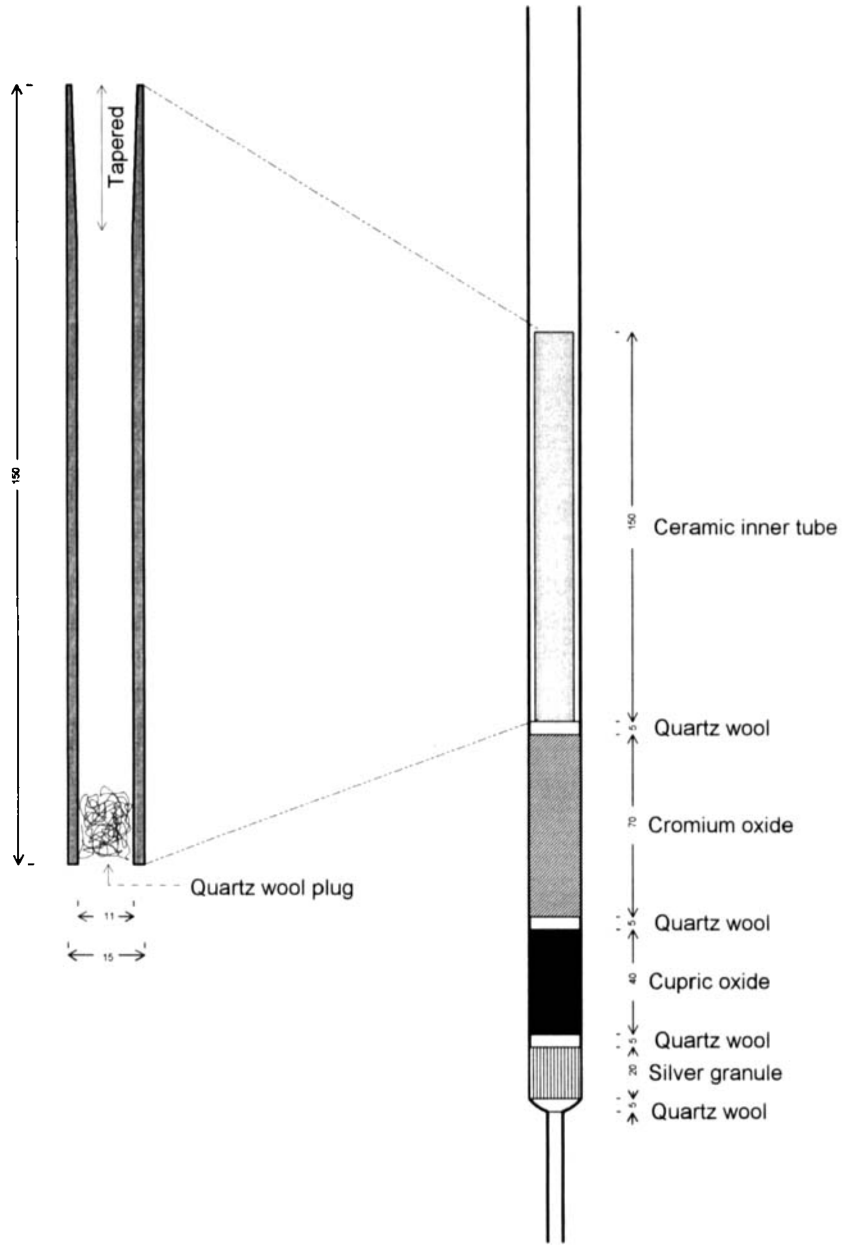

FIGURE 2 Setup of the combustion column with the ceramic inner tube for the Roboprep sample converter system

while the oxidation tube is in place at normal operating temperature of the furnace.

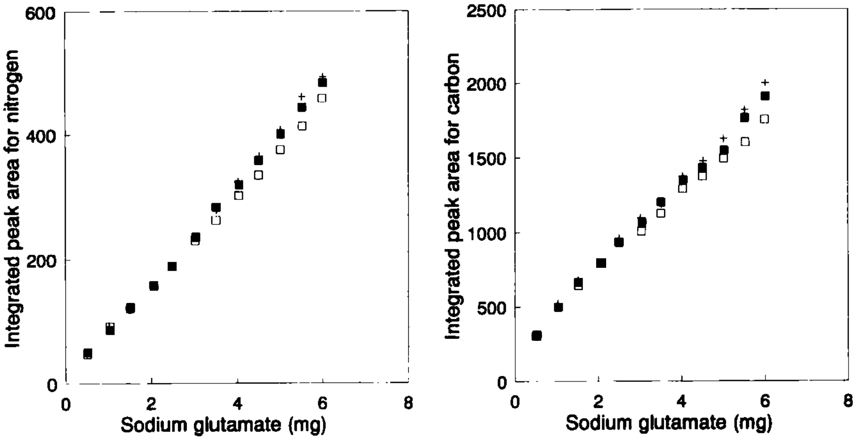

FIGURE 3 Integrated peak area for N_2 and CO_2 of sodium glutamate as a function of weight. Conventional sample preparation with tin capsule (+), tablet formation with 47-mm filter (☐), and tablet with 47-mm filter with 50-mg cobalt oxide (■) is compared

Integrated peak areas of N_2 and CO_2 of the tablet organic samples prepared with 47-mm GF/F filters were compared with those obtained with equivalent amount of organic matter in a conventional tin capsule. Filter tablets gave higher blank values than tin capsules, and the difference was 4–8 μg of nitrogen and 40–70 μg carbon. The blank was subtracted from the data. Figure 3 shows the carbon and nitrogen peak area for sodium glutamate as a function of its weight. The filter tablet samples yield lower peak areas at larger amount of sample for both carbon and nitrogen. For samples lower than 2 mg of sodium glutamate, the difference was small. The addition of 50 mg of cobalt oxide inside the sample tablet gave closer peak areas to those of samples applied in tin capsules. Linear regression analysis showed that the slope obtained for filter tablet samples was significantly different from the slope for tin capsule samples ($p<0.0001$ for both C and N), but the slope obtained for filter tablet with cobalt oxide was not significantly different from that of tin capsule at the level of $p=0.01$ ($p=0.0416$ for C and $p=0.0648$ for N).

The comparison was extended to other reagents. Table I shows the results as percentage of peak area relative to the tin samples. The sample weight was adjusted to give similar amounts of carbon as 4 mg of sodium glutamate. The filter tablet samples yielded significantly lower peak areas for same reagents and/or poor reproducibility. The addition of cobalt oxide was effective for elevating the yield but carbon of sulfathiazole gave a poor yield even with the addition of cobalt oxide.

TABLE I Relative integrated peak area of N_2 and CO_2 obtained by the present method for various organic reagents. The area was shown as percentage of the peak area with equivalent amount of samples loaded within a tin capsule. The ± values indicate standard deviation of triplicate analysis

	Nitrogen		*Carbon*	
	filter only	*with cobalt oxide*	*filter only*	*with cobalt oxide*
Sodium glutamate (4.0 mg)	95.0±3.8	98.0±0.6	96.8±0.8	99.5±0.9
Antipyrin (2.0 mg)	96.9±0.5	97.7±2.0	96.3±1.2	98.6±1.0
Glucose (3.5 mg)	-	-	90.3±7.6	96.2±1.4
Sulfathiazole (3.0 mg)	77.9±2.3	95.8±1.5	59.7±2.7	84.6±8.5
Caffeine (2.6 mg)	98.5±4.3	98.4±4.0	93.6±8.3	94.9±11.6
Cholesterol (1.5 mg)	-	-	96.7±2.8	103.6±11.2
Acetanilide (1.8 mg)	100.3±5.6	101.0±5.8	93.9±3.8	94.7±5.2
m-Dinitrobenzene (3.0 mg)	97.5±3.8	95.4±0.3	86.8±0.5	97.3±8.8
Spinach powder (3.0 mg)	87.0±1.0	93.3±3.2	89.2±7.9	96.6±2.2

The attainment of a lower yield with the filter depends on amount and nature of the sample. The reason for this lower yield may be the incomplete conversion yield of nitrogen and carbon to N_2 and CO_2 by the presence of excessive amount of melted glass fiber. It was also noticeable, however, that the filter samples shows longer retention time and slightly broader chromatographic peak resulting in some biased integrated peak areas.

We tentatively propose to limit organic amount in the tablet samples and to add cobalt oxide as a precaution. While the present method was excellent in reducing the sample volume and in preventing oxidation damage to the column, the possibility of incomplete combustion due to the existence of excessive amount of glass was not precluded for large amount of organic matter. A careful examination of the yield may be necessary depending on nature of the samples.

Acknowledgements

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