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AN IMPROVED MICROWAVE DIGESTION SYSTEM FOR RAPID WET ASHING OF --ETC(U)
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AN IMPROVED MICROWAVE DIGESTION SYSTEM FOR RAPID WET ASHING OF BIOLOGICAL FLUIDS AND TISSUES

September 1977

Progress Report for Period November 1976-May 1977

USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235

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This technical report has been reviewed and is approved for publication.

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An apparatus for the rapid, convenient, and safe wet ashing of most biological fluids and tissues in a microwave oven, used in combination with the acid scrubber and vapor exhaust system, is described. Exogenous contamination is minimal during the procedure and no special hood is required. The operator exposure to the hazards of noxious acid products of digestion is almost eliminated. The system described is suitable for microscale digestions by simply scaling down sample and reagent volumes. Most digestions are successfully completed in 10 to 20 min as compared to over 3 hr required for conventional ashing procedures.

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AN IMPROVED MICROWAVE DIGESTION SYSTEM FOR RAPID WET ASHING OF BIOLOGICAL FLUIDS AND TISSUES

INTRODUCTION

Most analytical methods for the determination of trace elements in biological specimens require separation of the analytes from their organic matrix. The destruction of biological matter is usually achieved by wet ashing with various acid mixtures or by dry ashing in a furnace at relatively high temperatures. This is the most unreliable step in the analysis for trace elements from the point of view of the introduction of exogenous contaminants. Problems frequently associated with various methods for the destruction of biological matrix prior to trace analyte determination have been discussed by many authors and covered excellently by Thiers (6) and Gorsuch (2, 3).

In spite of some obvious disadvantages, wet-ashing methods are most frequently chosen by a large majority of analysts undertaking trace- and ultratrace-element determinations. In wet ashing, the sample is subjected at low temperatures to the oxidizing action of liquid reagents, thus reducing the risks of negative contamination; however, losses can and do occur. (Perchloric acid digestion of biological samples for the determination of chromium is a well-recognized example of random analyte losses in the form of chromyl chloride even at temperatures as low as 250°C.) More serious disadvantages inherent in wet ashing include close and constant supervision by the analyst, the need for special fume hoods to handle perchloric acid, the dangers of explosions if established safety procedures are not strictly adhered to, and long elapsed times, particularly in case of short-life isotopes. All of these factors can be a severe limitation to the use of wet-ashing procedures.

Most of these problems are eliminated by wet ashing of biological samples in an adapted commercial microwave oven, as was proposed by Abu-Samra et al. (1). This report describes the modifications and improvements that we have made which permit convenient, rapid, and relatively hazard-free digestion of serum, urine, NBS 1577 Bovine Liver, and NBS 1571 Orchard Leaves Standard Reference Materials (SRM's), brewer's yeast, and molasses specimens.

MATERIALS AND METHODS

Apparatus

We used a 600-watt Litton Model 70-50 Menumaster microwave oven, cavity size approximately 24x35x39 cm, which is similar to many other brands for snack bar and cafeteria use. A Plexiglas box was fabricated

to fit compactly in the oven to protect the inner walls of the oven cavity from the extremely corrosive hot acid fumes. Large metal objects are not permissible inside a microwave oven, but small hinges and screws needed to fasten the door of the inner box have not caused any problems.

A 1-inch (2.54 cm)-diameter hole was cut in the left sidewall of the oven and the inner box to accommodate a Teflon exhaust vent. This outlet was connected through two offset right-angle elbows to an acid scrubber and an evacuation system. The two offset elbows were included in the exhaust line to effectively eliminate any chances of microwave radiation leakage. The digester system was checked with a Holaday Model 1500 electromagnetic leakage monitor and the system was free of detectable radiation leakage.

The Plexiglas cabinet used to protect the inner oven cavity from corrosive acid fumes became warped and developed blisters along the hinged end of the door of the inner cabinet. The damage apparently resulted from the high molecular water content of Plexiglas and it was evident that a more effective protective material would have to be used. We considered borosilicate glass (Pyrex) as an alternative but decided against its use on account of the relative difficulty of fabrication and its easy shattering characteristic. Polypropylene, which has a much denser molecular structure, with relatively low molecular water composition was finally chosen for fabrication of the inner cavity and has proven eminently satisfactory in routine use for over 6 months. The 20x30x36-cm polypropylene box was placed inside the oven cavity and connected to the acid scrubber-exhaust unit via a 1-inch (2.54 cm)-diameter hole drilled in the left side wall of the box. This exhaust port was fitted with a 1-inch (2.54 cm) O.D. polypropylene tubing which passed through the corresponding hole in the side of the microwave oven and was connected to the inlet port of the acid scrubber unit by means of a tight-fitting Teflon sleeve. A drain port with a stopcock, for the removal of any condensed acid that might accumulate in this connector, was provided in the tube connecting the oven to the scrubber.

Acid Scrubber Unit

The corrosive acid fumes generated during the digestion of biological materials must be efficiently removed from the oven to prevent rapid corrosion of the oven cavity. The spray fume scrubber described by Abu-Samra et al. (1) was only partially effective in removing the bulk of acid fumes and washing them into the drain. However, the scrubber severely limited the capacity of the blower to evacuate from the oven cavity when the sprayer was in concurrent operation. Even when the digestion system was located in a class I fume hood, considerable noxious fumes were retained in the polypropylene box and the operator is exposed to forced inhalation of these corrosive fumes.

We have designed an efficient mist-spray scrubber unit which is shown in Figure 1 and the scrubber-venting assembly is shown in Figur 2. The use of this scrubber has resulted in considerable attenuation of the operator exposure to acid vapor with a concomitant minimization of corrosion of the blower impellers. Some attack by residual acid fumes on the metal components of the fan housing was still visible. The installation of a more powerful blower fan for the rapid and efficient removal of the corrosive digestion fumes necessitated further modifications in the scrubber design. These alterations caused an upward deviation of the fumes to the top of the scrubber chamber where a fume diffusor directed them downward and caused a more even distribution of the corrosive fumes along the vertical axis of the scrubber. More efficient contact between the spray and the fumes resulted, and removal of acid in the fluid washing down into the drain was improved. A spray baffle was introduced about two-thirds down the length of the scrubber chamber to effectively eliminate the suction of the residual fine mist directly into the fan housing. Additionally, it was found desirable to provide a mist eliminator, consisting of a fine mesh polypropylene screen roll placed inside

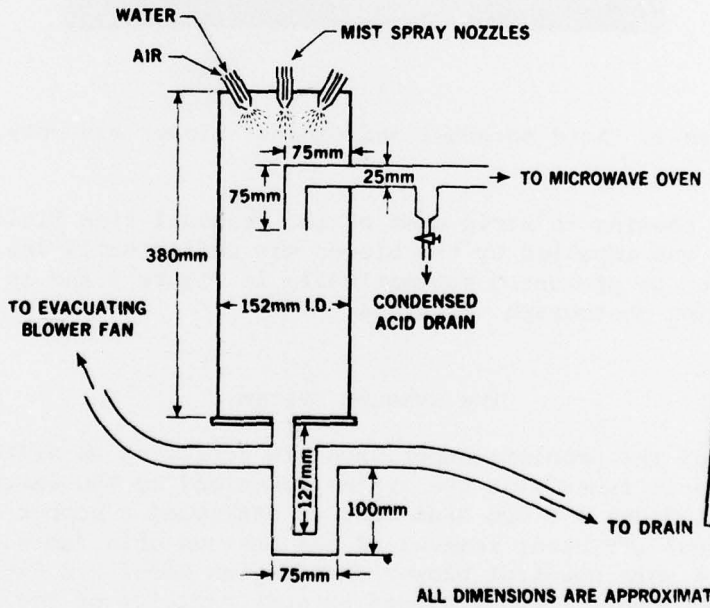

Figure 1. Schematic of the early acid scrubber.

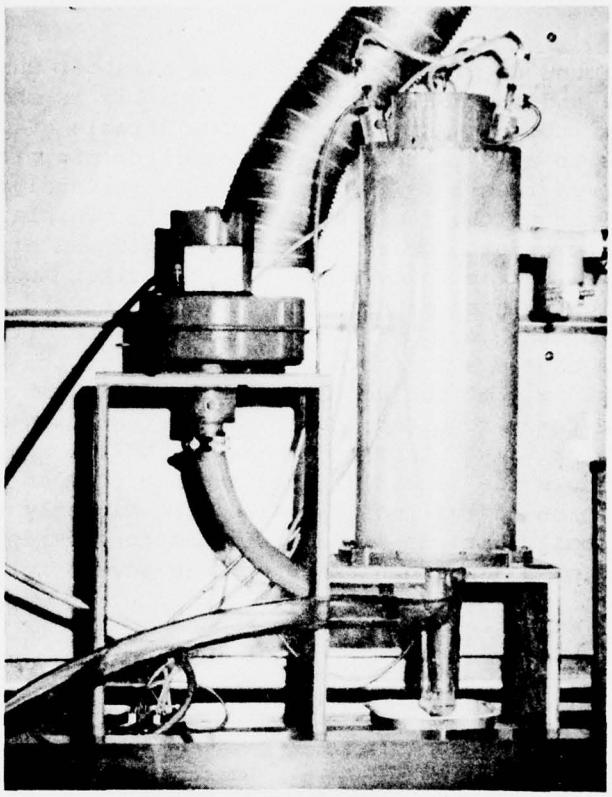

Figure 2. Acid scrubber and exhaust blower assembly.

a cylindrical housing to strip most of the residual fine fluid droplets. The resulting gas impelled by the blower was sufficiently dry. The improved scrubber is presented schematically in Figure 3 and is shown in the accompanying photograph, Figure 4.

The Exhaust System

In view of the problems experienced in achieving an efficient evacuation of the acid fumes with the system described by Abu-Samra et al. (1), using a Dayton Model 4 C 006 Drum Fan, we attempted a number of alternatives to achieve efficient removal of the noxious acid fumes. The initial approach was a more powerful blower fan (Dayton Model 2 C 610 drum fam with a 1/20 HP motor). The improved exhaust capacity of the system was, however, not significant. Since the presence of each bend or angle in an exhaust line results in an attenuation of the rate of vapor removal,

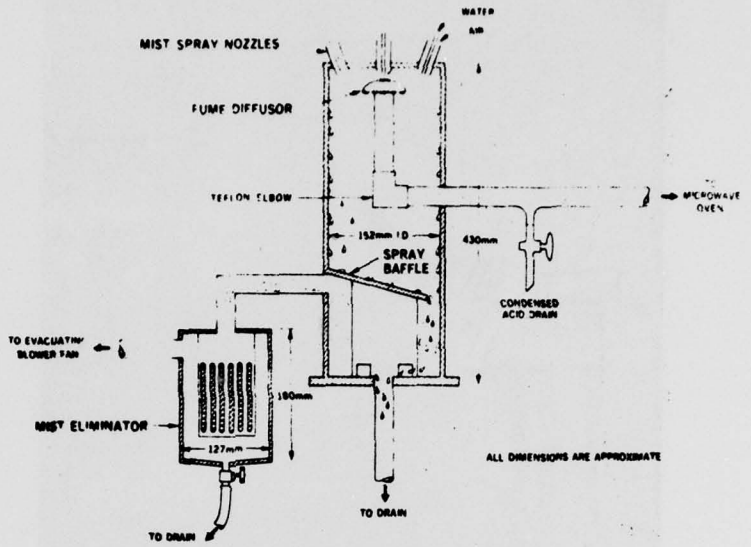

Figure 3. Schematic of the improved acid scrubber with a fume diffuser and mist eliminator.

we decided to take out all unnecessary angles in the venting system. This resulted in a significant improvement and there was no noticeable increase of the deleterious radiation leakage. However, the overall efficiency of vapor evacuation from the digestion chamber was still not entirely satisfactory. To achieve further improvement, we installed a Blo-Hard blower fan (Heat Systems-Ultrasonics, Inc., Long Island, N.Y.) with a rated capacity of 210 CFM at 6-inch (15.24 cm) static pressure of H_2O. The fan is of an all-welded rigid (poly)vinyl chloride (PVC) construction, with PVC-coated fiberglass tape rotor blades and has proven adequate, with only minimal traces of noxious fumes reaching the operator. We are in the process of acquiring a "Mystaire" (Heat Systems-Ultrasonics) scrubber unit which incorporates a waterweb mesh and a mist eliminator. This addition to the microwave digestion system would further improve the efficiency and considerably enhance useful life of the scrubber blower assembly. The complete digestion system in its final operational form is shown in Figure 5.

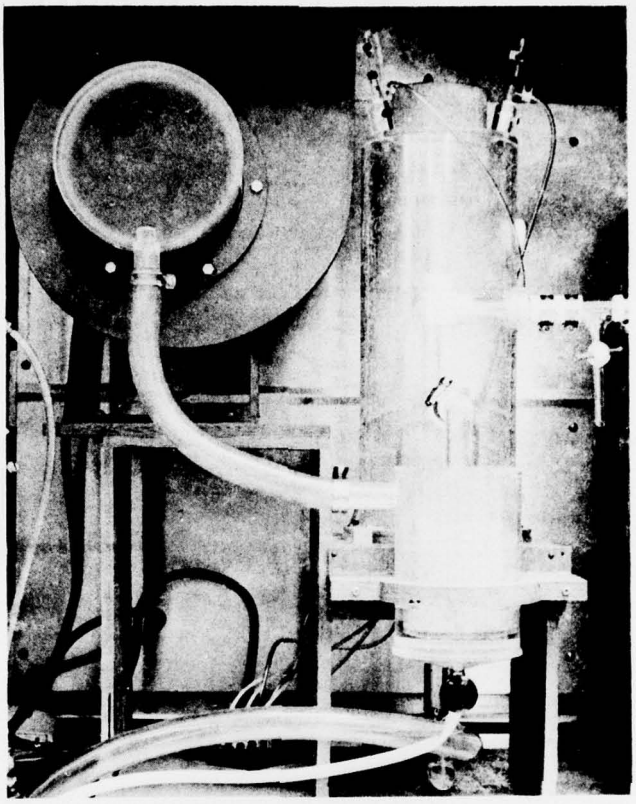

Figure 4. Improved acid scrubber-mist eliminator-vapor venting assembly.

Procedure

In our experience the nature and size of the sample has normally ranged from a few milligrams to 1 g of solids and 0.5 to 5.0 ml of fluids. The size of the digestion vessel, volume of the acids added, and the time usually required for ashing vary from sample to sample, but none of these factors has been found to be critical. Typically, 5 ml of a serum or urine samples are placed in a 50-ml Erlenmeyer flask and 5 ml of concentrated HNO_3 are added to each flask. After allowing any initial reaction to subside, the flasks are placed inside the oven and the contents allowed to simmer down to $\leq$ 1 ml. To each vessel 2 ml of 30 vol.% H_2O_2 are added and the contents are heated to incipient dryness. Progress of the digestion can be followed visually. The contents of the digestion flasks are then allowed to evaporate to complete dryness, cooled to the ambient temperature, and diluted to a predetermined volume with 0.5 N HNO_3.

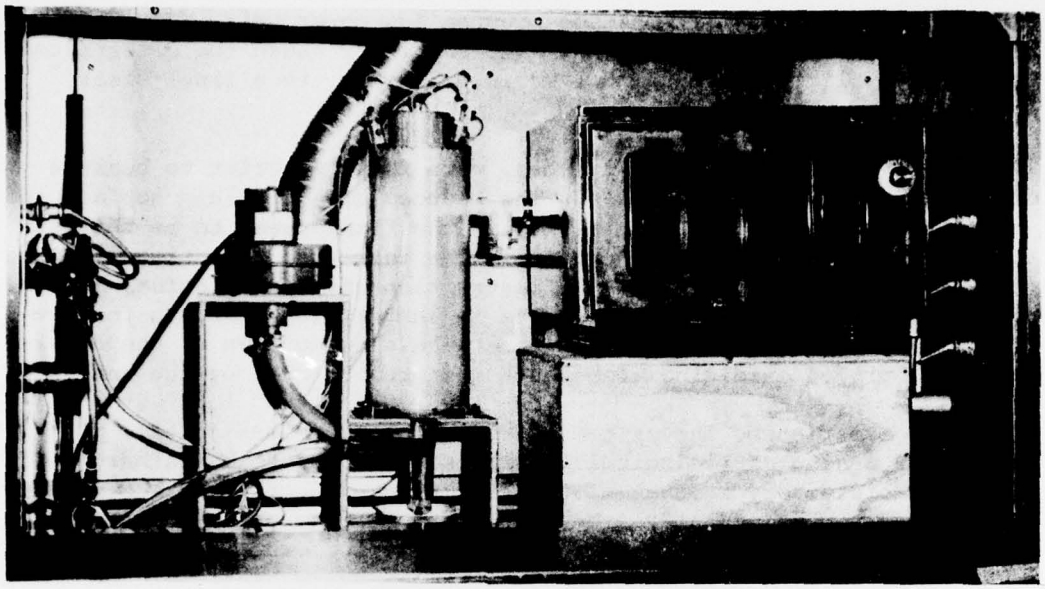

Figure 5. Improved microwave digestion system.

The resulting solutions are warmed on a hot plate and allowed to reflux gently for about 30 sec. This step insures that any residue that may have sputtered upon the upper levels of the digestion vessel is brought completely into solution.

The manufacturer of the microwave oven recommends that the units should never be operated empty or near empty for any extended periods of time. This recommendation is meant to avoid any risk of damage to the magnetron. As a standard operating procedure, a beaker containing 100 ml of distilled water is placed inside the cavity of the oven whenever the unit is used for digestion. This precaution is not necessary when the volume of acid is not small; it is necessary in our procedure since we carry the digests to total dryness in almost all cases.

RESULTS AND DISCUSSION

Wet ashing of samples in a microwave oven is a rapid, safe, convenient and nontedious procedure. Different sample sizes, as low as 0.1 g or 0.5 ml, can be easily brought into homogeneous or nearly homogeneous solutions in a few minutes and completely ashed with nitric acid-hydrogen

peroxide or with fuming perchloric acid in less than 10 min. The time required for digestion of complete ashing depends on the loading factor of the oven, particularly with the present low power unit. However, the time factor is not critical and we have routinely taken the digests to virtual dry solids, easily dissolved in 0.5 N HNO_3 to a final clear solution.

Erlenmeyer flasks, and even tubes, were found superior to beakers for ashing of biological samples. The reduced area of fluid surface and a markedly increased refluxing action in the flasks seem to be the main factors for this superior performance. The increased refluxing provides a better oxidizing ambient for the destruction of the biological matrix. However, this refluxing action results in longer elapsed times for complete digestion. An optimal balance between slowing down of the digestion and improved oxidizing atmosphere must be achieved by the analyst.

In our experience, the system described by Abu-Samra et al. (1) for the wet ashing of biological materials was found to be rather inefficient in satisfactory removal of the corrosive products of digestion and was a hazard to the operator. We also encountered problems with buckling of the Plexiglas box used to line the oven cavity. The improved design of the scrubber-venting system has reduced the exposure hazard of the operator to a tolerable minimum, and the use of polypropylene for the box to protect the inner cavity of the microwave oven has eliminated the twin problems of buckling and blistering experienced with Plexiglas.

It is possible to use smaller volumes of samples and the acid mixtures ($HClO_4$-HNO_3 or HNO_3-H_2O_2) for digestion. Microsampling can be done by simply scaling down the volumes of reagents in proportion to the sample size. For routine digestions, 10 ml of HNO_3 and 5 ml of H_2O_2 for a 5-ml sample of serum, blood, or urine provided a reasonable margin of safety without unduly prolonging the ashing time. Whenever we used 30 vol.% H_2O_2 alone, a flash usually resulted in the digestion vessel. Care must be exercised, therefore, in always adding peroxide to digestion samples with 1-2 ml of HNO_3 still present in the flasks. In digesting bovine liver and some other tissues, the final solutions obtained generally showed a residual yellow to deep brownish color. However, the solutions were homogeneous and sufficiently free of organic matter for analysis by flameless atomic absorption spectroscopy. We have not encountered frothing, bumping, or foaming for any of the samples ashed in the microwave digestion system, which has been in successful use for over 6 months now.

Studies using this procedure have consistently shown recoveries of 95%-105%. We present data for the analysis of NBS Standard Reference Materials, Orchard Leaves-SRM 1571 and Bovine Liver-SRM 1577, following wet digestion in the microwave digester, for chromium, copper, zinc, and cadmium. There is good agreement in the values for copper and cadmium with the NBS-certified values. The determined values for zinc show a systematic bias, attributable to positive contamination during the

digestion of the samples. There are no certified or suggested values for chromium in bovine liver (SRM 1577). The published values for chromium for this material range from 5-1500 ng/g (5). Using neutron activation analysis, McClendon (4), from the National Bureau of Standards, reports values of 163 and 210 ng/g, respectively, for chromium depending upon whether the samples were prepared by nondestructive or destructive methods. Our results are in fair agreement with the values for chromium in SRM 1577 bovine liver with those of McClendon using the nondestructive preparation of samples. Chromium levels we found in SRM 1571 (orchard leaves) are in good agreement with the suggested NBS values. Table 1 shows comparison of data obtained by us and the certified or suggested values for these four analytes.

TABLE 1. RESULTS OF ANALYSIS OF NBS 1577 BOVINE LIVER AND 1571 ORCHARD LEAVES BY FLAMELESS ATOMIC ABSORPTION AFTER WET ASHING IN THE MICROWAVE OVEN

	avg. (oven-FAAS)[a]	NBS value
	1577 Bovine Liver (µg/g)	
Cr	0.214 ± 0.04[b]	(.210)[c]
Cd	0.27 ± 0.03	0.27 ± 0.04
Cu	191.0 ± 4.8	193.0 ± 10.0
Zn	138.0 ± 3.1	130.0 ± 10.0
	1571 Orchard Leaves (µg/g)	
Cr	2.40 ± 0.2	(2.3)[d]
Cd	0.105 ± 0.01	0.11 ± 0.02
Cu	11.45 ± 0.23	12.0 ± 1.0
Zn	26.6 ± 2.3	25.0 ± 3.0

[a] Average of 3 replicate determinations each on 3-5 different days.

[b] ±1 standard deviation (day-to-day variability).

[c] Published INAA (nondestructive).

[d] Uncertified.

CONCLUSIONS

The major advantages in the use of a microwave oven for wet ashing are essentially the speed of operation and the resultant saving in time. Most digestions, which normally require 3 hr or longer with the traditional procedures, can be successfully completed in 10 to 20 min with the least amount of attention by the analyst. Additional advantages of the method are:

a) The system is safe; the samples are completely enclosed if an accident should occur; b) there is no need for a special hood for perchloric acid digestion; c) heating is uniform and damage from localized heating and carbonization is eliminated; d) bumping and frothing are almost completely eliminated; e) the risk of airborne contamination can be considerably reduced by using filtered air; and f) no special glassware is needed, although thorough decontamination of all glassware used is mandatory.

We have successfully digested many different types of biological specimens in the system described in this report, which has been in routine daily use in our laboratory for over 6 months and has proven quite effective for rapid clean digestion of biological fluids and tissues.

The microwave digestion system described in this technical report is a superior apparatus for wet ashing of a wide variety of biological specimens. We have made improvements over the preliminary design reported by other workers which have essentially eliminated exposure of the analyst to noxious acid vapors. Ashing of biological fluids and tissues can be accomplished safely, rapidly, and conveniently in less than 30 min (as compared to over 3 hr or more usually required in conventional procedures) with minimal exposure to hazard of either explosions or inhalation of dangerous acid digestion products.

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