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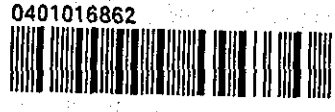

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CHEMICAL STUDIES OF THE DEGREE OF DECOMPOSITION AND DISSOLUTION IN MICROWAVE DIGESTS

by

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A Doctoral Thesis

Submitted in partial fulfilment of the requirements
for the award of

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Abstract

Most methods for the determination of trace elements in organic matter require the sample to be brought into solution, which may be achieved by heating with strong oxidising agents such as concentrated mineral acids, to decompose the organic matrix sufficiently to render the sample soluble, whilst not necessarily achieving complete decomposition. The use of microwave heating for this purpose, using sealed vessels under pressure, was investigated for a variety of food samples.

One drawback of this otherwise rapid method is the time taken to cool the vessels before opening. The use of liquid nitrogen to cool the vessel walls - before, during and after the heating cycle - was investigated, and its effect on the progress of the digestion was assessed. Another problem is the control of internal pressure, which can rise very rapidly when digesting samples of high organic content, and ways of controlling excess pressure when digesting larger samples were devised. These included the use of an open pre-digestion under reflux using a microwave-transparent coolant liquid, and the use of vessels capable of withstanding higher pressures. The latter proved more convenient as they enabled complete dissolution to be achieved in a single stage without pre-digestion, although cooling of these vessels was much slower than for the low pressure vessels, so overall sample preparation time was similar.

Various methods were developed for the complete dissolution of the samples using nitric acid and hydrogen peroxide. The degree of decomposition achieved for the different methods and sample types was monitored by measurement of residual carbon in the digests. A few samples were virtually completely decomposed with nitric acid alone, while the use of hydrogen peroxide, following a nitric acid digestion, was found to reduce residual carbon substantially in the others. The undecomposed material was analysed by various methods including ultraviolet/visible spectrometry, infrared spectrometry and thin layer chromatography. Decomposition products were found to include carboxylic acids, oxalates, phosphates, nitrates and nitro-aromatics.

Complete decomposition of milk powder, which proved most resistant to oxidation, was achieved by further treatment with perchloric acid. This was found necessary for anodic stripping voltammetric analysis, which required the destruction of interfering organic species for accurate determination of trace elements, unlike other techniques such as inductively coupled plasma- mass spectrometry, where complete decomposition was not required.

Publications and Presentations

Refereed Papers

Liquid Nitrogen Cooling in Microwave Digestion, Reid, H. J., Greenfield, S., and Edmonds, T. E., *Analyst*, 1993, **118**, 443.

Reflux Pre-Digestion in Microwave Sample Preparation, Reid, H. J., Greenfield, S., Edmonds, T. E., and Kapdi, R. M., *Analyst*, 1993, **118**, 1299.

Paper in Preparation

Investigation of Decomposition Products of Microwave Digestion of Food Samples, Reid, H. J., Greenfield, S., and Edmonds, T. E.

Presentations

Microwave Digestion of Food and Other Samples
Presented as a poster at the R & D Topics Meeting of the Royal Society of Chemistry (Analytical Division) at the University of Birmingham, 7 - 8 July 1992.

Microwave Digestion of Difficult Samples
Presented as a poster at the SAC '92 Conference at the University of Reading, 21 - 25 Sept 1992.

Reflux Pre-Digestion in Microwave Sample Preparation
Presented as a poster at the R & D Topics Meeting of the Royal Society of Chemistry (Analytical Division) at the University of Bradford, 13 - 14 July 1993.

Microwave Digestion: A Clear solution?
Presented as a lecture at the R & D Topics Meeting of the Royal Society of Chemistry (Analytical Division) at the University of Hertfordshire, 18 July 1994.

CHAPTER 1
THEORETICAL ASPECTS OF MICROWAVE DIGESTION & EQUIPMENT DESIGN

1.1 Introduction

The origin of the microwave oven can be traced back to the development of radar during the Second World War, and the chance discovery of the ability of microwaves to heat food very rapidly, when a chocolate bar was accidentally placed beside a radar vacuum tube at the Raytheon Company, a US manufacturer of radar equipment.[1] The idea was patented in 1945 by Percy LeBaron Spencer, a scientist at Raytheon, and the first commercial microwave unit, a cumbersome and expensive machine with a power output of 1600 W, was produced by them in 1947.[2] Further commercial appliances for cooking food appeared in the USA in the 1950's. However, it was not until the 1970's and 1980's that the domestic use of microwave ovens became widespread, with the advent of cheaper, globally marketed, Japanese-produced appliances.

The use of microwave energy as a heat source in sample dissolution for trace elemental analysis was first reported in 1975.[3] It has proved much quicker, less labour-intensive and potentially safer than conventional procedures, which involve prolonged heating of the sample in concentrated mineral acids, either in open vessels on hot plates, or in sealed vessels such as Teflon-lined steel bombs or Carius tubes in conventional ovens.[4,5] The development of microwave methods for the decomposition of a wide variety of sample matrices is discussed in Chapter 2.

Central to the successful development of safe, efficient microwave digestion procedures is a basic understanding of the theory of microwave heating and how it relates to the acid digestion mixtures and vessels being irradiated. This chapter will cover the important principles involved, and describe the design of microwave equipment to meet the heating requirements for acid dissolution.[6]

1.2 Theory of Microwave Heating

1.2.1 Microwave Radiation

Microwaves are electromagnetic energy with frequencies in the range 300 to 300,000 MHz (corresponding to wavelengths between 1 mm and 1 m) i.e. lying between infrared and radio frequencies in the electromagnetic spectrum. In order not to interfere with radar and telecommunications uses, domestic and industrial

microwave ovens must operate at one of the following frequencies: 915 ± 25, 2450 ± 13, 5800 ± 75 or 22,125 ± 125 MHz. The last two are of no use for microwave heating due to insufficient penetration of the sample (see Section 1.2.3). 915 MHz has a good penetration depth (about 4 inches for water), but as it corresponds to a wavelength (λ) of 32.5 cm, the size of cavity required would be impractical for domestic or laboratory use, so 2450 MHz (λ = 12.2cm) is most commonly used, and all home microwave units operate at this frequency.

Microwave energy is a non-ionizing radiation which causes molecular motion, but does not cause changes in molecular structure. Electromagnetic energy is converted to heat energy in a liquid or solid containing ions or polar molecules by two mechanisms: ionic conduction and dipole rotation. These will be dealt with separately below (see Sections 1.2.4 and 1.2.5). The microwave heating effect depends on frequency as well as power applied, and also on the nature of the sample. Variables associated with the sample are temperature and frequency dependent, and dictate the speed and homogeneity of the heating effect.

1.2.2 Dielectric Loss

Two parameters define the dielectric properties of materials. These are the dielectric constant, ε', which is a measure of the ability of the molecules to be polarized by the electric field, and the dielectric loss, ε'', which measures the sample's ability to dissipate the electromagnetic energy as heat. Both vary with frequency and temperature. The ratio of the dielectric loss to the dielectric constant is the dissipation factor of the sample, $\tan \delta = \varepsilon''/\varepsilon'$, which defines the ability of a material to convert electromagnetic energy into heat energy at a given frequency and temperature. Tables giving dissipation factors for various materials at 3000 MHz and 25°C are available.[7]

1.2.3 Penetration Depth

When microwave energy penetrates a sample, the energy is absorbed by the sample at a rate dependent upon its dissipation factor ($\tan \delta$). Penetration is infinite in materials that are transparent to microwave energy ($\tan \delta \approx 0$), and effectively zero in reflective materials, such as metals. Tan δ is a finite amount for absorptive samples; the greater the dissipation factor, the less the penetration of the microwave energy (i.e. penetration depth is inversely proportional to dissipation factor). Penetration can be characterised by the half-power depth for a given sample at a given frequency, which is that distance from the surface of a sample at which the power density is reduced to half that at the surface. For example, the half-power depth for water is about 1 inch for 2450 MHz at 25°C.

In large samples with high dissipation factors, microwave heating will be relatively superficial, and though heating will occur beyond the penetration depth due to thermal conductance, temperatures at or near the surface will be higher. Thus, the relatively small sample sizes used in most analytical dissolutions are advantageous. However, one disadvantage is that a considerable amount of microwave energy is unabsorbed (reflected), and steps must be taken to protect the magnetron from damage due to such reflected energy. (See Section 1.4.2 (v)).

1.2.4 Dipole Rotation

Dipole rotation (or dipole polarization) refers to the alignment, due to the electric field, of molecules in the sample that have permanent or induced dipole moments. As the electric field increases, the molecules rotate into alignment, and as the field decreases, disorder is restored and thermal energy released, in the dielectric relaxation time. This process occurs 4.9×10^9 times per second at 2450 MHz, and results in very rapid heating.[6] This heating effect is very dependent on the frequency of the electromagnetic radiation: at low frequencies, the time taken by the electric field to change direction is longer than the response time of the dipoles, and the dielectric polarization keeps pace with the electric field. Thus transfer of energy to random motion is very slight and the temperature hardly rises. If the dielectric field oscillates very rapidly (i.e. at high frequencies), it changes direction faster than the response time of the dipoles, and thus the dipoles do not rotate and there is no heating. At microwave frequencies, the time in which the field changes is comparable with the response time of the dipoles. They rotate, but the resulting polarization lags behind the changes in the electric field. The lag means that the sample absorbs energy and is heated.[8]

The efficiency of heating by dipole rotation depends on the sample's dielectric relaxation time which depends in turn on temperature, and the viscosity of the sample.

(i) Effect of dielectric relaxation time:

The maximum energy conversion per cycle due to dipole rotation is given by $\omega = 1/\tau$ where ω is the angular frequency of the microwave energy in radians per second ($\omega = 2\pi f$; f = microwave frequency) and τ is the dielectric relaxation time of the sample, defined as the time taken for the molecules in a sample to achieve 63% of their return to disorder. It is important to appreciate that the optimal frequency for maximum heating rate would result in very low penetration depths and inefficient heating of the sample interior. A frequency of 2450 MHz, whilst not producing maximum heating rates, nevertheless produces rapid heating due to a suitable balance between dielectric loss and penetration depth.

For water, $1/\tau$ is greater than ω for 2450 MHz at 25°C. As the sample is heated, $1/\tau$ increases as the dielectric relaxation time decreases, and therefore the difference between ω and $1/\tau$ becomes greater, resulting in a decrease in the dissipation factor and therefore an increase in the penetration depth with increased temperature.

(ii) Effect of Viscosity:

Increased viscosity at lower temperatures restricts molecular mobility and results in a lowering of the dissipation factor, as the molecules are less able to align with the microwave field. For example, ice has a very low dissipation factor (and thus absorbs microwaves very poorly). Raising the temperature above freezing point decreases the viscosity and causes the dissipation factor to increase. Thus the viscosity has a much greater impact on the dissipation factor at low temperatures than the dielectric relaxation time, which would tend to lower the dissipation factor as the temperature rose. However, at higher temperatures, the water becomes more fluid and the dielectric relaxation time is the dominant factor, causing a six-fold decrease in the dissipation factor between 5°C and 95°C and a consequent increase in penetration depth.

1.2.5 Ionic Conduction

Ionic conduction is the conductive migration of dissolved ions in the applied electromagnetic field. This induced current results in heat production due to resistance to current flow, so-called I^2R losses. Factors affecting ionic conduction are size, charge, concentration, mobility and conductivity of the ions in solution, their interaction with the solvent and the temperature of the solution; many of these factors are interrelated. An increase in ion concentration increases the dissipation factor (i.e. increases absorption of the microwaves), which is also affected by the temperature of the solution, because temperature affects ion mobility and concentration.

The effects of different concentrations of various ion species have recently been studied.[9] The addition of sodium chloride was found to increase the heating rate of water more than sodium hydroxide and much more than sulphuric acid.

1.2.6 Relative Contributions of Dipole Rotation and Ionic Conduction

For a given sample, the relative contributions of the two heating mechanisms are largely determined by temperature. For small molecules, such as water and other solvents, the dielectric loss due to dipole rotation decreases as the sample temperature increases, as explained above. In contrast, heating due to ionic conduction increases with sample temperature, and therefore as an ionic sample

is subjected to microwave energy, heating is initially dominated by dipole rotation effects. As the temperature increases, ionic conduction becomes the dominant factor.

The relative contribution of the two mechanisms will depend on the mobility and concentration of the sample ions. If these are low (e.g. deionized water at low temperature), sample heating will be entirely dominated by dipole rotation. Therefore, the heating time will depend on the difference between the reciprocal of the relaxation time of the sample ($1/\tau$) and the input microwave angular frequency (ω). The larger the difference, the slower the heating rate. However, for a sample of high ionic content, such as concentrated mineral acids or solutions with high salt content, microwave heating will be dominated by ionic conduction, particularly as ion mobility increases with increased temperature, and heating rates will be independent of the relaxation time of the solution.

1.3 Effects of Microwave Heating

1.3.1 Comparison of Microwave and Conventional Heating

Using microwaves instead of conventional heating typically cuts the time required for an open-vessel acid dissolution from hours down to minutes. This reduction is due to several factors; because vessels used in conventional heating are usually poor conductors of heat, it takes time to heat the vessel and transfer the heat to the solution; also, excess energy is dissipated through convection currents and vaporization at the liquid surface. By contrast, because microwave heating is so rapid and direct, without heating the vessel, substantial localized superheating can occur. Microwave heating is also said to effect better contact between acids and sample, due to the intense internal heating mechanically agitating and rupturing sample surface layers, thereby exposing new surfaces to the attacking acid.[10,11] Another factor is the acid volume, which is usually small, allowing complete penetration of the sample by the microwaves, thus avoiding problems due to superficial heating of large volumes.

1.3.2 Superheating Effects at Atmospheric Pressure

It has been shown that microwave irradiation of polar liquids results in superheating above their normal boiling points at atmospheric pressure.[8,12] In the case of some organic solvents, these effects can be dramatic, and involve general superheating of the solvent and not just localized effects. For example, ethanol can be maintained at an equilibrium temperature of 20°C above its normal boiling point for many hours.[13]

It has been suggested that such superheating occurs because the vessel walls are relatively cool (only being heated by conduction from the vessel contents and constantly cooled by convective air flow), which means there is insufficient energy available to break down the surface tension at the vessel walls and allow boiling, until the liquid has been heated to well above its normal boiling point.[14] However, this theory does not explain why a liquid with a high surface tension such as water only superheats to ca. 5°C above its boiling point, whereas organic solvents with much lower surface tension superheat much more (typically 13 - 26°C above their conventional boiling points).

An explanation for this phenomenon has recently been offered by Baghurst and Mingos.[13] They propose a model adapted from conventional boiling theory. Conventional nucleate boiling relies on the existence of cavities, pits and scratches, with dimensions of the order of microns, on the inside vessel surface, in which bubbles can form. When the temperature of the surrounding liquid is at least equal to the saturation temperature corresponding to the pressure in a tiny vapour bubble (a "vapour embryo"), then bubble growth occurs. The energy for evaporation at the bubble-liquid interface is supplied by a superheated liquid layer surrounding the bubble, and boiling occurs when the forces holding the bubble in place are overcome and the bubble is released from the cavity.

In conventional heating, thermal energy is supplied to the vessel walls, which in turn heat the liquid in the vessel. Boiling occurs from the surface of the vessel at suitable nucleation sites where superheated layers exist. The inversion of the temperature profile during microwave heating accounts for the high equilibrium temperatures (nucleation limited boiling points, NLBPs) reached. Because the outer walls of the flask are continuously cooled, the layer of solvent immediately adjacent to the walls may be somewhat below the temperature of the bulk liquid. Only when the bulk temperature rises to the NLBP does the liquid layer adjacent to the vessel walls reach the saturation temperature of the vapour embryo sites.

This model can be used to explain the difference in behaviour between water and organic solvents. The number of vapour embryos trapped in the surface of the vessel is determined in part by the ability of the solvent to wet the surface. Organic solvents generally wet surfaces well and diminish the vapour trapping capabilities, thus reducing the number of nucleation sites, which means a high NLBP is required before boiling can occur. By contrast, water wets surfaces poorly and there will be large numbers of active sites available. Baghurst and Mingos tested this hypothesis by the addition of detergents, and elevation of NLBPs of up to 18°C were observed, while reducing the wetting properties of the

glass surface by silylation reduced the NLBP of water to 102°C.

It is apparent from the above study that the extent of superheating depends on the condition of the vessel surface (in this case glass) and the wetting properties of the solvent. Teflon has poor wetting properties which may well discourage superheating, as would surface scratches on older vessels, though newer vessels with smooth surfaces would encourage it. It also seems likely that suspended particles in the acid digestion mixture could provide sites for boiling to occur and discourage superheating effects.

1.3.3 Superheating Effects at Elevated Pressures

For reactions in closed vessels, the increased pressure results in an elevation of the boiling point above its conventional temperature. However, the temperature reached cannot be predicted from partial pressure data accumulated under equilibrium conditions, as the gas phase is not in thermal equilibrium with the liquid phase.[15] This is because the loss of heat from the vessel reduces the temperature of the volatilized solvent to below that of the liquid phase. Because the gas phase is cooled by the vessel walls and absorbs little heat from the microwave field, condensation occurs at the top of the container, thus reducing the internal pressure. The non-equilibrium conditions in the microwave vessel make it difficult to directly relate pressure to temperature. However, it can be concluded that much higher liquid temperatures are reached for a given pressure than with conventional heating.

1.3.4 Interaction of Mineral Acids with Microwave Radiation

The apparent power absorbed by a sample subjected to microwave radiation is given by

$$P_{abs} = KC_p m\Delta T/t \qquad (1)$$

where P_{abs} = apparent power absorbed by sample in watts (1 W = 1 $J.s^{-1}$)
K = conversion factor from calories s^{-1} to watts (4.184 $J.cal^{-1}$)
C_p = heat capacity ($cal.g^{-1}.deg\ C^{-1}$): 0.9997 for water, 0.5728 for nitric acid (70.4%) at 25°C
m = mass of sample in grams
ΔT = final temperature – initial temperature (deg. C)
t = time in seconds

For 1 litre of water heated at full power for 2 min from an initial temperature of 20°C , the equation simplifies to:

$$P_{abs} = 35 \times \Delta T$$

This relationship can be used to measure the power output of a microwave oven, as the volume of water is large enough to absorb all the microwave energy.

Equation (1) can be used to calculate the power uptake of any quantity of material for which the heat capacity is known and for which the temperature rise can be measured.[15] Having calculated the power absorbed, rearrangement of equation (1) for time enables the heating times required to reach particular temperatures for a given volume of acid to be predicted. However, there are potential sources of error, namely the changes in heat capacity and dielectric behaviour with increasing temperature, and the heat loss during microwave absorption, which are not addressed in equation (1). This means that the predicted values only hold true for relatively large volumes of pure acids (>200 g), where heat losses are minimal and temperature increases are small, thus minimizing changes in heat capacity and dielectric behaviour. The addition of sample matrices alters the dielectric behaviour, as well as potentially producing heat from exothermic reactions, making accurate predictions of temperature (and pressure in closed systems) very difficult for "real" digestion mixtures.

The effects of acid type and volume on microwave absorption have been studied.[16] Quantities smaller than 500 g absorbed energy proportional to their total mass. Very small quantities, such as 5 ml (7.2 g) of nitric acid, absorbed as little as 40 W of the available 574 W, though such volumes got hotter than larger volumes for the same power setting and exposure time, as there was less mass to heat. Above 500 g, nearly all the available power was consumed. On a mass basis, the acids tested had absorption efficiencies similar to, but slightly lower than, water. Nitric acid was found to be nearly as efficient an absorber of power as water, followed by hydrofluoric, sulphuric and hydrochloric acids. The heat capacity of each acid increases as the concentration decreases and approaches that of pure water. Because of this property, the authors say, power absorption increases as the acid becomes more dilute. However, factors other than heat capacity are involved in comparing heating rates of equal volumes of dilute and concentrated acids, such as density changes and differences in dielectric behaviour due to large variations in ionic strength, viscosity etc.

A linear relationship exists between applied power and absorbed power, so predictions can be made about microwave absorption at partial power settings if the absorption at full power is known. For instance, if a sample absorbs 400 W at a full power setting of 600 W, 200 W will be absorbed if the power is reduced to 50%. (It is *not* true to say, however, that 200 ml of acid will absorb twice as much power as 100 ml).

1.4 Equipment Design

1.4.1 Basic Microwave Instrumentation

Domestic microwave ovens are often used for acid digestions, but as these appliances were designed to heat relatively large quantities of food, they are not ideally suited to analytical work, and often require modification. They can suffer corrosion damage from acid fumes, they often need to be operated inside a fume hood, turntables may be absent or slow, the cycling of the magnetron is slow (see below), and, perhaps most importantly, the magnetron can be damaged by reflected microwaves unabsorbed by small analytical samples (necessitating the placing of containers of water in the oven to absorb excess power). That said, domestic microwaves are now so cheap that they may be regarded in some laboratories as disposable items to be replaced at regular intervals, and provided they work for the required digestions and suitable safety precautions are taken,[17] they can be useful laboratory tools.

1.4.2 Commercial Oven Systems

To overcome the problems associated with the use of domestic appliances for analytical work, various modifications were incorporated into a purpose-built oven for microwave acid digestion introduced by the CEM Corporation (Matthews, NC, USA) in 1985. Various updated models have been produced since, but all operate on the same principles.

The basic components of a commercial microwave oven for acid digestion (shown schematically in Fig. 1) are:

i) the magnetron

Microwave energy is produced by the magnetron, the microwave field radiating from an antenna enclosed in the vacuum envelope of a tube. The output frequency is tuned to 2450 MHz, and 600 - 700 W of microwave power is delivered from an electrical input of 1200 -1400 W. The remaining energy is converted to heat which must be dissipated by air cooling. The power output is controlled by "cycling" (i.e. switching on and off to obtain an average power level). The time base of the cycle in analytical instruments is 1 second, compared with 10 seconds for a typical domestic oven. This means that at a 50% power setting, the magnetron would be switched on and off every 0.5 s, compared to 5 s on/5 s off for a domestic oven. The rapid switching is important to avoid significant heat losses from small analytical samples during the long off time.[6]

ii) the microwave cavity

This is of similar dimensions to a domestic oven, with fluoropolymer (Teflon)

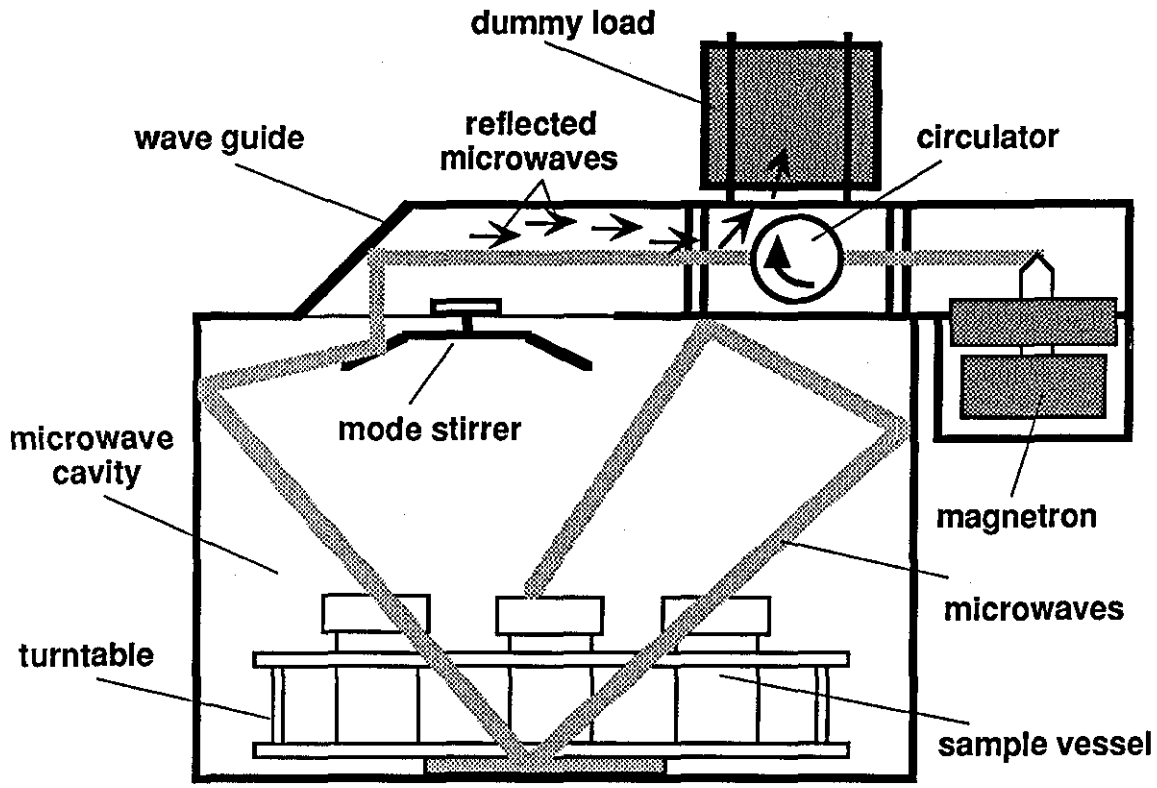

Fig. 1 Schematic Diagram showing operation of oven

coated walls to protect against corrosion of the underlying metal. Microwaves entering the cavity are repeatedly reflected from wall to wall, losing energy by interaction with absorptive samples in their path. The pathways of the microwaves form criss-cross patterns (or modes) so that microwave exposure is dependent on position in the cavity; a sample may be located at a position of reinforcement of the criss-crossing waves or at a position of partial cancellation, which leads to non-uniform heating if a turntable is not used (see (vii)).

iii) the wave guide

The wave guide is made of a reflective material, such as metal, and directs microwaves emitted by the magnetron into the microwave cavity.

iv) the mode stirrer

This is a rotating fan-shaped blade, located inside the roof of the oven, which reflects and mixes the energy entering the microwave cavity from the wave guide, to distribute the incoming energy so that heating is more independent of position.

v) the terminal circulator

This device is incorporated into commercial analytical microwave systems (but not in domestic ovens) to protect the magnetron from damage due to reflected microwaves which can cause overheating and failure of the magnetron. It is

located in the path of the microwaves emitted by the magnetron. It uses ferrites and static magnetic fields to allow microwaves to pass in the forward direction but diverts the reflected waves into a dummy load where the energy is dissipated as heat. This enables the smallest of samples to be safely heated, and the oven may even be operated empty. It avoids the use of power-balancing water loads necessary when using domestic ovens, which are wasteful of power, as well as being inconvenient.

vi) the exhaust fan

An exhaust fan is used to effect air cooling and to draw acid fumes out of the cavity and into a fume hood via a corrosion resistant hose. In domestic ovens, the fan is usually single-speed and operates automatically when the oven is on, whilst in analytical ovens, it is adjustable to allow optimisation of cooling and acid removal.

In addition, modern analytical systems usually incorporate the following:

vii) the turntable

A reversing turntable (i.e. turns 360° clockwise, then 360° anticlockwise, to avoid tangling of monitoring tubes) holds up to 12 sample vessels and enables more uniform heating (i.e. to counteract inhomogeneity of the microwave field). It is usually set to rotate at a faster speed than a domestic oven turntable (6 - 10 rpm instead of 3 rpm).

viii) pressure/temperature monitoring facilities

A transfer port at the rear of the oven, incorporating two small holes leading into the cavity via a metal wavelength attenuator cutoff (to prevent microwave leakage), allows monitoring of pressure and/or temperature. Pressure is monitored via a length of Teflon tubing, and temperature via a fibreoptic temperature probe or shielded thermocouple wire, linking a sample vessel to an external measurement device. The latest models have feedback loops which shut off the microwave power during a pressure digestion when a selected pressure is reached, then restart when the pressure has dropped to a lower level.

1.4.3 Vessel Designs

Microwave oven systems as described above are suitable for both open and closed vessel digestion, using a variety of vessels. The main requirements are that the vessel is made of a material which is microwave transparent (or absorbs microwaves only weakly), is a poor conductor of heat (to provide good insulation), has good chemical resistance to acids and good thermal stability. Reference to published tan δ values for a variety of possible materials[7] enables a suitable vessel material to be selected. The lower the tan δ value (dissipation

factor), the more microwave transparent the material is. Quartz and borosilicate glass both have low tan δ values (although Pyrex is higher), as do many polymers (except nylon). Table 1 shows some tan δ values for various materials at 25°C and 3000 MHz.

Teflon PFA (polytetrafluoroethylene, PTFE, with perfluoroalkoxy sidechain) is an appropriate vessel material for microwave acid digestion, as it is essentially microwave transparent, is resistant to all acids, has a melting point of just over 300°C and is an extremely poor conductor of heat. The only digestions for which it is unsuitable are those using phosphoric or sulphuric acid, as these have boiling points above the melting point of Teflon PFA (although it is possible to use sulphuric acid as a minor component of the digestion mixture). This limitation has recently been overcome by the use of vessels made of Teflon-TFM (tetra fluorometoxil),[18-20] which reportedly has an operating temperature of up to 350°C.

While Teflon vessels are ideal for microwave use, all other vessels normally used for open-vessel, conventional digestions, except metal vessels, can be used for open-vessel microwave digestions, e.g. glass beakers, boiling flasks etc. Generally, those offering a good degree of refluxing (e.g. long-necked vessels such as Kjeldahl flasks) are preferable, to avoid high evaporation losses.

Table 1. Dissipation Factors of Different Materials

Material	tan δ (x 10^4)
Water	1570.0
Fused Quartz	0.6
Porcelain No. 4462	11.0
Borosilicate Glass	10.6
Soda-borosilicate (Pyrex)	54.0
Soda-lime-silicate glass	126.0
Nylon 66	128.0
Polystyrene	3.3
Polyethylene	3.1
Polyvinyl Chloride	55.0
Teflon (PTFE)	1.5

Measurements made at 25°C and 3000 MHz. Data from Reference (7)

The disadvantages of open-vessel systems (loss of volatile species, introduction of environmental contamination, use of large volumes of acid, temperatures limited to the boiling point of the acid at atmospheric pressure) has led to the

development of closed vessels, incorporating pressure relief mechanisms to prevent the pressure rising too high and risking vessel rupture. Losses of volatile elements and airborne contamination problems are eliminated, less acid is required, the fumes are contained, and the high pressures result in higher temperatures and therefore quicker reactions than in open systems.

Several types of microwave pressure digestion vessel have been produced. They vary greatly in the sample sizes they can accommodate, and the pressures they can withstand.

The CEM Corporation have marketed a range of vessels,[21] the original ones being of all-Teflon PFA construction (initially 60 ml capacity, more recently 120 ml), designed for relatively low internal pressures of less than 120 psi (830 kPa). More recently, CEM have produced a medium pressure, lined digestion vessel consisting of a Teflon PFA liner in a polyetherimide body and cap, having a maximum operating pressure of 200 psi (1380 kPa), and improved pressure relief system. These vessels are recommended as more suitable for the digestion of samples of high organic content than the low pressure vessels. (See Chapter 3 for more detailed description and diagrams of these vessels, which were used in this work). CEM also produce a heavy duty vessel incorporating a Teflon PFA liner in a thick outer body capable of operating at pressures up to 600 psi (4 MPa).

Another medium pressure microwave system is the Floyd RMS 150 system (Floyd, Lake Wylie, SC, USA).[22] A double-walled digestion vessel operates at up to 300 psi (2070 kPa) with pressure control and without the need for a capping station.

A pressure vessel capable of withstanding much higher pressures - to 1200 psi (8.3 MPa) - has been produced by the Parr Instrument Company (Moline, Illinois, USA). Designed for use in a standard microwave oven, the vessel has a covered, inner Teflon sample cup, which is sealed in a very thick, high-strength, outer case. A safety pressure relief disc is incorporated in the construction. The main drawback of these vessels (apart from their cost) is the limitation to small sample sizes. The larger of the two available vessels only has a cup capacity of 45 ml and the maximum sample size which can be digested is only 0.2 g for organic samples.

The new PMD (Pressurized Microwave Digestion) system (Paar, Graz, Austria) also operates at pressures up to 1200 psi (8.3 MPa). It uses high-purity quartz vessels (with Teflon seals) and has a pressure control sensor to shut off power when the maximum operating pressure is reached. However, the small oven can

only accommodate two vessels at a time.

Another high-pressure microwave digestion system is the Milestone MLS-1200 system, which uses high-pressure vessels constructed of Teflon-TFM, which allows the use of high boiling acids such as sulphuric acid up to 350°C, and can withstand pressures of up to 1400 psi (10 MPa) before the safety rupture disc vents. Up to 10 vessels are accommodated in a rotating safety shield in a high-powered oven (rated at 1200 W) with an unpulsed microwave mode reported to give a homogeneous field. Previously, the main disadvantage of this system was reported to be the inability to monitor internal temperature and pressure,[23] but the new Milestone system, the MLS-1200 Mega, offers pressure and temperature control options, with a choice of vessels (Teflon-TFM or quartz), of capacity up to 330 ml and operating pressures from 300 - 1400 psi (2 - 10 MPa).[24]

A new and interesting development in vessel design has recently been reported.[20] This new vessel combines the advantages of pressure digestion and focused microwave heating (see Section 1.4.4). The prototype system consists of a closed, Teflon-TFM inner vessel (28 ml capacity) surrounded by a stainless steel casing with a screw cap and compression plate for mechanical strength, which can withstand up to 20 MPa or 2900 psi. Between the vessel and casing is a microwave antenna system, enabling up to 100 W of microwave power to be focused onto the sample/acid mixture. Safety disks rupture if the internal pressure exceeds 16 MPa or 2300 psi. The design enables vessel cooling by water or fluid (up to –10°C) in situ both pre- and post-digestion. Complete oxidation of bovine liver with nitric acid in a single-step procedure is reported for the new vessel, although sample size is limited to 0.1 g. This system has recently been favourably compared with an on-line microwave system (see Section 1.4.5) in terms of decomposition efficiency for biological samples, as measured by residual carbon content of the digests.[25]

1.4.4 Focused Microwave Systems

Another approach to microwave digestion is to use focused microwave digestors such as the Prolabo systems (Rhône-Poulenc, Paris, France),[26] rather than ovens. These relatively compact instruments allow single sample digestion at atmospheric pressure. Whilst only being able to digest one sample at a time, and without the benefits associated with closed, pressure digestion, there are three major advantages of this technique. The first is the ability to handle large samples (several grams can easily be accommodated in the long, cylindrical glass reflux vessels) without any problems of pressure build-up. Secondly, the instruments lend themselves very easily to automation. The more sophisticated models allow

programming, not only of power and time, but of automated addition of reagents at different stages in a digestion, and multiple samples are sequentially digested via an automated turntable system. Thirdly, as the microwave energy is focused directly into the sample and acid mixture, it is potentially much more energy efficient than the oven systems, where much of the microwave energy is reflected and wasted, being converted to heat by the dummy load.

1.4.5 Flow Injection Systems

A recent development in microwave digestion has been the advent of on-line systems whereby the sample/acid mixture is introduced as a slurry into a coil of Teflon tubing (or, in the earliest system, a Pyrex coil) in a microwave oven[27-29] or focused microwave digestor.[30,31] A novel, flow-through glass digestion device has recently been designed for use with a commercial focused microwave system.[32] Digestion systems may be coupled directly with flame, cold vapour or hydride generation atomic absorption spectrometry, or, alternatively, the digest solutions may be collected in suitable vessels for later analysis. The microwave exposure is controlled by adjusting the flow rate, or by stopping the flow whilst the digestion is accomplished.[33]

An automated, continuous flow microwave digestion system is now on the market (CEM SpectroPrep system), enabling up to 180 samples to be prepared with unattended operation. The system is self-cleaning and has the advantage of operating at low (5 - 10%) acid concentrations. The manufacturers claim rapid (2 - 3 min per sample), complete digestion of samples such as wastewater, soil, sediment, biologicals etc. (although the inclusion of a filtration step implies dissolution is not always complete).

It seems an elegant approach to sample preparation, but at present suffers from being limited to small samples and short digestion times, with problems of back pressure (due to build-up of evolved gases) and incomplete decomposition. It is, however, becoming more popular as further refinements are made and the technique becomes more flexible (see Section 2.8).

CHAPTER 2

DEVELOPMENT & APPLICATIONS OF MICROWAVE DIGESTION

2.1 Introduction

Despite recent interest in solid and slurry sample introduction methods for chemical analysis, especially for spectrometric techniques such as inductively coupled plasma-atomic emission spectrometry (ICP-AES)[34] and electrothermal atomization atomic absorption spectrometry (ETAAS),[35,36] most quantitative analytical techniques still require that samples be introduced in liquid form,[5] and with modern computerized instruments capable of ever faster measurements and interpretation of results, sample dissolution is often the rate-determining step in the overall time taken for an analysis.

Conventional wet digestion techniques for organic materials and minerals are often time-consuming, involving prolonged heating with mineral acids, such as hydrochloric, nitric, sulphuric and perchloric acids, requiring frequent operator attention, and a knowledge of the explosion hazards, particularly when potentially powerful oxidants such as hot perchloric acid are involved. Open-vessel digestions run the risks of loss of volatile analyte species, introduction of environmental contamination, and concentration of impurities from the relatively large volumes of acid used. Temperatures are limited to the boiling points of the acids, and provision must also be made for the safe removal of the corrosive acid fumes generated. Digestions in closed vessels such as Teflon lined, steel-jacketed bombs or Carius tubes can solve these problems, but they are time-consuming, may be hazardous due to the pressures generated, and can be expensive.

To address such shortcomings, the use of microwaves to accelerate the acid dissolution of samples for trace element analysis was proposed as a modern alternative to these conventional procedures.

2.2 Early Experiments with Microwave Digestion

Microwave digestion as a sample preparation technique for trace element analysis was first reported in 1975 by Abu-Samra et al.[3] They digested biological samples with a nitric-perchloric acid mixture in open vessels in a modified domestic oven. Digestions in domestic microwave ovens (usually at atmospheric pressure) were carried out occasionally over the following 10 years for biological and environmental materials.[37-40]

Early researchers realised that open-vessel work was not ideal, involving the risks

of contamination, analyte loss and corrosion of oven interiors, as well as being limited to the boiling points of the acids. Attempting to counteract these drawbacks, workers tried digestions in sealed vessels, using polycarbonate bottles[41,42] and Teflon PFA vessels,[43,44] but without any means of monitoring or relieving the pressure inside the vessels, the danger of explosion was a major safety worry. Also, the domestic ovens used in this early work were designed for cooking relatively large food samples; to avoid damage to the magnetron from reflected microwaves, beakers of water had to be placed in the oven to absorb excess microwaves.

2.3 The Microwave Digestion Boom

Having remained something of a curiosity for a decade, the advent of purpose-built, commercial analytical systems in the mid 1980's, with specially designed ovens and vessels incorporating safety features (as discussed in Chapter 1), has produced a rapid rise in the popularity of microwave digestion, with many applications being reported. A number of commercial systems have proved popular, notably the CEM low/medium pressure systems, the Prolabo focused microwave digestors (ambient pressure) and the Parr high pressure microwave bombs, although domestic ovens and improvised vessels continue to be used as cheaper alternatives by some workers. Developments in on-line microwave digestion systems have also generated much interest recently. Microwave digestion has been favourably compared with other decomposition techniques, successfully applied to a wide variety of sample types and found to be particularly useful for small numbers of samples, when digestion times can be very short.

Sample types successfully digested (usually in a fraction of the time required using conductive heating) have included food, plants, soils, metals, and various biological, geological and environmental samples. Reported applications have been reviewed by Matusiewicz and Sturgeon[45] and, more recently, by Kuss.[46] The latter provides a comprehensive coverage of published work in the field up to the end of 1991, although gives fewer practical details than the earlier review. Applications are listed in Tables 2 and 3.

Table 2. Applications of microwave digestion to the analysis of mineral, environmental and metallurgical samples

Sample type/size	Elements, technique	Digestion system	Acids[a,b]	μwave heating[c]	Ref.
Coal, fly ash, oil shales, sediments, rocks; 200 mg	22 elements by ICP-AES	Domestic oven + open Teflon or polycarbonate beakers in vacuum desiccator	Aqua regia (5ml) + HF (2ml)	3 min at 625W for 1 sample	39
Coal fly ash, sediments, rocks; 100 - 200 mg	16 elements by ICP-AES; Na, K by FAAS[d]	CEM MDS-81 + 120ml Teflon vessels with relief valve (80psi)	Aqua regia (15ml) + HF (5ml)	19 min at 300W - 600W[e] for 4 samples	47
Coal, fly ash, sediments, rocks, sewage; 250 - 500 mg	24 elements by ICP-AES and ETAAS	CEM MDS-81 + 120 ml Teflon vessels with relief valve (80psi)	Aqua regia-HF mixtures	20-25 min at 300 - 600W for 6 samples	48
Coal; 0.1 - 1.15 g	Fe, Ni, V by ETAAS	Domestic oven + 200ml Pyrex test tubes + polyprop. screw caps, in 2L plastic jar	HNO_3 (5ml) + HCl (2ml)	3 x 1 min at 600W (cooling between)	49
Sediment, soil; 0.5 - 1.0 g	Up to 17 elements by ICP-AES	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	1:1 HNO_3 (10ml) + HCl (2.5ml) + 30% H_2O_2 (2ml)	2-stage, total 95 min at 450W	50
Soils; 500 mg	Hg by CVAAS[f]	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	HCl (6 ml) + HNO_3 (2 ml) + H_2O (10ml)	15 min at 200W - 600W 12 samples	51
Soils; 500 mg	Cu, Zn by FAAS	Domestic oven + 60ml sealed, screw-cap Teflon PFA vessels in 4L polythene containr	HNO_3 (4ml) + HF (7ml)	7 min at 400W for 8 samples	52
Peat; 500 mg	Cu, Zn, Mn, Li, Al, Fe, Ca, Mg, K, Na by FAAS	CEM MDS-81 + 60 ml Savillex Teflon PFA jars (75 psi)	HNO_3 (5 ml)+ $HClO_4$ (2 ml) + HF (4 ml)	75 min at 190W, 4 samples (predign on hotplate)	53
Soil, sediment; 500 mg	Ag, As, Ba, Cd, Cu, Cr, Ni, Pb, Se, Tl & Zn by ETAAS; Hg by CVAAS	Domestic oven + CEM 120ml Teflon PFA vessels with relief valve (100 psi)	HNO_3 (10 ml)	12 min at 480 - 600W for 12 samples	54
Soil, sediment; 100 - 200 mg	9 elements by ICP-AES	Domestic oven, sealed 44 ml PTFE vessels	HNO_3/H_2O_2 /HF mixture	3 min at 500W	55
Mineral soil, clay	Si, Al, Fe, Mg, Ca, Na, K, Ti by ICP-AES	Domestic oven + 60 ml Teflon bombs	HF/HNO_3	7 min at 350W	56
Soils, sediments, sludges; 0.5 - 1.0 g	Cd, Cr, Cu, Fe, Mn, Pb & Zn by FAAS, ETAAS &/or ICP-AES	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	Aqua regia (16 ml)	60 min at 600W for 12 samples	57
Marine sediment; 300 mg	19 elements by ICP-AES or ETAAS	Domestic oven + sealed Teflon vessels (60 ml, no relief valve)	HNO_3 (4.5 ml) + HCl (1.5 ml) + water (1 ml)	15 min at 720W for 25 samples	58
Solid waste; 500 mg	19 elements by ICP-AES; As, Se by ETAAS	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	HNO_3 (10 ml)	10 min at 574W for 6 samples	59

Sewage sludge; 150 - 500 mg	Pb by FAAS	Domestic oven, continuous flow thro' tube, direct injection of slurries	HNO_3 (25 ml)	5 min at 650W per sample	28
Oily waste; 2 ml	As, Sb, Se by HGAAS[g], Hg by CVAAS	Milestone system: MLS-1200 oven + HPV80 vessels (up to 350°C, 1400 psi)	H_2SO_4 + HNO_3 (3 + 1), 5 ml	11 min at 240W-600W for 4 samples	23
Marine sediment, granite; 200 mg	Al, Zn, Si by FAAS; Cr by ETAAS	Domestic oven (700W) + 20 ml Teflon bombs (250 psi, press. relief) in pressure cooker	HF (6 ml) + aqua regia (1 ml)	60 s at 700W for 1 sample	60
Sandstone, granite & sediments; 2 g	U, Th by radiochemical separation & α-spectroscopy	Domestic oven + sealed Teflon bombs & Parr μwave high pressure bomb	HNO_3 + HCl + HF (1+1+1), 2 ml	4 - 8 min at 600W per sample	61
Red mud (from bauxite processing); 100 mg	Cd, Cr, Pb, Ag, Au by ETAAS	Domestic oven (600W) + screw-cap 100 ml PTFE bottles (no relief valve)	HCl (4.5 ml) + HF (2.5 ml) + HNO_3 (3 ml)	10 cycles of 30 s at 600W + 1 min rest, 6 samples	62
Feldspar, rock, zircon, glass wafers; up to 500mg	Pb, U by isotope dilution analysis	CEM MDS-81 + 60 ml Savillex Teflon vessels - no relief valves - in 300 ml Teflon jar	HNO_3 (1 ml) + HF (7 ml) + 50% $HClO_4$ (0.5 ml)	20 min at 90-140W, add HF then1 h - 5 h at 140W	10
Rocks; 10 - 100 mg	Fe, Mn, Na, K & Mg by FAAS; Si by uv/vis spectrophotom.	Domestic oven, Parr 4781 high pressure bomb (1200 psi, 23 ml)	HNO_3 (0.3 - 1.0 ml) + HF (0.1 - 0.7 ml)	45 - 110 s at 500W, (pressure <1000 psi)	63
Phosphate rock; 100 - 500 mg	(general method for AAS, ICP-AES etc)	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	HBF_4 (5 ml) + HNO_3 (5 ml)	2 min at 600W per sample	64
Carbonate rock; 200 mg	Si, Al, Fe, Ca, K, Mg, Na: FAAS; Si, Al by N_2O-C_2H_2 flame	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	HNO_3 (3 ml)+ HF(1 ml); hot-plate pre-dign + acetic acid[h]	33 min at 570W - 630W for 12 samples	11
Minerals; 300 mg	As, Se, Cu, Zn by FAAS	CEM MDS-81D + sealed Savillex Teflon PFA vessel (75 psi) - no vent in cap	HNO_3 (3 ml) +HCl (5 ml); H_2O (22 ml) added part-way through	3 min with HNO_3 at 280W ; 8 min at 280-600W, 18 samples	65
Geological SRMs ; 100 mg	42 elements by ICP-AES (solns), & dc arc spec. (residues)	Domestic oven + 250 ml screw-cap polycarbonate bottles in plastic food contnr	HNO_3 (2 ml) + 7:3 HCl/HF (5 ml)	2.5 min at 650W for 12 samples	41
Sulfide minerals; 0.5 - 1.0 g	Cu, Zn by FAAS	Domestic oven + sealed, lab-made Teflon PFA vessels	1.5 g $KClO_3$ 10 ml HNO_3 + 5 ml HF	3 min at 477W for 4 samples	44
Soils, rocks, paints; 50 mg	up to 36 elements by ICP-AES	CEM MDS-81 oven + 100 ml Pyrex beaker covd with watch glass	5:4 HNO_3/ $HClO_4$ (10 ml)	20 min at 150W - 450W	66
Steel; 1 g	Al, Mn, P, V, Ti, As, Cu, Ni, Cr, Mo, Sn, Si, W by dc plasma AES	Domestic oven (625W) + Savillex 60 ml Teflon vessels (75 psi)	HNO_3 (3 ml) + HCl (3 ml) + HF (2 ml)	80 s at 625W for 1 sample	43
Platinum; 0.1 - 1 g	Au, Pb, Pd by ETAAS	CEM MDS-81D + CEM lined vessels (200 psi)	Aqua regia (10 ml) ; Vessel purged with Ar	2 h at 300W or 450W for 6 or 12 samples	67

Jewellery; 10 mg	Au by FAAS	Domestic oven, sealed Teflon vessels	Aqua regia (2 ml)	5 min at 325W per sample	68
FeCr, FeMn alloys; 500 mg	Al, Co, Cr, Cu, Mn, Mo, Ni, Si, Ti, V by ICP-AES	Domestic oven (600W) + open PTFE beaker	1:1 H_2SO_4 (10 ml) + H_3PO_4 (25 ml); FeCr: +10ml HNO_3	10 min at 330W per sample	69
FeSi alloys; 100 - 300 mg	Multi-element analysis by ICP-AES	Domestic oven (600W) + open PTFE beakers	H_3PO_4 (50ml) + HNO_3 (10ml)	10-15 min at 330W per sample	70
Steel; 200 mg	(general method for AAS, ICP-AES etc)	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	HCl (6 ml) + HNO_3 (2 ml); Post-dign: add 1 ml HF	30 s open, 30 s sealed at 300W per sample	64
Ceramic automotive catalysts; 5 g	Pt, Rh, Ce, Ni, Ba, Pd, La, Fe, Pb, S, P, Zn, Mn, Ca by ICP-MS	CEM MDS-81D + Teflon PFA vessels (100 psi: relief valve)	5 ml HCl + 5 ml HF	25 min at 600W, in 3 cycles, cooling bet.	71
Petroleum industry catalysts; 100 mg	Fe, Ni, Co, Mo by FAAS	Domestic oven , (650W) + sealed Teflon reactors (125 ml)	HNO_3 (2 ml) + 2 drops isoamyl alcohol + 2 ml H_3PO_4	6 - 15 min at 650W	72

See Notes at foot of Table 3

In addition to the above, further applications of microwave heating have been reported for the decomposition of steels and nickel-based alloys for ETAAS,[73] dusts, ashes and sediments for ICP-AES,[74] dust for lead and cadmium by DPASV and ETAAS,[75] and soils for cadmium by ETAAS,[76] and an evaluation of four different microwave digestion procedures has been reported for the decomposition of a range of environmental reference materials for ICP-AES or ICP-MS analysis.[77]

Table 3. Applications of microwave digestion to the analysis of biological, botanical and clinical samples

Sample type/size	Elements, technique	Digestion system	Acids[a,b]	μwave heating[c]	Ref.
Orchard leaves, bovine liver; 500 mg	Zn, Cu, Pb by FAAS[d]; As, Se, Co, Cr, Ni by neutron activation analysis	Domestic oven (600W) lined with Plexiglas, exhaust to acid fume scrubber + 125 ml Erlenmeyer flasks	4:1 HNO_3 + $HClO_4$ (10 ml)	15 min at 600W for 12 samples (to $HClO_4$ fumes)	3
Human teeth, 5 - 15 mg; swordfish, 5 - 10 g	Hg in fish by CVAAS[f], teeth anal. by ASV[i]	Domestic oven (600W) + open Pyrex test tubes (teeth), or 125 ml flask + funnel to reflux (fish)	HNO_3/ $HClO_4$/ H_2SO_4 mixtures	600W; teeth: 10 min/30 samples; fish: 3 min/ 3 samples	37

Biological SRMs; 200 mg	Ni by ICP-AES	Domestic oven, Parr microwave bombs (1200 psi, 45 ml)	HNO_3 (4 ml) + HCl (2 ml); Ni-DPTH[j] ext. into 1-butanol	14 min at 180 - 360W[e]	78
plants, fish, soil, bovine liver; 250 mg	Cu, Fe, Zn, Cd, Cr, Pb, Ni by FAAS	Domestic oven + Savillex Teflon PFA 60ml screw-cap vessels	H_2SO_4 (1.5 ml) + HNO_3 (1.5 ml)	60 s at 700W	79
Bovine liver; 150 mg	Cd, Cu, Fe, Mn, Pb, Zn by ICP-AES	Domestic oven (600W, no power regulation) + open conical flasks	HNO_3 (5 ml) + $HClO_4$ (1 ml)	20 min at 600W	38
Tomato leaves, pine needles, bovine liver; 500 mg	19 elements by ICP-AES and ETAAS	CEM MDS-81 + 120ml Teflon vessels with relief valve (80psi)	Aqua regia (20 ml)	25 min at 300 - 600W for 6 samples	48
Animal tissue; 100 mg x 2 (2 digests per vessel)	24 elements by ICP-MS	Domestic oven (700W) + Parr microwave bomb (1200 psi, 23 ml)	HNO_3 (2 ml) + HNO_3 (1 ml) for 2nd digestion	75 s, medium power, per 100 mg sample	80
Chorella, mussel, bovine liver, sargasso, pepperbush; 50 - 500 mg	Ca, Fe, Mg and Zn by FAAS (on-line)	CEM MDS-81 + flow inj. system: 0.8 mm ID PTFE tubing (20 m); pressure regulator + back-flush filter	Slurry of sample + 5% v/v HNO_3 (25 - 250 ml); 1 ml injectd	525W, flow rate 4 - 6 ml min^{-1} (1 - 2 min per sample)	29
Feeds, plant & animal tissue; up to 2 g	Se by ETAAS	Prolabo A300 automated system: open, cylindrical glass flasks with sequential addition of acids	(1) HNO_3 (20 ml) (2) 2:3 H_2SO_4 /$HClO_4$ (5 ml) (3) H_2O (5 ml)	Total 78 min at 15 - 40% power. Se-DDDC[k] extd in $CHCl_3$	81
Dried biological samples; 150 mg	Al, Cu, Fe, Mn, Zn by ICP-AES.	Domestic oven + sealed PTFE containers	HNO_3 (3 ml), HCl (0.3 ml), $HClO_4$(0.3 ml) + HF (0.15 ml)	8 min 30 s at 200W	82
Pine needles, lobster hepato-pancreas; 250 mg	Ca, Zn, Cu & Fe by dc plasma-AES	Floyd RMS-150 (600W) + double-walled Teflon PFA vessels (80 ml, 200 psi + relief discs)	HCl 6 ml) + HNO_3 (6 ml) + HF (3 ml)	15 min at 540W for 12 samples	83
Bovine liver, mussel, hair, pepperbush, tea leaves; 100 mg	Zn, Cu, Fe, Ca, Mg, Mn by "one-drop" FAAS	Domestic oven (500W) + closed double PTFE vessel (7 ml inner), NaOH bet. vessels, in polyprop. jacket.	HNO_3 (2 ml) + $HClO_4$ (0.3 ml) + HCl (0.1 ml) + HF (0.1 ml)	14 min at 200W	84
Lobster hepato-pancreas; 50 - 250 mg	Cu, Fe, Sr by FAAS; As, Cd, Co, Cr, Mn, Mo, Ni, Pb, Se, V, Zn by ETAAS	CEM MDS-81 + pressure bombs: (A) CEM (100 psi) (B) Berghof (370 psi) (C) Parr (1200 psi)	HNO_3 + 30% H_2O_2 (1:1): (A) 8 ml (B) 6 ml (C) 2ml	A: 3 min at 600W, for 12 samples; B,C: 1 min, 600W, for 1 sample	85
Bovine liver; 200 mg	As, Se by HGAAS[g]	Domestic oven + 50 ml sealed Teflon centrifuge tubes	HNO_3 (7.5 ml) + H_2SO_4 (2.5 ml) + H_2O_2 (0.3 ml)	5 x 6 min,100 - 650W; pre- & post-dign: 1 h at 90°C, 5 samples	86
Wheat flour, tuna; 150 - 200 mg	Mn, Zn, Fe, Cu, Mg, Na, K by FAAS	CEM MDS-81 + Savillex Teflon PFA 60 ml vessels (with relief valves)	HNO_3 (5 ml)	8 min at 150W per sample + hotplate 30 min (open)	87

Biological & botanical standards; 200 mg	18 elements by ICP-AES	Domestic oven + open Teflon or polycarbonate beakers in vacuum desiccator	Aqua regia (5 ml) + HF (2 ml)	3 min at 625W for 1 sample	39
Marine samples: (a) biolog. tissue, (b) sediment; 250 mg (dry)	As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Zn: ICP-AES, FAAS &/or ETAAS	CEM MDS-81D + 120 ml Teflon PFA vessels, with relief valve (100 psi) & completely closed,+press. monitor	(a)HNO_3, 6 ml $HClO_4$, 0.5 ml (b)HNO_3,3 ml HF, 3 ml + $HClO_4$, 1 ml	600W to 65 psi; power reduced so 60 - 65 psi for 20 min	88
Shellfish; 12.5 g (wet)	Pb, Cd, Cu, Cr, Zn by FAAS	CEM MDS-81D + 120 ml Teflon PFA vessels, with relief valve (100psi)	9 ml HNO_3	200W/Off 15 min ea. till clear; open predig. 200W 6-12 samples	89
Mussels; 250 mg (dry) or 1 g (wet)	As by ETAAS	Domestic oven (750W)+ lab-made PTFE vessels, 120 ml, with screw caps	2 ml HNO_3 + 2 ml 30% H_2O_2	1 min at 555W then 4 min at 300W per sample	90
Fish; 200 mg	Pb by ETAAS	Domestic oven (600W) + Parr 4782 bomb (45 ml, 1200 psi)	HNO_3 (2.5 ml)	90 s at 600W	91
Diet & fecal samples; 0.5 - 1 g	Ca, Cu, Fe,K, Mg, Mn, P, Zn by ICP-AES	CEM MDS-81D + 120 ml Teflon PFA vessels, with relief valve (100psi)	HNO_3 (5 ml) + 6M HCl (2ml)	Alternate heat (300 - 450W) & cool: 30min, 12 samples	92
Bovine liver, botanical reference materials; 350 mg	Al, Ba, Cd, Cu, Fe, Mg, Mn, Zn by ICP-AES	Sample slurry + acid pumped into 4.2 m coiled Teflon PFA tubing (i.d. 4 mm) in domestic oven (720W)	20 ml water + 20 ml HCl, HNO_3, aqua regia or 4 : 1 HNO_3 : H_2O_2	2 min at 720W whilst flow stopped	33
Foods; 0.5 - 1.0 g	Ca, Cu, Fe, Mg, Mn, K, Na, Zn by FAAS	Prolabo Microdigest A-300 (automated system at atmospheric pressure)	HNO_3 , (15-25 ml) + H_2O_2 (30%), 5 ml + H_2SO_4, 0-10ml	30 - 45 min per sample 30 - 45% power	93
Citrus juices; 25 g	B, Ca, Cu, Fe, K, Mg, Mn, Na, P, Si, Sn, Sr, Zn by ICP-AES	CEM MDS-81D + 120 ml Teflon PFA vessels, with relief valve (100psi)	Aqua regia (15 ml)	2 x 8 min at 600W with 5 min rest in between	94
Pig liver, wheat flour, mixed diet; 200 - 300 mg	Ca, Mg, Cu, Fe, Mn, Zn by sequential ICP-AES	Commercial oven (500W) + closed 44 ml PTFE vessels	HNO_3 (2 ml) + 30% H_2O_2 (1 ml)	2 min at 500W for 6 samples	95
Bone, bovine liver; 5 - 100 mg	Al, Fe, Cu, Co, Pb & Se by ETAAS , Zn by FAAS	Commercial oven (1400W) + Parr dign bomb with 23 ml liner modified to 4.5 ml	HNO_3 (1 - 2 ml)	40 - 90 s at full power	96
Bovine liver, mouse tissues; 25 - 250 mg	Fe by FAAS	CEM MDS-81D + 60ml Teflon PFA vessels, with relief valve (100psi)	HNO_3 (1 ml) + 30% H_2O_2 (1 ml)	15 min at 150W for 4 - 12 samples	97
Fat-rich foods; 100 - 200 mg	Zn, Fe, Cu detd by FAAS, Ni by ETAAS, Se & Hg by HGAAS	Domestic oven (700W) + screw-cap 50ml PTFE vessels with relief disc (150 psi) & Parr bombs (1200 psi)	HNO_3 (2 ml) + 30% H_2O_2 (1 ml) added for last stage	4 x 10 min at 280 - 560W, cooling in ice between heat cycles	98

Octocorals; 1 g (dry weight)	Cd, Cu, Ni, Pb, Zn by ETAAS	Domestic oven (600W) + 200ml thick-walled Pyrex test tubes sealed with screw cap	HNO_3 (10 ml)	4 - 6 x 1 min at 240W, cooling after each cycle, per sample	99
Food; 200 mg	Various elements by ETAAS	Domestic oven + 25ml PTFE bombs (Max. pressure 10 bar)	HNO_3 (4 ml)	3 min at 650W	100
Diet composites, bovine liver, wheat flour; 250 mg	Se by ETAAS	CEM MDS-81 + Savillex Teflon PFA 60- & 120-ml vessels, pressure & temp. monitored	HNO_3 (5 ml)	Hotplate, 1 h 60°C + two-stage µwave at 170 - 290W to 180°C	101
Milk 0.1 - 0.5 g (dry weight)	Zn by ICP-AES	CEM MDS-81D, Floyd 150 RMS, Prolabo M 300 + suitable vessels	HNO_3 + H_2O_2 and/or H_2SO_4	Various	102
Dried milk, flour, bovine liver, oyster, serum; 100mg	Hg by CVAAS	Domestic oven (700W) + sealed Teflon PFA vessels (no relief valve) in lidded box	HNO_3 (1 ml)	20 min at 140 - 420W for 6 samples	103
Food crops, seafood; 200 - 250 mg	Cd by ETAAS	Domestic oven (600W) + Parr 4782 bomb (45 ml, 1200 psi)	2.5 ml HNO_3 + V_2O_5 (few µg) as catalyst	90 s at 600W	104, 105
Food crops, fish; 200 mg	Hg by CVAAS	Domestic oven (600W) + Parr 4782 bomb (45 ml, 1200 psi)	HNO_3 (2.5 ml)	90 s at 600W	106, 107
Vegetable samples; 200 mg	As by HGAAS	Domestic oven (600W) + Parr 4782 bomb (45 ml, 1200 psi)	2.5 ml HNO_3 + 50 µg V_2O_5 as catalyst	90 s at 600W	108
Agricultural crops; 500 mg	P, K, Ca, Mg, Fe, Mn, Zn, Cu by ICP-AES	CEM MDS-81D + Teflon PFA vessels (100psi)	HNO_3 (10 ml) + HCl (5 ml)	15 min at 600W for 12 samples	109
Botanical samples; 500 mg	Ba, Ca, Mg, Mn, P, K, Na, S, Zn by ICP-AES	CEM MDS-81 + open, 100 ml Kohlrausch glass flask	HNO_3 (20 ml) +H_2O_2 (1 ml) in 2 stages	60 min,540W (1-2 ml left), 12 samples	40
Botanical, biological and food samples; 2.0 g	Ba, Ca, Mg, Mn, P, K, Na, S, Zn by ICP-AES	CEM MDS-81 + open, 100 ml Kohlrausch glass flask	HNO_3 (25 ml) + H_2O_2 (2 ml) added in 3 stages	5 min at 300W, 55min at 600W, 12 samples	1
Botanical, biological and food samples; 1.0 g	Al, Si by ICP-AES	CEM MDS-81 + semi-closed Teflon vessel with reflux top (round-bottom Teflon tube)	HNO_3 (25 ml) + H_2O_2 (2 ml) +HF (1.5 ml) (3 stages)	90 min at 200 - 600W; last 30 min with HF & reflux top	1
Pine needles, citrus leaves; 250 mg	15 elements by dc plasma AES; Fe, K, Mg, Mn also by FAAS	MWS 1200 system (AGW) + pressure vessel (HPV-80: modified PTFE, 80ml, 200 bar).	5 ml HNO_3, 2 ml 40% HF + 0.5 ml 30% H_2O_2 (3 stages)	27 min at 300 - 600W, cool /add reagent between stages	110
Plant tissue; 200 mg	Hg, Se by HGAAS	Domestic combination oven (750W µwave + 1500W convection) + sealed 60 ml Teflon vessels	HNO_3 (4 ml)	20 min at 75 - 375W (convection used for drying)	111
Flour, hay; 0.3 g	Mo by thermionic quadrupole MS	CEM MDS-81D + Teflon PFA vessels (120ml, 100 psi)	HNO_3 (10 ml) + 30% H_2O_2 (2 ml) for 3rd stage	30 min,180W open predig; 3 x 10 min at 420 - 600W, 12 samples	112

Citrus leaves; 200 mg	Ca, Mg, Ba, Mn, Zn, Cu by ICP-AES	CEM MDS-81D + Teflon PFA vessels, with relief valve (100 psi) + IR probe temp. monitor	HNO_3 (5 ml) + HF (2 ml)	20 min at 240 - 600W, with temp. control, for 6 samples	113
Vegetable tissue; 100 mg	P,Ca, Mg, K & S by ICP-AES.	Domestic oven (750W) + 50 ml Teflon bottles in unsealed Pyrex container	HNO_3 + $HClO_4$ (10 + 4), 2 ml	560W - 750W 15 - 25 min for 25 samples	114
Drugs; 0.5 - 1.2 g, blood, 1 ml	Gd by ICP-AES, Cu by FAAS, Cr by ETAAS	CEM MDS-81D + open, 30ml Kjeldahl flasks	HNO_3 (5 ml)	7 - 12 min at 120 - 480W for 3 samples	115
Diluted blood; 70 - 100 µl	Cu, Zn, Fe by FAAS	Domestic oven (700W) + flow injn system: 50 cm coiled Pyrex tube in oven	0.3M HCl + 0.4M HNO_3 (100µl)	15 - 26 s at 700W (cont. flow: 1.8 ml min^{-1})	27
Blood, urine, milk powder, tissues; 100 mg	Pb, Cd & Cu by ASV; As by HGAAS	High pressure PMD system (Paar, Austria) 1 - 2 quartz pressure vessels (1200 psi)	HNO_3/ $HClO_4$/ H_2SO_4 mixtures	80 - 100% power for 10 min	116
Tap water for haemo-dialysis (Venezuela); 5.0 ml	Al by DPP[l] (of Al-Solochrome Violet RS complex)	CEM MDS-81D + Teflon-PFA vessels with pressure relief valves (100 psi)	HNO_3 (5 ml) + conc. H_2O_2 (2 ml)	6 x 5 min at 600W, (cool/ vent betwn), 12 samples	117
Urine and environ-mental waters; 3 ml	As, Bi & Pb by HGAAS, Hg by CVAAS	Prolabo MX 350 + automated on-line flow inj. system linked to AA	Various mixtures depending on element	75W, 30 - 40 samples per h	30, 31, 118
Bone & brain tissue; 200 - 800 mg	Al by ETAAS	CEM MDS-81D + Teflon PFA vessels, with relief valve (100 psi)	HNO_3 (3 ml)	6 min at 300 - 600W for 12 samples (hot-plate predign, 1 h)	119
Biological tissue, marine sediment; < 250 mg	15 elements by FAAS and ETAAS	CEM MDS-81D + lined CEM bomb (200 psi), sample in 4 ml Teflon cup above acid	HNO_3 (Vapour phase digestion)	25 min at 60 - 150W	120
Blood, tissue, botanical CRMs; 1 ml /1.5 mg	Pb by FI-ETAAS[m]	Sample + acid mixture pumped into PTFE coil (2 m) in domestic oven (700W)	HCl + HNO_3 (1+1, each 0.5 mol l^{-1}) 500 µl acid: 200 µl sample	Sample in oven 25 secs at 700W; flow rate 1.5 ml min^{-1}	121
Honey; 0.2 - 0.3 g	Al, B, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb & Zn by ICP-AES.	1200W oven + sealed PTFE vessel (10 ml)	30% H_2O_2 (2ml) + 65% HNO_3 (2ml)	9 min at 240W - 600W	122
Biological & environmental ref. materials; 10 - 30 mg	Pb, Cd, Cu, Mn & Fe by ETAAS	Domestic oven + covered Teflon vials inSterilite container covered with plastic film	100µl HNO_3 + 100µl water + 20 µl H_2O_2	46 min at 70 - 250W + 1 min at 600W; **100 samples at a time**	123
Blood plasma; 1 ml	Cu by DPASV	CEM MDS-81D + Teflon PFA vessels, with relief valve (100 psi)	2 stages: (i) HNO_3 (5 ml) ; (ii) $HClO_4$ (5 ml)	(i) 30 min at 180 - 600W (ii) Repeat	124
Rats' organs, human blood; 100mg dry, 400mg wet	up to 36 elements by ICP-AES	CEM MDS-81 oven + closed, 500 ml Teflon bottle	HNO_3 (3 ml)	6 x 1 min at 150W, cooled 1 min bet. cycles	66

Notes to Tables 2 & 3:

a All acids were concentrated unless otherwise stated.

b All digestions for ICP analysis using HF involved post-digestion treatment with boric acid - with hot-plate or further microwave heating for 5 - 30 min - to remove the HF (as HBF_4) which would otherwise attack any glass or quartz components of the ICP system.

c Where domestic ovens are used, a beaker of water (typically 100 ml) is normally placed in the oven to absorb excess microwaves and avoid damage to the magnetron due to reflected microwaves. This results in longer heating times/higher powers than are needed for similar samples using analytical microwave ovens.

d FAAS = Flame atomic absorption spectrometry, with air-acetylene flame unless otherwise stated

e Where a range of microwave power is given, applied power was varied over several stages within the total heating time given.

f CVAAS = Cold vapour atomic absorption spectrometry

g HGAAS = Hydride generation atomic absorption spectrometry

h The powdered rock samples were heated with 0.5M acetic acid in open vessels on a hot-plate until evolution of CO_2 ceased, thus preventing excess pressure build-up in the subsequent closed digestion, and inhibiting the formation of insoluble calcium fluoride, which was otherwise formed by reaction of undissolved carbonate material with HF.

i (DP)ASV = (Differential pulse) Anodic stripping voltammetry

j DPTH = 1,5-bis(di-2-pyridylmethylene)thiocarbonohydrazine

k DDDC = diethylammonium N,N-diethyldithiocarbamate

l DPP = Differential pulse polarography

m FI-ETAAS = Flow injection electrothermal atomic absorption spectrometry

In addition to the above, further applications of microwave heating have recently been reported for the decomposition of plant materials and milk products for analysis by ICP-AES, FAAS and ETAAS;[125] blood for the determination of arsenic by FI-HGAAS,[126] and mercury by CVAAS;[127,128] bone and teeth for ETAAS analysis;[129] samples from wine production for lead by ETAAS;[130] fish for selenium by DPP[131] and HGAAS;[132] biological tissues and serum for ruthenium by ETAAS;[133] plant tissue for aluminium, silicon and iron by ICP-AES;[134] and plant and animal tissue for polonium by alpha-spectrometry.[135]

2.4 Comparison with Other Sample Preparation Techniques

Microwave digestion has been directly compared with other sample decomposition techniques for a variety of sample matrices as indicated in Table 4. Microwave conditions were optimised to give recoveries comparable to - and sometimes better than - non-microwave methods. Precision and limits of detection were usually better with closed vessel microwave methods, due to more reproducible heating and lack of contamination leading to lower blanks. However, open vessel microwave digestion can cope with larger samples, and may effect more complete decomposition.[136]

2.5 Problems Encountered with Microwave Digestion

As can be seen from the above, microwave digestion has become a popular method of sample preparation for trace element analysis in recent years. However, the technique is not without its problems. Greenberg et al.[137] studied various dissolution methods, including microwave pressure digestion, for botanical reference materials and found that significant fractions of some elements (especially Cr) were lost to the Teflon PFA vessel walls and insoluble particles (even when digest solutions appeared clear).

Whilst it was initially claimed by the manufacturers that the all-Teflon PFA pressure vessels of the type available for the first part of this project (i.e. those rated to 120 psi (830 kPa)) are suitable for the digestion of 1 - 2 g samples with a high organic content,[138] it is noticeable from the literature that most workers using these vessels in the MDS-81D have been digesting much smaller samples, typically 250 mg (see Tables 2 & 3), larger samples often requiring lengthy open-vessel pre-digestions,[89,119] or several heat/cool/vent cycles to avoid too much pressure being generated.[139] According to Pratt et al.[140] and Kingston and Jassie[15] these vessels can withstand the pressure build-up resulting from complete oxidation of *up to* 250 mg of organic sample. One method,[92] reportedly suitable for the single-stage digestion of 0.5 - 1 g diet samples left "residue" in the vessels.

Due to their thermal insulation properties, post-digestion cooling of the Teflon vessels is often a lengthy process, but a necessary one, to allow the internal pressure to decrease before opening the bomb. Apart from the usual procedure of leaving the vessels to cool to room temperature in air, reported post-digestion cooling methods include water,[43,47,60,63,85,100] ice-water,[41,44,79,90] a freezer,[80,104] or, rarely, liquid nitrogen.[15,101] However, the need for a more effective cooling method has been recognized.[63] Recent work by Matusiewicz[20] attempts to address this problem in the design of a new high pressure/temperature focused

microwave heated Teflon bomb, which enables cooling by water or fluid (to –10°C) in situ both pre- and post-digestion.

Table 4. Microwave digestion versus other sample preparation techniques

Method	Sample type	Reference	Comments
Fusion ($LiBO_2$ or $Li_2B_4O_7$)	coal fly ash, geological samples botanical SRMs	47,141 137	Microwave method rapid, accurate for most elements, giving salt-free matrix, but low recoveries for Cr; fusion good for difficult minerals (eg Zr-containing ores) but loss of volatiles a problem.
High-pressure digestion with conventional heating	coal & coal fly ash paints biological tissue steel water soils milk blood,urine, fish, sediment	39,47 66,142 38,66 43 117 51,66 102 127	High pressure digestion in Parr bomb slow and contamination risk from metal casing,[39] but good for volatiles compared to open-vessel microwave digestion.[66] However, microwave pressure digestion faster and can cope with larger samples than Parr bomb.[51]
Dry ashing	biological samples plant tissue shellfish, mussels bone & bovine liver citrus juices peat meat milk honey grapes, vine leaves	38,87 109,143,144 89,90 96 94,145 53 146 102 122 130	Results comparable; microwave much faster and better for volatile elements.
Conventional open-vessel wet ashing	coal; peat soils; solid waste steel & platinum paints biological samples plants food shellfish, mussels marine sediment petroleum catalysts mineral/geological	49,53 50,52,54,57,66 59 67,147 66,142 38,66,87,119 109,137,144, 148,149 93,102,150 89,151 58 72 44,51,141	Closed vessel microwave digestion much faster, less labour intensive, avoids use of $HClO_4$ and minimizes airborne contamination and volatile analyte losses. But limited to small samples (< 500 mg) when organic content is high, whereas samples of several grams can be handled by conventional methods.
Oxygen flask combustion	plastics, for Cd & Pb	19	Closed microwave better sensitivity & precision , and can be automated, but slower & more expensive than O_2 flask.
Slurry preparation	biological samples	87	Slurry method very convenient and rapid, but only suitable for certain analytical techniques.

2.6 Study of the Chemistry of Microwave Decomposition

As most workers have been interested in preparing samples for analysis by AAS or ICP-AES, the difference between complete dissolution and complete decomposition has rarely been addressed, although for AAS the issue may be of some significance. However, it has been noted by several workers[16,80] that digestion with nitric acid alone is insufficient for complete oxidation.

Kingston and Jassie[16] monitored amino acid levels and found them reduced by a factor of 10^5 in human urine, and to less than 5×10^{-3} μg g^{-1} in wheat flour, after microwave digestion. Pratt et al.[140] have identified some of the organic residues from nitric acid digestion as isomers of nitrobenzoic acid. A detailed study of organic residues from biological materials after thermal pressure digestion with nitric acid has been carried out[152-154] and it may well be that the chemistry of microwave digestion follows a similar course. It was reported that phenylalanine in the biological samples reacted to give a mixture of isomeric nitrobenzoic acids, histidine to give imidazole-4-carboxylic acid, linoleic acid to give cyclopropane-1,2-dicarboxylic acids and tryptophan to give a mixture of aromatic compounds.

Several workers have measured the residual carbon content (RCC) of solutions after pressure digestion using microwaves[25,85,88,102,136] and conventional heating,[66,152] in order to assess the degree of decomposition. Interference from residual carbon has been reported in ICP-AES[66] and ICP-MS.[80] Hee and Boyle[66] found it necessary to correct for carbon interference (in the range 3000 - 10,000 μg of C per ml) in ICP-AES analysis, while Friel et al.[80] found a post-digestion, open beaker, treatment with H_2O_2, involving 30 min on a hot-plate at 90°C, effectively removed the problem.

Nakashima et al.[88] found that oxidation efficiency for nitric/perchloric acid digestion of a marine biological reference material, as measured by residual carbon levels, rose from 64% for a single-stage microwave digestion at 70 psi (480 kPa), to 91% when this was followed by a hot-plate open digestion. A 2-stage microwave digestion, whilst still only having an oxidation efficiency of 76%, was still felt superior to hot-plate digestion, as it was much faster and not subject to contamination and loss of volatiles. Matusiewicz et al.[85] reported oxidation efficiencies of 77 - 86% when digesting the same reference material using nitric acid/hydrogen peroxide mixtures in 3 types of microwave pressure vessels.

More recently, Krushevska et al.[102] compared RCCs of milk samples digested by hot-plate, high pressure ashing, closed microwave (low and medium pressure) and open focused microwave, using various acids and programs, and found them

to vary widely. For samples with a fat content >1%, HNO_3 alone was insufficient for complete decomposition. The addition of H_2SO_4 improved decomposition (i.e. lowered RCC) especially for the low pressure microwave method. The high pressure asher and open microwave methods using a mixture of HNO_3, H_2SO_4 and H_2O_2 gave the lowest residual carbon content values (<1%, compared with up to 25% for other methods). Further studies[136] confirmed the superior oxidation efficiency of the open focused microwave and high pressure ashing methods over a closed medium pressure microwave system for the decomposition of milk, biological tissues (mussel, kidney, oyster and bovine liver) and urine. The problem of low solubility sulphates formed by some elements with sulphuric acid was also addressed, by the addition of various reagents (HNO_3, HCl and NH_4EDTA) in the final digestion step, which resulted in improved recoveries for Sr, Ba and Pb. However, the effectiveness of decomposition attributed to this open focused microwave method must be weighed against the drawbacks discussed earlier and the losses of volatile elements reported by other workers.[98]

Very low RCC levels (<1%, i.e. decomposition efficiency >99%) have also recently been reported[20,25] for biological samples using the new high pressure/high temperature focused microwave heated Teflon-TFM bomb described earlier (see Section 1.4.3).

2.7 Temperature Measurements during Microwave Heating

Whilst monitoring pressure in a closed vessel during microwave heating is a relatively straightforward matter, using an external pressure transducer, the measurement of temperature during closed or open-vessel procedures has been more of a problem, as this cannot be carried out by conventional means, such as by thermocouples (unless specially shielded) or resistance thermometry because of the metallic nature of such probes. Fibre optic techniques offer one (albeit rather expensive) solution, and have been used to study superheating effects.[13]

Several workers have developed alternatives, including a miniature glass bulb device, attached to a pressure transducer,[155] which measures temperature via the pressure rise in the fixed air volume. It has been used to measure temperatures in the range 273 - 500 K ± 0.5 K in Pyrex reaction vessels. Vessels coated with thermochromic paint have also been used to monitor temperature and study superheating effects.[12] Temperature in closed Teflon pressure vessels has been monitored using an infrared probe mounted under a hole drilled in the bottom of an MDS-81D oven. The probe sensed each vessel as it passed over the hole as the turntable rotated.[113] The data was collected on an Apple II computer, which

provided feedback control, shutting off microwave power when the desired temperature was reached.

Specific decomposition temperatures (as witnessed by a sharp rise in pressure at almost constant temperature) of the three basic components of biological and botanical matrices in nitric acid have been measured as 140°C for carbohydrates, 150°C for proteins and 160°C for lipids.[156] This compares with a boiling point of ca. 180°C for nitric acid in microwave pressure vessels, but only 120°C at atmospheric pressure with conventional heating; hence microwave pressure digestion can be expected to greatly enhance the decomposition of biological samples.

2.8 Flow Injection Systems

The development of on-line microwave digestion systems incorporating flow-injection methodology has been a popular area of recent research. Such methods have often been designed for use in direct combination with atomic absorption spectrometry (flame,[27-29,157,158] electrothermal,[121] hydride generation,[31,118,126] and cold vapour[30,31,118,128]). Colorimetric detection has also been employed, after on-line treatment with suitable reagents, for the determination of lead[159] and phosphate.[160,161]

Samples digested have included food,[158] milk[32] sewage sludges,[28,158] blood,[27,32,126,128,162] biological tissues,[157] urine[30,32,118] and waste waters.[160,161] Continuous[28,29,158] and stopped-flow[32,33,128] systems have been designed.

2.9 Computers, Robots and Microwave Digestion

Recent advances in microwave digestion include the development of robot systems,[65,163,164] expert-database systems[165,166] and the application of chemometric procedures such as principal components analysis etc.[83,167,168] A database with some 780 digestion procedures, using an open focused microwave system, has been compiled and analysed to give preferred methods.[169]

2.10 Conclusions

In general, the difficulties associated with larger (1 g) organic samples, incomplete decomposition and lengthy cooling times do not appear to have been properly addressed. The aims of this project include the investigation of chemical and physical processes associated with microwave pressure digestion, and the development of methods suitable for the rapid digestion of larger organic samples, effecting complete dissolution of the sample (preferably using nitric acid alone), and complete decomposition where required.

CHAPTER 3

EQUIPMENT & MATERIALS USED IN THIS PROJECT

3.1 Digestion Apparatus

3.1.1 Microwave Oven

The microwave oven used in this work is a CEM MDS-81D model supplied by CEM (Microwave Technology) Ltd (Buckingham, UK), part of the CEM Corporation (Matthews, NC, USA). It has a power rating of 630W ± 70W, adjustable in 1% increments and programmable for 3 heating stages of differing power, the time for each stage being adjustable in 1-second increments. The oven incorporates the features described in Section 1.4.2, including a removable Teflon turntable holding up to 12 vessels, and is illustrated in Fig. 2. A microswitch in the door catch causes the automatic switch off of microwave energy (and an audible warning) if the door is accidentally opened during a heating program.

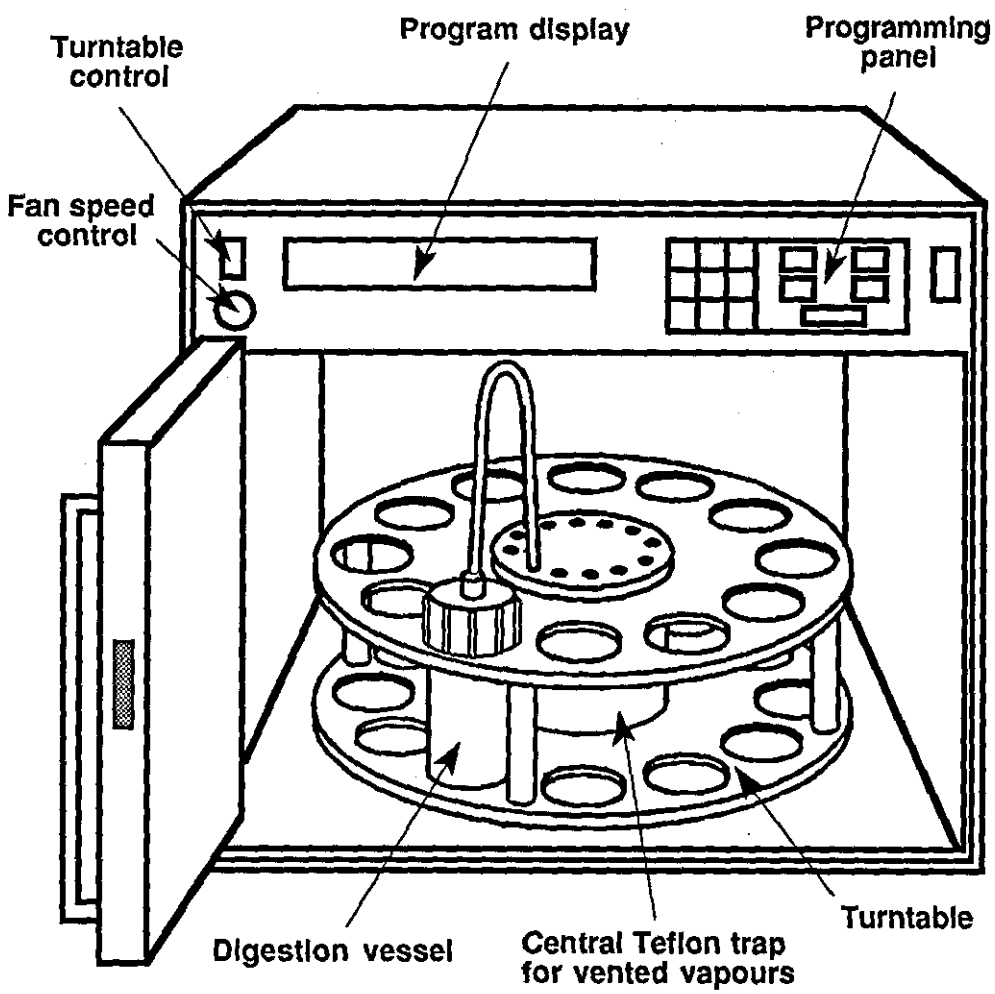

Fig. 2 CEM MDS-81D Microwave Oven

The power output of the oven was measured by heating 1 l of water in a polypropylene beaker (placed in the front right hand corner of the microwave cavity, as recommended in the CEM Operation Manual), for 2 min at full power (see Section 1.3.4). The initial and final temperatures were measured and the power calculated as

$$\text{Power} = 35 \times (\text{final temperature} - \text{initial temperature})$$

Using this method, the maximum power of the oven was measured at 675 W ± 15 W (9 determinations).

3.1.2 Pressure Vessels

The CEM digestion vessels used in this project were of two types:

i) Teflon PFA Vessels

The vessels used in the earlier part of the project were of all-Teflon PFA construction, having a capacity of 120 ml and incorporating safety relief disc valves designed to open if the internal pressure reaches 120 psi (830 kPa) above atmospheric. The design of these vessels and operation of the pressure relief mechanism is shown in Fig. 3. The vessels were sealed to a reproducible torque of 16.3 N.m using a separate capping station, also supplied by CEM. Manual venting of these vessels at the end of the heating cycle was accomplished by pressing on the side of the venting nut to slightly flex the cap and release the seal.

Although rated to 120 psi (830 kPa), the vessel caps gradually became slightly deformed with use, and the sealing lip of the relief valve was prone to fine scratches, which affected the seal, causing the pressure relief mechanism to operate unpredictably at lower pressures.

ii) Lined Vessels

Following difficulties experienced with the all-Teflon PFA vessels, new vessels designed to withstand higher pressures - and recommended as more suitable for the digestion of larger organic samples - were purchased. These have a capacity of 100 ml and consist of a Teflon PFA liner, cover and venting system (with disposable rupture discs) contained in a polyetherimide body and cap, having a maximum operating pressure of 200 psi (1380 kPa), the caps being fitted and tightened by hand. Fig. 4 shows the construction of these vessels.

The pressure relief system proved more reliable than that of the all-Teflon PFA vessels, as it is not reliant on a pressure-dependent ring seal, but instead uses

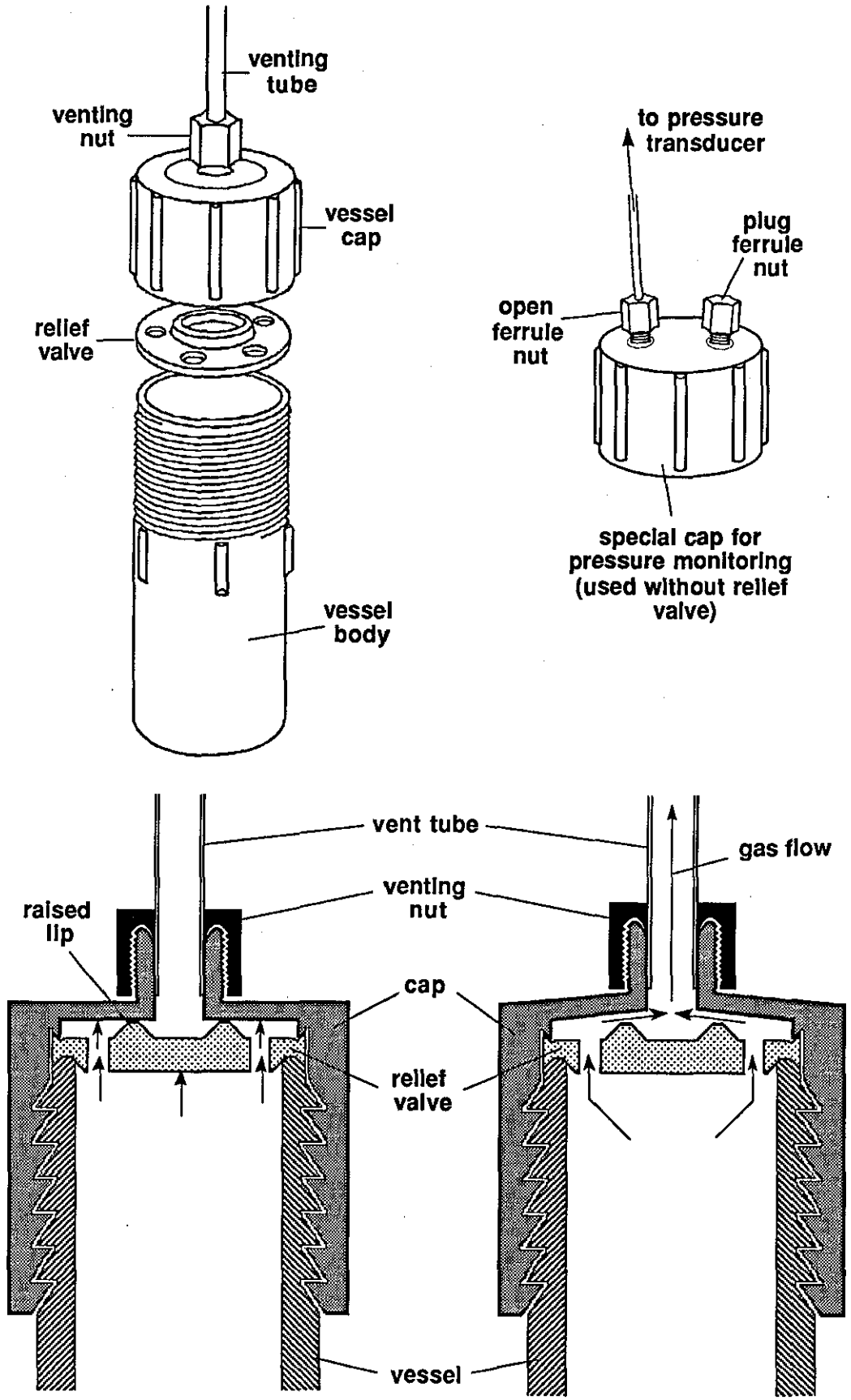

Fig. 3 Construction & operation of low pressure Teflon PFA vessels (CEM)

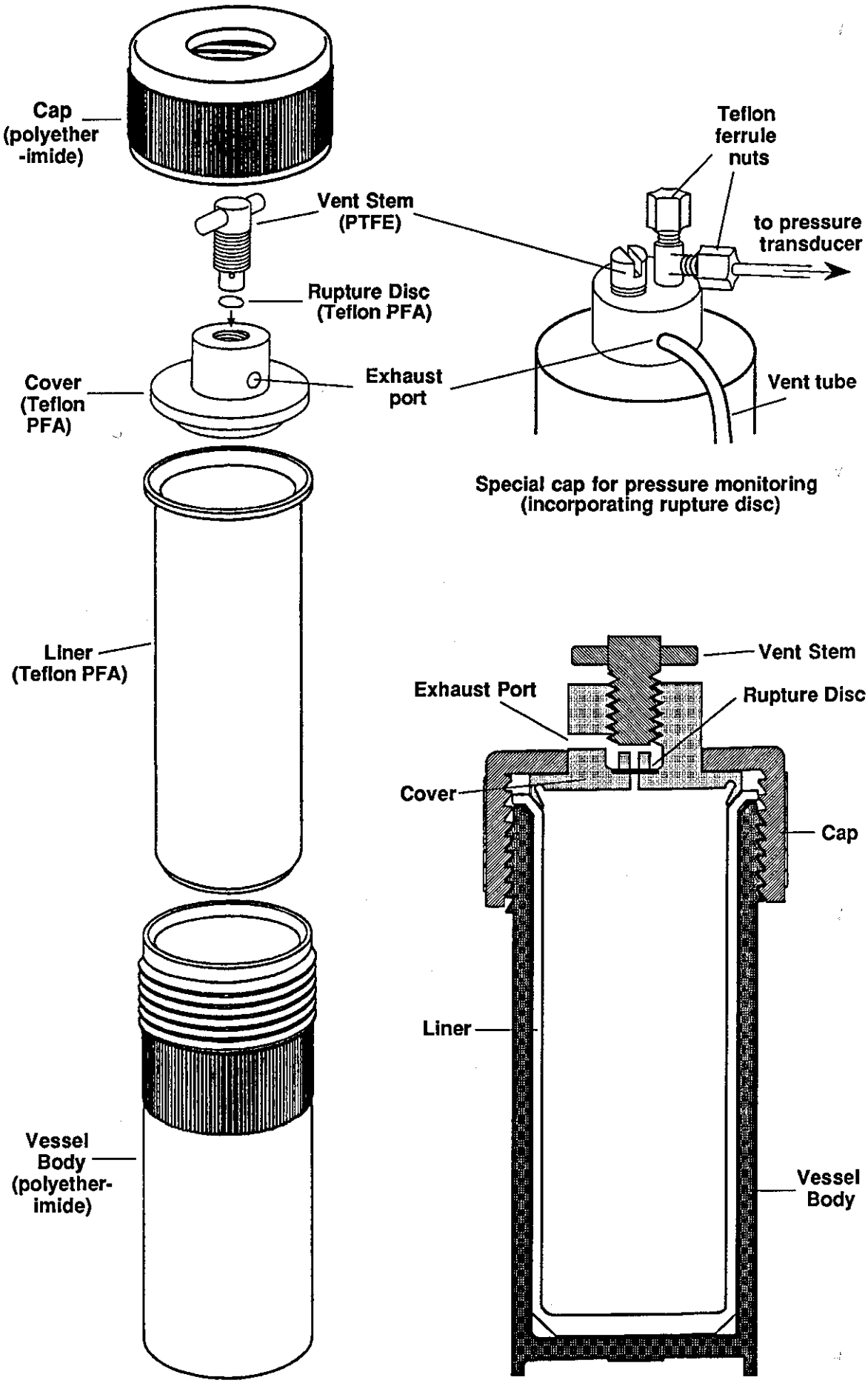

Fig. 4 Construction of lined digestion vessels (200 psi/1380 kPa, CEM)

disposable Teflon discs designed to rupture if the pressure rose too high (although these are more time-consuming to fit than the relief valves in the other vessels). Manual venting of these vessels was also more controllable, being accomplished by unscrewing the vent stem. The main disadvantage from the point of view of this work is that the outer polyetherimide shell, whilst designed for mechanical strength, is not nearly as thermally robust as Teflon PFA and therefore unsuitable for liquid nitrogen cooling. It is also attacked by perchloric acid, which could not be used with these vessels.

3.1.3 Pressure Monitoring Equipment

In order to monitor the pressure rise during digestions, one vessel in each batch was fitted with a special cap, as shown in Figs. 3 and 4, having two transfer ports (designed to enable simultaneous temperature and pressure monitoring); one was sealed with a Teflon plug ferrule nut, the other was attached via a Teflon open ferrule nut to a length of 1/8 in. (3.2 mm) o.d. Teflon tubing. This passed out of the microwave cavity, via a waveguide attenuator designed to prevent the escape of microwave radiation - incorporating narrow bore metal piping running along the inside wall of the unit between the cavity and the outer casing - and through an inlet/outlet port (diameter 6.2 mm) at the rear of the unit. The tubing was connected to a differential pressure transducer, as shown in Fig. 5.

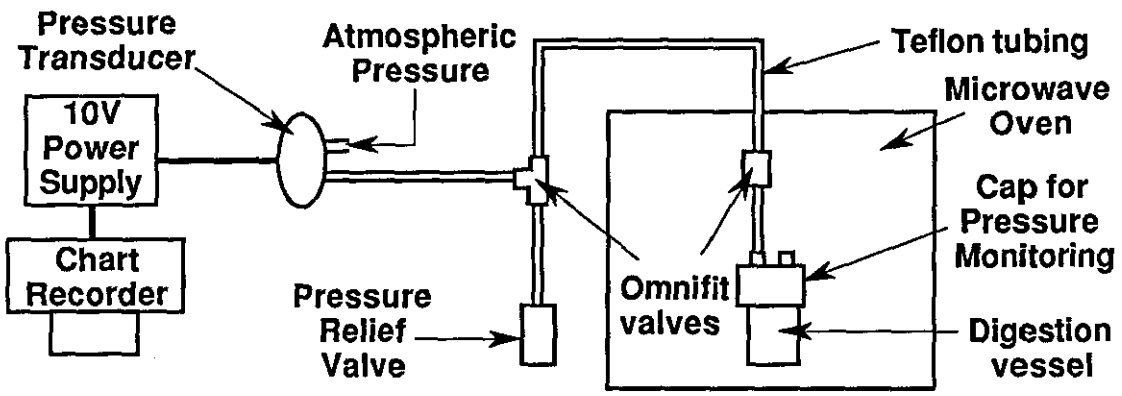

Fig. 5 Schematic Diagram of Pressure Monitoring Apparatus

For earlier work using the all-Teflon PFA vessels, a Sensym SX150DN pressure transducer, rated to 150 psi (1030 kPa), was used, and as the pressure monitoring vessel had no pressure relief valve in its cap (unlike the standard vessels), a brass safety valve, set to open at 120 psi (830 kPa), was incorporated into the pressure line, via a three-way Teflon valve (Omnifit, Cambridge, UK). The latter served to prevent corrosion and enabled easy disconnection for flushing the tubing with water between digestions.

For later experiments, a pressure transducer with an operating range up to 200 psi (1380 kPa), manufactured by Data Instruments, Acton, MA, USA, was installed. This was of a more substantial construction than the previous transducer, having a stainless steel housing and being more suitable for corrosive environments. It was connected to the pressure line tubing via stainless steel Swagelok fittings, and the power supply was modified from 10 V to the recommended 5 V. The new transducer was suitable for either the low or medium pressure vessels. The latter did not require a pressure relief valve in the pressure line, as the special cap for pressure-monitoring of these vessels incorporated a rupture disc for pressure relief, unlike its counterpart for the low pressure vessels.

A two-way Omnifit Teflon valve was installed in the pressure line close to the pressure monitor vessel in the oven, to enable easy and safe removal of vessels from the oven whilst maintaining pressure in the monitoring vessel. The valve, incorporating a Teflon body and a rotating Tefzel valve key and having a pressure rating of 500 psi (3450 kPa), joined a short length (ca. 10 cm) of tubing from the monitor vessel to the main pressure line tubing. The Teflon-Teflon seals at each side of the valve were made using gripper fittings (Omnifit), rated to 1000 psi (6900 kPa) and designed to allow frequent connecting and disconnecting, via finger-tight screw caps, without damaging the seals. After completion of a heating cycle, the valve was closed by a 90° rotation of the valve key and disconnected from the pressure line. The vessel, with the short tubing and valve still attached, could then be removed from the cavity, cooled and safely vented in a fume hood by returning the valve key to the open position.

This valve proved very useful at the operating pressures of the all-Teflon PFA vessels, but for the higher pressures reached in the lined vessels, intermittent leakage problems were encountered with the valve, and its use was discontinued for the later higher pressure work. The problem appeared to be caused by warming of the valve causing sufficient expansion of the stainless steel part of the gripper fittings to release their grip on the Teflon tubing (which resulted in the tubing blowing off on two occasions at 140 - 150 psi (970 - 1030 kPa)).

To prevent cross contamination of samples, the Teflon tubing was rinsed with deionized water between digestions. The tubing was then left partially filled with water to isolate the transducer and safety valve from acid fumes, and thus prevent corrosion.[16,85,88]

The pressure response of each transducer, as measured on a chart recorder, was calibrated by application of increasing pressures, in 10 psi (70 kPa) increments (as

indicated by the cylinder head gauge), from a nitrogen cylinder attached to the end of the pressure line. Each transducer showed a reproducible, linear response over its operating pressure range. The Sensym transducer gave a response of 1.3 mV psi^{-1} (with 10 V power supply), and the chart recorder sensitivity was adjusted to give a suitable reading over the required pressure range (full scale deflection of 200 mV, or 150 psi (1030 kPa). The Data Instruments transducer, operating with a 5 V power supply, gave a response of 0.3 mV psi^{-1}, and the chart recorder was adjusted accordingly (full scale 50 mV, or 200 psi (1380 kPa)). Both transducers gave responses within the manufacturers' specifications at the recommended power supply voltages.

A check on the readings using a second cylinder gauge indicated this calibration was sufficiently accurate for the purposes of this work. When using the Data Instruments transducer, signals appropriate to both low (100 psi, 690 kPa) and medium (200 psi, 1380 kPa) pressure vessels could be recorded by switching between sensitivity settings on the chart recorder. The calibration of the original (Sensym) transducer was checked with the tubing filled with water and found to be unaffected by the presence of the water up to 125 psi (860 kPa), when the pressure relief valve in the pressure line opened.

3.1.4 Turntables to Hold Vessels in Oven

i) Air Cooling

The removable 12-position carousel, as supplied by CEM with the oven, was used where no liquid nitrogen cooling was required in the oven.

ii) Liquid Nitrogen Cooling

A container made from polystyrene was used as a combined turntable/liquid nitrogen containment vessel in the microwave oven (see Section 5.2).

In both cases, the turntable was set to rotate alternately clockwise and anticlockwise (turntable switch to "ALT"), to avoid tangling the pressure line tubing.

3.2 Analytical Instrumentation

3.2.1 Atomic Absorption Analysis

Analysis for copper and manganese was accomplished with a Philips PU 9100 Atomic Absorption Spectrometer using an air/acetylene flame.

3.2.2 Infrared Analysis

Infrared spectra of organic residues from the digests were recorded on a Perkin-Elmer 1600 series Fourier Transform Infrared Spectrometer.

3.2.3 Elemental Analysis

Measurements of residual carbon content were carried out using a Perkin-Elmer 2400 CHN Analyzer.

3.2.4 Voltammetry

Voltammetric analysis was carried out using a Metrohm 626 Polarecord with 663 VA stand, with a hanging mercury drop electrode, saturated Ag/AgCl (3 mol l^{-1} KCl) reference electrode and glassy carbon auxiliary electrode.

3.3 Reagents & Samples

Samples of tomato puree (labelled P), tomato puree spiked with 10 $\mu g\ g^{-1}$ Cr and 50 $\mu g\ g^{-1}$ Al (Q), dried pet food (K), milk powder (M), dried sweet bay leaves (S) and sodium alginate (T) were provided by the Metallic Impurities in Organic Matter Subcommittee of the Analytical Methods Committee (AMC) of the Royal Society of Chemistry (Analytical Division). Results of previous analyses of these samples (hereafter referred to as the AMC samples) are available for comparison with this work.

Initial experiments were carried out using AnalaR grade concentrated nitric acid (SG 1.42, BDH, Poole, Dorset, UK and Fisons Scientific, Loughborough, UK). This grade of acid was also used for cleaning vessels via "acid blank" heating cycles in the microwave oven. Where analysis of the digests was carried out by ICP-MS, for digestions involving pre-digestion reflux, and for all experiments using the lined digestion vessels, samples were digested in AristaR concentrated nitric acid (70%, BDH), and made up to volume using triply deionized water from a Liquipure Modulab system (Liquipure Europe, Bicester, UK).

Spectrosol grade hydrogen peroxide (30% m/v) (BDH,) and AR grade perchloric acid (71%) (Fisons) were used for post-digestion treatments. HiPerSolv (HPLC grade) methanol (BDH) was used for extraction of residues.

CHAPTER 4

AIMS OF PROJECT AND INITIAL STUDIES

4.1 Broad Aims

The general aims at the commencement of this project were as follows:

i) To determine the degree of decomposition arising from microwave digestion of a variety of samples, using a range of analytical techniques.

ii) To develop microwave methods which produce complete dissolution and sufficient decomposition for trace element determination in these samples by different techniques.

iii) To examine the feasibility of using liquid nitrogen as a vessel coolant during the digestion process.

4.2 Hot-Plate and Ultrasonic Digestions

As a prelude to a detailed examination of microwave digests, in terms of their appearance and chemical composition, it was thought useful, for comparative purposes, to carry out some simple ultrasonic and hot-plate digestions, monitoring their progress visually and by ultraviolet (UV)/visible spectrometry. Digestions of tomato puree were carried out in open beakers on a hot-plate and in an ultrasonic bath; UV/visible spectra were run on aliquots of the digests at various stages.

For each experiment, a 1 g sample of tomato puree (AMC sample P) was weighed into a glass beaker (using disposable plastic spoons to transfer the puree) and 25 ml of concentrated nitric acid added. Three 15 min cycles in the ultrasonic bath failed to dissolve the sample completely, but after 5 min simmering on the (pre-heated) halogen hot-plate, the solution was deep yellow but fairly clear, and a completely clear yellow solution was obtained after 10 min. Heating was continued for a total of 60 min, making up to volume with nitric acid to replace evaporation losses, and taking aliquots at intervals of 5 - 10 min.

UV/visible spectra of the early solutions, for both the hot-plate and ultrasonic digestions, showed a strong broad absorbance at ca. 380 nm and a sharper peak at ca. 340 nm. (Due to the strong absorbance of nitric acid at wavelengths < 330 nm, the absorbance of these solutions could not be measured at shorter wavelengths). These peaks could be due to nitro substituted aromatics or conjugated aliphatics such as carotene.

In the case of the hot-plate treatment, as digestion progressed the broader peak shifted to shorter wavelength, eventually disappearing as a shoulder on the sharper peak. After 30 min digestion, no further change was apparent in the spectra except for a gradual diminution of the sharp absorbance maximum at 338 nm. The digests became progressively paler yellow with increased heating time. The changing absorbance in the visible region with the shift to shorter wavelength and gradual decrease in absorption intensity (with corresponding fading of the colour of the digest solution) could be due to the gradual breakdown of long conjugated double bond systems (from e.g. carotene) and nitro substituted aromatics.

In the case of the ultrasonic digests, the UV/visible spectrum showed virtually no change, even after 45 min treatment, the two strong absorbance maxima at 380 and 340 nm remaining, indicating that heat was needed for the digestion to proceed.

4.3 Initial Studies of Microwave Digestion

4.3.1 Testing of Pressure Vessels

The pressure capabilities of the all-Teflon PFA vessels (section 3.1.2 (i)) were tested by loading them with water (25 ml), capping them with a pressure relief disc inserted, and heating in the microwave oven (2 - 3 vessels at a time), along with a pressure-monitoring vessel. Of 13 vessels tested, only two reached the manufacturer's specified pressure (120 psi ± 10 psi (830 kPa ± 70 kPa)) before the disc valve opened. One reached 105 psi (720 kPa), but the others all vented at less than 90 psi (620 kPa): 7 of the 13 released the pressure at 70 - 80 psi (480 - 550 kPa) and one vented as low as 45 psi (310 kPa). The latter was exchanged for a spare disc and cap and achieved a pressure of 90 psi (620 kPa) before venting. By swapping the discs, caps and bodies of the vessels, and rejecting any discs with scratches on the sealing lip, the best combinations in terms of pressure capability were found. (These did not necessarily correspond to the vessels of best visual appearance). The best six vessel assemblies were then used for further work.

4.3.2 Digestion of Samples

Initial experiments were carried out using the Teflon PFA vessels and concentrated nitric acid to digest the samples. Digestions were carried out with four vessels in the microwave cavity - two vessels loaded with 1 g of sample and 10 ml acid, and two with 10 ml acid only. The internal pressure in one of the sample digests was monitored while microwave power was applied, initially at

100%, then at a reduced level as the pressure rose. A number of problems became apparent.

4.3.3 Problems Encountered

(1) Rapid pressure rise

In the case of the milk powder, pet food and sweet bay samples - especially the latter - the pressure rise was very rapid and difficult to control, as the pressure often continued to rise even after microwave power had been switched off. A typical pressure/time curve is shown in Fig. 6. Such pressure rises often resulted in a vessel's pressure relief valve opening, with loss of sample into the central vent trap.

(2) Incomplete dissolution in single heating cycle

Because of the need to prevent the internal pressure rising above about 100 psi (690 kPa), it was necessary, for all the samples except tomato puree, to carry out two or three heating/cooling/venting cycles to dissolve the sample sufficiently for analysis. This was a time consuming procedure, as the vessels had to be cooled to room temperature before they could be vented to release the accumulated carbon dioxide without risk of loss of sample. The sweet bay proved the most difficult sample to digest in this respect.

(3) Unreliable pressure performance

Even after selecting the "best" vessels and caps (see Section 4.3.1), the vessels were still prone to venting below their rated pressure, and this severely restricted the pressures at which they could be operated, in order to minimize the risk of premature venting leading to sample loss as an aerosol during digestions.

Because of this problem, the vessels were systematically tested using a method recommended by the manufacturers: each vessel was loaded with 30 ml water and heated individually for 10 min at 45% power. Under these conditions, venting between 6 and 8 min from the start of heating indicates satisfactory operation. It was found that some of the vessels vented early (4 - 6 min) and some late, but by exchanging rupture discs between vessels, all except one vessel performed within specification. This test, however, produces a steady pressure rise with gradual heating. It was concluded that the problem stems from the rapid pressure rise at relatively low temperatures encountered with the AMC samples (especially the sweet bay).

It was also found that slight disturbance of the vent tubing whilst the vessel was hot and under pressure often caused the vessel to vent, so care had to be taken

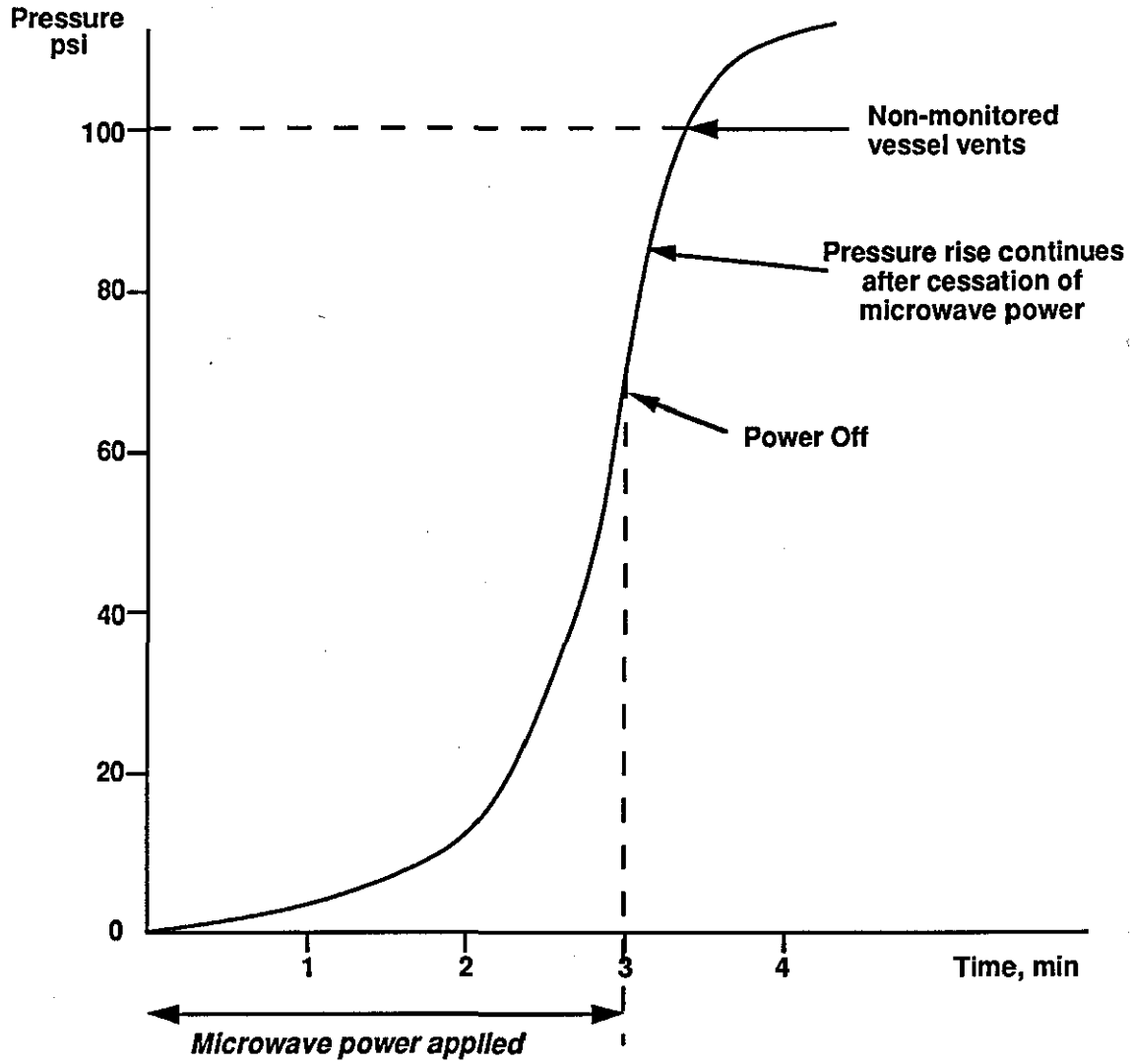

Fig. 6 Pressure rise in microwave digestion of sweet bay

when removing hot vessels from the oven for cooling. In addition, it was found that vessels which had been used for pressure-monitoring changed shape over time such that the standard caps would no longer fit them, so certain vessels had to be kept for use only with the pressure-monitoring cap.

(4) Incomplete digestion

Whilst complete dissolution could sometimes - though not always - be achieved, with multi-stage digests if necessary, (small amounts of siliceous material remained in the sweet bay digests and undissolved fatty residue in the milk powder and pet food digests: see Section 6.2), in no case was decomposition complete, as witnessed by the yellow colour of the digests, and UV/visible spectrometry. This could lead to potential interferences in analysis, particularly using techniques susceptible to organic interferences, such as electroanalytical methods.

4.3.4 Possible Solutions

(1) Control of microwave power

As the power of the microwave unit was controllable in 1% increments, the applied power was varied during a digestion in response to the monitored pressure, in order to try and control the pressure rise. Rest periods (i.e. 0% applied power) were also employed. However, it was found that pressure build-up occurred even with gentle heating, due to the formation of carbon dioxide during the decomposition process, and the temperatures reached were not high enough for complete decomposition. A typical pressure/time profile showing the effect of varying the applied power during a digestion is shown in Fig. 7.

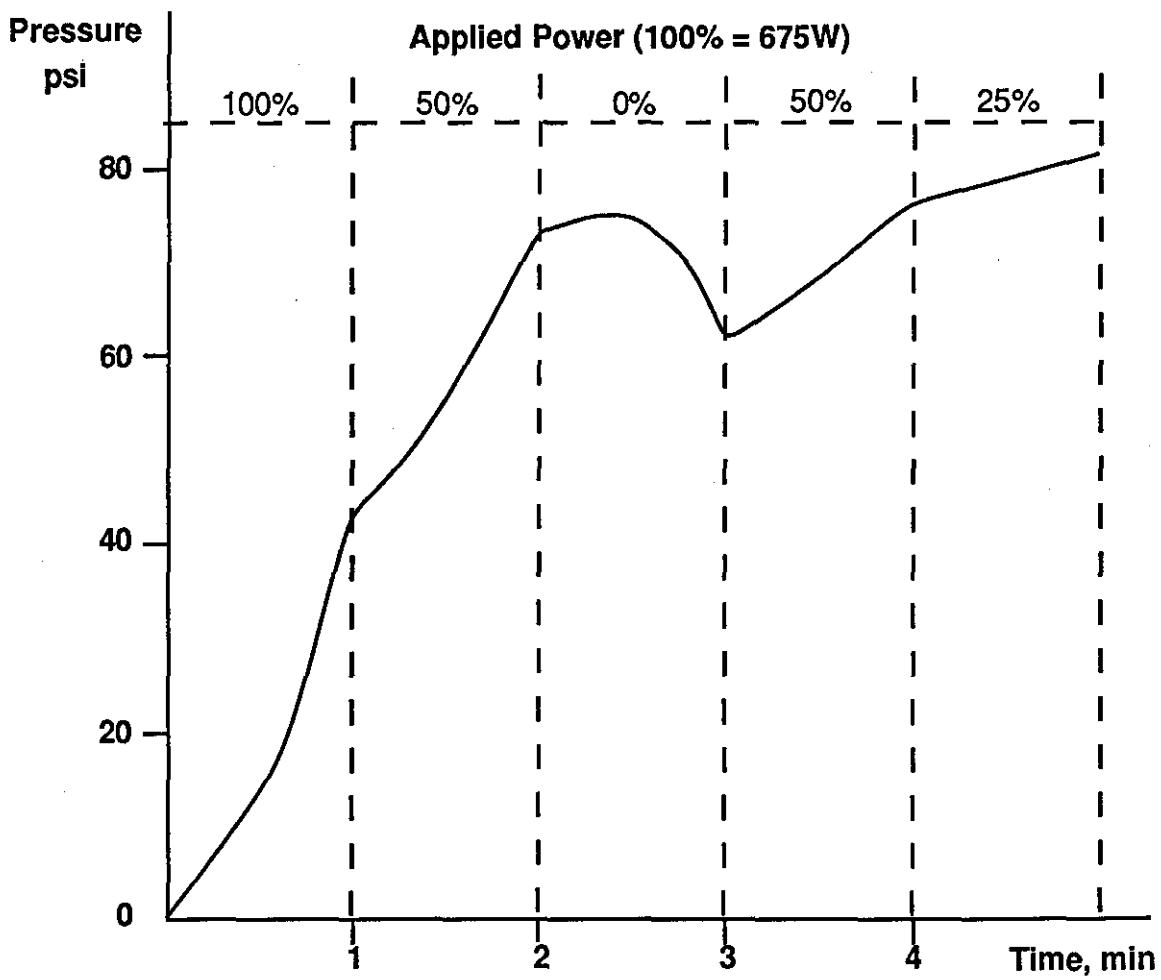

Fig. 7 Variation of applied power during the 2nd stage of a two stage digestion of sweet bay

(2) Use of diluted acid

It was suggested by the equipment manufacturers (CEM) that the use of diluted acid e.g. 8 ml conc. HNO_3 + 2 ml water, instead of 10 ml conc. HNO_3, could ameliorate problems of excess pressure. This was tried, but although the onset of the rapid pressure rise was delayed, it was not prevented, and premature venting

was still a problem.

(3) Liquid nitrogen cooling

The use of liquid nitrogen as a vessel coolant, to control the pressure rise and to effect rapid cooling at the end of a heating cycle, was investigated. The results of these investigations are presented in Chapter 5.

(4) Open-vessel pre-digestion

The incorporation of an open-vessel microwave pre-digestion stage into the procedure was investigated, the aim being to release the carbon dioxide formed from easily-oxidised materials, before sealing the vessels and carrying out a pressure digestion. After experimenting with covered, unsealed vessels for the pre-digestion, a reflux technique was developed to minimize evaporation losses. This work is detailed in Chapter 7.

(5) Use of higher pressure vessels

Though some of the problems associated with the all-Teflon PFA vessels were overcome by the means described above, and methods for the dissolution of the AMC samples were subsequently developed (see Chapter 6), vessels capable of withstanding higher pressures seemed preferable. The lined vessels obtained part way through this project (see section 3.1.2 (ii)) would effectively solve the problems associated with excess pressure encountered with the lower pressure vessels, single stage digests being sufficient for complete dissolution in all cases (except for the small amount of siliceous material remaining in the sweet bay digest, as before). However, the manufacturers recommend a maximum loading of 0.5 g when digesting organic samples in these vessels, so sample size is limited. The cooling process is also much slower with these vessels as liquid nitrogen cannot be used. The development of methods using lined vessels is discussed in Chapter 8, and an investigation of the degree of decomposition resulting from their use is presented in Chapter 9.

CHAPTER 5

EXPERIMENTS WITH LIQUID NITROGEN COOLING

5.1 Introduction

A major limitation of microwave digestion concerns the necessary delay in opening up the digestion bombs, as the contents must be cooled to room temperature before opening, for safety reasons as well as to avoid loss of volatile analytes. The digests retain their heat for a considerable time after microwave power is switched off, partly due to the nature of the microwave heating effect (the equivalent of "standing time" in microwave cookery) and partly because the Teflon digestion vessels are such poor conductors of heat. In addition, for larger (ca. 1 g) organic samples, the internal pressure often continues to rise after the cessation of microwave power, due to the evolution of carbon dioxide from continuing decomposition reactions. Hence the delay to allow the vessel contents to cool and the internal pressure to reduce to a safe level adds greatly to the overall time for sample preparation by this technique.

Various post-digestion cooling methods have been reported, as discussed earlier (Section 2.5), including the occasional use of liquid nitrogen.[15,101] It was felt that its potential usefulness as a coolant had not been fully explored, in particular for cooling before and during digestions, as well as after the end of a heating cycle.

As liquid nitrogen is transparent to microwaves, it will only experience heating through contact with the hot vessel walls (which are, in turn, indirectly heated through contact with their contents), and thus the cooling effect should be retained for the duration of a typical digest. The Teflon PFA vessels used in this project may be safely immersed in liquid nitrogen, and hence it was decided to investigate the feasibility of its use for vessel cooling *in situ*.

5.2 Design of Vessel to Hold Liquid Nitrogen

A containment vessel for holding liquid nitrogen in the microwave unit was made from moulded polystyrene (from packaging material), as it is a good insulator and effectively transparent to microwaves ($\tan \delta = 3.3 \times 10^{-4}$, see Table 1 in Chapter 1). The vessel, of square cross-section, could accommodate four evenly-spaced pressure bombs plus central venting trap (a fifth uncapped digestion vessel). The vessel walls were 3 cm thick, and the outer surface was wrapped in several layers of thin polythene film to minimize evaporation of the nitrogen through the porous walls, polythene also having a low $\tan \delta$ value of 3.1×10^{-4}. Four indentations were made in the base of the container to enable it to locate in

the turntable drive mechanism in the floor of the oven, and thus be rotated. Microwave irradiation of the empty container at 100% power for 5 min did not cause it to warm up, thus confirming its microwave transparency.

5.3 Initial Experiments with Acid Blanks

5.3.1 Unsealed Vessels

Four uncapped digestion vessels were placed in the polystyrene container, after loading two with 10 ml water and two with 10 ml conc. HNO_3. After liquid nitrogen was introduced, the water took 3 min to freeze and the acid 4 min. The container, complete with vessels and frozen contents and containing liquid nitrogen, was placed in the microwave cavity and heated for 2 min at 100% power. The water remained frozen, but the acid melted and reached a temperature of 60°C. After cooling to room temperature, more liquid nitrogen was added and the assembly was quickly transferred to the oven and again heated for 1 min at 100% power. The temperature of the nitric acid following this procedure was measured at 70°C, though it had probably been higher than this, as it was being rapidly cooled by the liquid nitrogen, reaching 20°C in under 1 min. The water remained frozen throughout the process, indicating the poor coupling of ice with microwave energy as mentioned earlier (Section 1.2.4 (ii)).

It was thus shown that very cold (even frozen) nitric acid will couple with the microwave energy to produce rapid heating, and on cessation of microwave power, the cooling is very rapid. The liquid nitrogen did boil away fairly rapidly in the polystyrene container (inside or outside the oven), making it necessary to "top up" every 3 - 4 min, so without better insulation this set-up would only be convenient for short digestion times.

5.3.2 Sealed Vessels

Microwave heating of four sealed Teflon vessels containing conc. HNO_3, with and without immersion in liquid nitrogen, led to the following observations:

a) The internal pressure rise in the vessels was slowed significantly by the presence of liquid nitrogen during the heating cycle. The peak pressure after heating for 3 min at full power was reduced from 70 psi (480 kPa) to 20 psi (140 kPa) (This, of course, may be an advantage for some samples).

b) The liquid nitrogen boiled off fairly quickly as the acid heated up; the 700 ml needed to immerse the vessels up to their caps evaporated within 5 min at 100% power. To avoid overheating (and possible melting of the polystyrene),

it was necessary to top up the container periodically with liquid nitrogen. This was done while the container was in the oven, using a Pyrex glass funnel and flexible PVC tubing passed through the open door, taking care not to crack the tube when very cold.

c) Liquid nitrogen cooling at the end of the heating cycle lowered the temperature and pressure very rapidly and enabled safe opening of the vessels within a few minutes.

5.4 Digestion of AMC Samples with Liquid Nitrogen Cooling

5.4.1 Cooling During Digestions

Microwave digestions were carried out on 1 g samples of tomato puree and sweet bay powder. These two samples showed very different behaviour when subjected to standard (non-cooled) microwave pressure digestion, the tomato puree producing a modest pressure rise and rapid dissolution, the sweet bay producing a rapid pressure rise, requiring multiple heating, cooling and venting cycles for complete dissolution. (This difference is probably at least partly due to the high water content of the tomato puree, which meant that only about 250 mg of organic material had to be decomposed).

In each case, four sealed vessels, three containing 1 g of sample and 10 ml of conc. nitric acid, the fourth an acid blank, were immersed in liquid nitrogen in the microwave unit and subjected to microwave heating.

In the case of tomato puree, 100% power was applied till the pressure reached 100 psi (690 kPa). This took 3 min 20 s, about twice the time taken without cooling. More liquid nitrogen was added for cooling, the monitoring vessel disconnected and the vessels removed from the oven. All the tomato puree digests had blue solid (frozen) deposits around the top of the liquid (the blue colour could be due to frozen N_2O_3 which is blue and freezes at ca. -100°C). On warming to room temperature, the blue colour disappeared to leave a yellow-green solution, the UV/visible spectrum of which showed significant amounts of organic residues (see below), indicating that although complete dissolution had been achieved in this single stage digestion, decomposition was incomplete.

Performing the sweet bay digestions whilst the vessels were immersed in liquid nitrogen reduced the pressure rise dramatically by comparison to an uncooled digestion, and enabled stabilisation of the pressure at 70 psi (480 kPa) at an applied power of 25% (170 W), following initial heating at 75% (510 W), on the

first heating cycle. Such pressure stabilisation could not be achieved with uncooled digests, even at low applied powers. However, two 10 minute heating cycles failed to produce complete dissolution, so it appeared the rate of digestion was being decreased.

It was found that when liquid nitrogen was used for cooling during the microwave digestion, vapour from the digestion mixture froze onto the walls of the vessel instead of refluxing back into the liquid. As microwaves only heat the liquid and not the vapour, the latter rapidly cools and freezes. Nitrogen dioxide, said by Würfels et al.[152] to play a vital role, in vapour form, in nitric acid pressure digestions, will freeze at these temperatures (f.p. –10°C). This will naturally slow down the rate of digestion.

5.4.2 Pre-Digestion Cooling

In an attempt to reduce the pressure rise without slowing the digestion unduly, vessels were cooled in liquid nitrogen before the start of sweet bay digestions. This postponed the onset of the pressure rise, but once started it was again rapid, and no improvement in digestion efficiency was found.

5.4.3 Post-Digestion Cooling

The cooling of hot pressure vessels after digestion by immersing them for 1 min in liquid nitrogen in the polystyrene container proved a very efficient means of cooling the digests down to (or below) room temperature, without freezing them. The vessels could then be safely vented and returned to the microwave unit for further heating, or opened and transferred to volumetric flasks for dilution with the minimum delay. Using this cooling technique, four 1 g tomato puree samples could be prepared in 7 minutes (from the start of heating to opening the first vessel). Samples such as pet food and milk powder which required a two-stage digestion, with cooling and venting to relieve excess pressure between two heating periods, could be processed in 20 min.

5.4.4 Decomposition Progress

To investigate the progress of decomposition of the tomato puree samples, using microwave heating and liquid nitrogen cooling, in comparison with the hot-plate digestions carried out earlier, multi-stage digests of 1 g tomato puree samples in 10 ml conc nitric acid were carried out as follows, with cooling, venting and opening of the vessels between the stages:

Stage (1): Digestion as described above, heating at 100% power for 3 min 20 s with four vessels immersed in liquid nitrogen in the polystyrene container.

Stage (2): The digests were heated at 100% power for a further 2 min in the turntable (without liquid nitrogen cooling during the digestion). The pressure only rose to 45 psi (310 kPa). Liquid nitrogen cooling enabled rapid opening of the samples (within 5 min of the end of heating).

Stage (3): Repeat of (2). The pressure rose to 20 psi (140 kPa).

Stage (4): The samples were heated at 100% power till one of the vessels vented at 90 psi (620 kPa) after 5 min, when power was stopped and the vessels transferred to the polystyrene container and liquid nitrogen added to cool. The monitoring vessel was vented and opened when the pressure dropped below 10 psi (70 kPa) 6 min from the end of heating, by which time the digests had cooled to around room temperature. The digests were now all pale yellow (after allowing the nitrogen dioxide to evaporate).

The progress of the multi-stage digestion was monitored as for the hot-plate digestions (see Section 4.2) by recording the UV/visible spectra of the digest solutions after each stage. After stage (1) the solution gave strong absorbance maxima at 440 nm, 380 nm and 335 nm. (i.e. an extra peak at 440 nm by comparison to the hot-plate digests discussed in the previous chapter). As decomposition progressed through stages (2) - (4), the digests showed absorbance maxima shifting to shorter wavelength as before (the "extra" peak gradually disappearing), eventually ending as a strong (but diminishing) peak at 335 nm with a shoulder at 340 - 350 nm, after stage (4). The extra peak compared to the hot-plate digests was probably due to dissolved NO_2, present in the pressure digests due its much increased solubility at elevated pressures (see Section 6.2); the similarity of the spectra in other respects suggests the microwave pressure digests follow a similar, though more rapid, course to digests using conventional heating at atmospheric pressure. (for explanation of absorbances, see Section 4.2).

5.5 General Observations

As explained above, the use of liquid nitrogen cooling during the microwave digestion slows down the digestion rate and may render complete digestion impossible due to the freezing of the reactants. A better solution may be to use liquid nitrogen for cooling after digestion is complete, where it reduces the delay in opening the vessels and processing the digests, or for slowing down the initial stages of digestions which produce rapid pressure rises (if reduction of microwave power is insufficient to control the pressure rise).

An illustration of the effect of liquid nitrogen cooling during a digestion is shown

in Fig. 8, where the initial pressure rise is slowed by immersion in liquid nitrogen; when this has boiled away, the pressure rises rapidly, but is controlled by the addition of further liquid nitrogen.

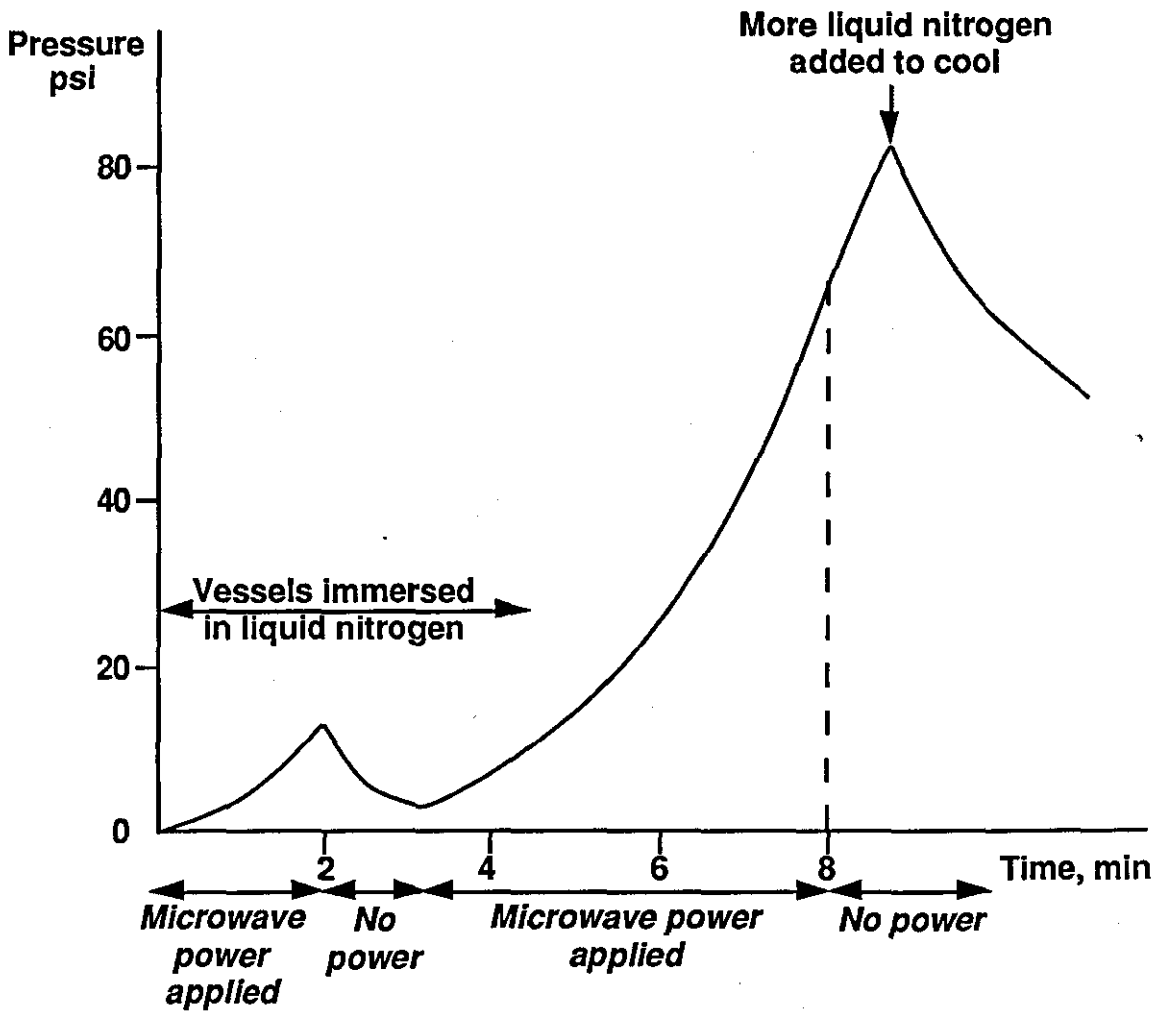

Fig. 8 Liquid nitrogen cooling during digestion of organic samples

Although simple and convenient to make, the polystyrene container was not ideal for holding vessels in the cavity for several reasons. Firstly it is limited to four vessels, secondly the vessels often become hot enough to melt the polystyrene when no coolant is present, thirdly the polystyrene acts as insulation preventing the air cooling of the vessels recommended by the manufacturers, and lastly the constrained position of the vessels' vent tubes, which is necessary for them to fit into the cavity, renders the pressure relief valves liable to open prematurely. This last effect was tested with several vessels and it was found that the position of the vent tube had a marked effect on the vent pressure. With the tube bent into the position required for the polystyrene container, the vessel vented 1 - 2 min earlier than with the vent tube rising vertically from the cap.

Undoubtedly these problems could be overcome by an alternative design in a

different material such as Teflon. However, the container was useful for immersing four vessels in liquid nitrogen for post-digestion cooling, following their transfer from the Teflon turntable. It was also successfully used for short digestions involving rapid pressure rises at relatively low temperatures, when the quick addition of liquid nitrogen in situ was effective in controlling the pressure rise and thus stopping the relief valves opening. It also proved particularly useful where several heating, cooling and venting cycles were required, greatly reducing the overall digestion times.

5.6 Conclusions

The use of liquid nitrogen for cooling during microwave digestion slows down the digestion due to the freezing of the reactants, although the moderation of the pressure rise when digesting samples of high organic content may be useful. An alternative is to use the liquid nitrogen for post-digestion cooling, when it counteracts any continuing pressure rise and greatly reduces the delay in opening the vessels and processing the digests.

CHAPTER 6

DEVELOPMENT OF DIGESTION METHODS FOR ORGANIC SAMPLES USING LOW PRESSURE VESSELS

6.1 Introduction

In the development of methods for the digestion of the AMC samples, the main aim was to minimise the time taken to achieve complete dissolution of 1 g samples. By varying the duration and level of applied power (using lower power settings and rest periods) it was hoped to develop a simple, rapid method for the digestion of each sample type. Several problems were encountered, as detailed in Chapter 4, the main one being the difficulty of adequately controlling the rapid pressure rises in the digestion vessels, and the tendency for premature venting to occur, thus compromising the digestion.

6.2 Appearance of Digests

It was found that all the samples gave vivid, deep green solutions (often cloudy) after digesting for a few minutes and reaching pressures over about 70 psi (480 kPa) (the green colour did not appear at lower pressures). The deep green colour is apparently due to NO_2 which is dissolved in the acid solution under pressure;[15] this colouration gradually disappeared on leaving the digests covered but unsealed at room temperature, and also on diluting the digests. As the digestion time increased and further heating cycles were carried out, the solutions became gradually paler and more yellow in colour.

Of the five matrices, complete dissolution was achieved in only two cases - tomato puree and sodium alginate, the latter sometimes requiring a second heating cycle. The pet food, milk powder and sweet bay all failed to give completely clear solutions, even after lengthy, multi-stage digestions. One of the problems was the difficulty of stabilising pressure in the vessels which would enable a longer heating time and thus more complete dissolution.

The pet food and milk powder both left small amounts of undigested fatty residues adhering to the sides of the vessel. Several workers have reported such residues when digesting biological materials,[92,99,119] and have found no detectable trace elements in these residues. Thus it was concluded that small amounts of fatty residue could be tolerated, as these could quite easily be separated from the digest solutions, as they adhered to the vessel walls, enabling the clear solutions to be decanted off.

The sweet bay left some undissolved particles, the siliceous nature of which was confirmed by IR analysis. (This is to be expected for botanical samples where HF has not been employed). If these were left to settle, a clear supernatant solution was obtained for analysis.

6.3 Digestion Methods

The following programs were found to produce clear, or almost clear, solutions, when used to digest four 1 g samples with 10 ml concentrated nitric acid, whilst keeping pressures below 100 psi (690 kPa). Total digestion times are measured from the start of heating to opening the first vessel (i.e. including cooling time). The letters in brackets after each matrix refer to the samples' identifying labels as they were received in the laboratory.

a) Tomato Puree (P & Q):

100% power for 1 min, then 75% power for 1 min 45 s.
Total digestion time : 7 min

b) Sodium Alginate (T):

Single stage:
100% power for 1 min, 50% power for 1 min 15 s, 0% power for 3 min.
Total digestion time: 11 min

Two stage:
(1) 100% power for 1 min, 50% power for 1 min 15 s, 0% power for 3 min.
Cool and vent, then
(2) 100% power for 2 min, 0% power for 1 min 30 s, 50% power for 30 s, 0% power for 3 min, then alternating 25% power & 0% power for 2 min each for a further 8 min (maintaining pressure at 70 psi (480 kPa)).
Total digestion time: 32 min

c) Pet Food (K):

(1) 100% power for 15 s, 50% power for 1 min, 0% power for 3 min.
Cool and vent, then
(2) 100% power for 30 s, 50 % power for 2 min, 0% power for 2 min.
Total digestion time : 21 min

d) Milk Powder (M):

(1) 100% power for 30 s, 50% power for 30 s, 0% power for 2 min.
Cool and vent, then
(2) 100% power for 1 min, 50% power for 1 min 30 s, 0% power 2 min.
Total digestion time : 20 min

e) Sweet Bay (S):

(1) Pre-digestion in unsealed vessels: 100% power for 30 s, 25% power for 5 min.

Allow to cool slightly, seal caps, then

(2) 100% power for 1 min, 25% power for 1 min, 0% power for 5 min, 25% power for 2 min, 0% power for 1 min.

Cool and vent, then

(3) 100% power for 2 min, 0% power for 2 min, 25% power for 5 min, 0% power for 5 min.

Cool and vent, then

(4) 100% power for 2 min, 25% power for 1 min, 50% power for 2 min, 0% power for 30 s, 25% power for 2 min.

Total digestion time: 75 min

The vessels were cooled with liquid nitrogen at the end of each heating cycle, until the contents reached room temperature (though the pressure was often still quite high, up to 50 - 60 psi (340 - 410 kPa)). The vessels were then vented in the fume cupboard to release excess gases before opening or returning to the microwave cavity for further heating. Such venting of the vessels had to be done carefully to minimise the risk of loss of sample as an aerosol,[80] but the alternative of further cooling the digests to below freezing before opening[104] increased the overall sample preparation time considerably.

6.4 Discussion of Methods

6.4.1 Speed of Sample Preparation

When considering the overall speed of a sample preparation method, factors which should be taken into account include

(a) number of samples prepared simultaneously
(b) time taken to weigh samples/load vessels
(c) heating time
(d) cooling time
(e) time for transfer/dilution of digests

Adding together the times (b) - (e) inclusive and dividing the result by (a), gives the average preparation time per sample. For the above methods, (a) = 4, (b) = 15 min, (e) = 10 min and (c) + (d) = total digestion times given above. This translates to the following average sample preparation times for the various matrices:

Tomato Puree: 8 min

Sodium Alginate:	9 min (1-stage); 14.25 min (2-stage)
Pet Food:	11.5 min
Milk Powder:	11.25 min
Sweet Bay:	25 min

It should be noted that the relatively short heating cycles employed in these methods resulted in digests whose quality was not always ideal in terms of digestion completeness as indicated by appearance - colour, undissolved residues etc. However, the use of longer heating cycles was limited by the difficulty of maintaining a stable pressure in the vessels. Better solutions could be obtained, but this necessitated further heating cycles, which added considerably to the sample preparation time.

6.4.2 Variation in Pressure/Time Profiles

There was considerable variation in pressure/time profiles recorded under the same apparent conditions (i.e. same sample type and size, acid volume, number of vessels and microwave program), making it difficult to predict what pressure rise would be produced by a particular heating program, thus making reproducibility a problem. This was especially true for the pet food and milk powder, where pressure rises varied greatly, and the above methods were a compromise, which sometimes resulted in a rather modest pressure rise in the first stage, but erred on the side of safety to prevent any runaway pressure rise.

When carrying out individual digestions, adjustment of the above methods, as necessary, according to the observed pressure rise, ensured adequate pressure rises whilst preventing over-pressurisation leading to venting. One factor in the pressure variability was the ambient temperature in the laboratory, which was found to have a significant effect on pressure rises in the early stages of a digestion (on colder days, pressure rises were up to 50% less than at warmer temperatures).

6.4.3 Notes on Individual Matrices

a) Tomato Puree

The above digestion program gave complete dissolution (yellow solution) and the peak pressure reached was 65 psi (450 kPa) above atmospheric. The high water content (measured at 74.3%) obviously contributed to the ease of this digestion , as only 250 mg of solid material had to be digested (in contrast to the other samples, which are all powders).

b) Sodium Alginate

A single stage digest was not always sufficient to produce a completely clear solution, as a white precpitate often remained, which disappeared after a second heating cycle.

c) Pet Food

As explained above, unpredictability of the pressure rise was a problem with this matrix. During the first heating stage, the pressure often continued to rise after the power was switched off, making adjustments during digestions difficult. However, pressure rises in the second heating stage were much more controllable, enabling adjustments to be made at that stage if necessary. The two stage method above gave a yellow-green solution with some fat residues.

d) Milk Powder

Again, care had to be taken to avoid a runaway pressure rise in the first stage of the digestion; liquid nitrogen cooling at the end of the heating time proved useful in preventing any continued pressure rise. The above method produced clear solutions with a thin film of fat residue on the vessel walls.

e) Sweet Bay

This proved the most difficult matrix to digest satisfactorily, due to the very high pressures produced. Various approaches were tried in an attempt to control the pressure rise whilst still heating the samples sufficiently to digest them. These included liquid nitrogen cooling before and during digestions, as detailed in Chapter 5, using diluted acid (see Section 4.3.4 (2)), and carrying out multi-stage digestions using higher pressure in the last stage.

However, the most successful approach was to carry out an open vessel pre-digestion. The vessels were loosely capped and heated for various times and powers, to allow gaseous reaction products of easily-oxidised material to escape before pressure digestion. Care had to be taken not to lose sample or acid through spitting or evaporation, and the relatively mild conditions detailed above were found to be suitable in these respects. The pressure rise in the subsequent closed digestion was much more controllable, although three further heating/cooling/venting cycles were needed to dissolve all but the siliceous material.

Alternatively, leaving the acid and sample to pre-digest at room temperature overnight (covered but unsealed) also helped to alleviate pressure rise problems in subsequent pressure digestions, but was not as effective as the heated pre-

digestion (and obviously took longer). Ultrasonic pre-digestion was also tried, to improve the mixing of the sample and acid (which was visibly poor for sweet bay), but a 10 minute immersion in an ultrasonic bath produced little improvement in mixing.

6.4.4 Effect of Reduced Sample Size

Although methods suitable for digesting larger (ca. 1 g) samples were sought, some smaller samples of sweet bay were digested, to study how great a part sample size played in the problem of excessive pressure rises. It was found that reducing the sample weight to 250 mg per vessel, and the volume of acid per vessel to 5 ml, enabled the digestion to be carried out easily in a single stage, 50% power producing a smooth, controllable pressure rise. Once the pressure had reached 70 psi (480 kPa), which took around 5 min, it could be maintained at this level (for, say, 5 min) by reducing the power to 25%. The digests produced in this manner were similar in appearance to those which had taken four heating/cooling/venting cycles using 1 g samples. The total digestion time (including cooling) was reduced from 75 min to 15 min using the smaller samples.

6.4.5 Effect of Hydrogen Peroxide

In an attempt to produce a clear solution after nitric acid digestion of the sweet bay, hydrogen peroxide (5 ml, 30%) was added to two such digests. One was subjected to a closed microwave digestion and produced a very rapid pressure rise which was difficult to control, although dissolution was quickly rendered almost complete. The other was heated in an unsealed vessel in the microwave oven, which produced only a very gradual improvement in dissolution. It was clear that the addition of hydrogen peroxide resulted in more complete oxidation than nitric acid alone, as previously reported,[80,85,97] and its use to oxidise residual organic material following nitric acid digestion offers some advantages over other post-digestion treatments such as perchloric acid (some of the safety precautions required when using $HClO_4$ can be avoided when 30% H_2O_2 is used, and there is no introduction of potentially interfering Cl).

The rapid pressure rise observed when using hydrogen peroxide in the pressure digestion mirrored a recent study[170] in which a spontaneous reaction was found to occur when using hydrogen peroxide and nitric acid to digest gram quantities of organic material. The volume of H_2O_2 added and the heating time/power must be carefully controlled to make sure the reaction does not go out of control. In view of this, it was decided not to pursue the use of hydrogen peroxide with the all-Teflon PFA vessels, due to their poor pressure-handling capabilities. Hydrogen peroxide treatment using the newer, lined vessels would be

investigated later (see Chapter 8).

6.5 Preparation of Digests for ICP-MS Analysis

Digests (in duplicate, with blanks) of all the AMC samples were prepared following the general procedures described in Section 6.3 above. A strict vessel-cleaning protocol was adhered to: vessels used for the samples were cleaned with detergent in an ultrasonic bath for 10 min, rinsed with water and soaked overnight in dilute nitric acid (10%). Immediately before use, all vessels were pre-blanked by loading with 10 ml conc. nitric acid, sealing and heating for 4 min in the microwave oven at full power. The acid was discarded before loading vessels with 1 g sample and 10 ml nitric acid, or 10 ml nitric acid for the blanks.

For all except the tomato puree digests, the power/time settings were modified during each digestion, as the pressure was monitored, to produce a suitable pressure (of around 70 psi (480 kPa)), and premature venting of vessels was not a major problem, although it did occur on occasions. The sweet bay digestion incorporated an open vessel pre-digestion followed by 3 pressure digestion stages, as shorter digestions left residues adhering to the vessel walls.

The following cooling protocol was followed: at the end of each heating cycle, the pressure monitor vessel was immersed in a Dewar of liquid nitrogen for ca. 30 s, until any digest solution in the tubing had returned to the vessel. The two-way valve was then closed, the monitor vessel disconnected from the pressure line and the turntable removed from the oven. The three standard vessels were then transferred to the polystyrene container into which liquid nitrogen was poured. After 30 s, the pressure monitor vessel was placed in the polystyrene container for a further 30 s cooling. All four vessels were then removed and the remaining liquid nitrogen poured back into the Dewar.

This cooling method was found to rapidly cool the vessel contents to room temperature or below, without freezing the digests. Care had to be taken when transferring the hot vessels not to physically agitate the vent tubes, as this tended to disturb the seal in the vessel cap causing venting. As this happened when removing the end of the vent tube from the central trap of the turntable, it was found convenient to use empty uncapped vessels in spare holes in the turntable as traps, thus enabling swift removal of the vessels without disturbing the seals.

Due to possible problems with chromium analysis if there are any organic residues in the digest - as interfering ArC^+ ions may be formed in the plasma - it was decided to do a short and a long digestion for one of the samples for

comparative purposes. To this end, sodium alginate was digested over both one- and two- stage programs.

The digests and blanks were diluted to 25 ml. A 2 ml aliquot of each digest was removed and diluted to 100 ml with the addition of indium nitrate at a concentration of 0.1 ppm indium as internal standard. A representative set of blanks were similarly diluted. These diluted digests and blanks were sent to Mr J Franks at ICI Wilton for ICP-MS analysis.

6.6 Results of ICP-MS Analysis

The results of the ICP-MS analyses are presented in Table 5. These generally show fairly good agreement between duplicate digestions of the same sample, given the measure of uncertainty of the semi-quantitative analysis. There are, however, some anomalies which require explanation.

The first column of results for each sample (i.e. T1, T3, S1, M1, P1, Q1 and K1) are for the digests in vessels attached to the pressure monitoring line, and therefore none of these samples vented during the digestion. The results in the second column of each pair are for the digests in vessels with standard caps and pressure relief valves. Some of these - Q2, S2 and K2 - vented during heating, and hence these digestions may be more incomplete. Although there is little evidence of loss/incomplete recoveries of elements by comparison to the unvented digestions, the vented samples were cloudier in appearance than the unvented samples, which were clearer with little or no undissolved residues.

The most obvious, and consistent, differences between the first and second columns are shown in the levels of Cu, Zn and Pb, which are all much higher in the first (pressure-monitored) digest than the second, except for T3 and T4 which are in good agreement. The reason for this would seem to be contamination from the pressure line, and in particular the brass safety relief valve and the brass fitting linking the pressure transducer to the tubing (as the original brass T-junction had been replaced by a Teflon valve, that potential source of contamination had been removed). Only T3 and T4 do not follow the pattern, and they were the only digests where no "acid blank" (as detailed in Section 6.5) was carried out (as they followed on from the previous sodium alginate digests T1 and T2, it was felt rinsing the tubing with deionized water would be sufficient). This implies that the blank run with acid extracted copper and zinc from the brass fittings into the pressure line (through acid vapour entering the tube) and this subsequently contaminated the sample digest.

Table 5. ICP-MS Semiquantitative Analysis Results

	Sodium Alginate, 1-stage dign		Sodium Alginate, 2-stage dign		Sweet Bay		Milk Powder		Tomato Puree		Spiked Tomato Puree		Pet Food	
	T1	T2	T3	T4	S1	S2	M1	M2	P1	P2	Q1	Q2	K1	K2
Mg	15	18	17	17	670	660	780	750	480	480	450	470	920	900
Al	18	19	13	11	42	60	2.8	ND	6.6	5.8	64	99	29	20
P	190	180	180	180	1200	1100	8500	7800	840	860	740	840	11K	10K
Ca	960	1000	900	900	10K	10K	13K	12K	460	460	420	460	11K	10K
Ti	0.73	2.0	0.38	0.52	ND	ND	0.41	0.78	1.2	1.4	1.5	0.74	2.3	6.7
V	ND	ND	0.36	<0.13	1.0	0.77	0.23	0.41	0.33	<0.13	0.36	<0.13	0.90	0.27
Cr	ND	ND	1.4	0.99	1.0	0.14	0.51	<0.13	1.0	0.54	12	14	1.4	1.3
Mn	39	40	39	40	29	28	0.14	ND	3.9	3.6	3.3	3.9	26	27
Fe	43	50	40	46	96	73	<10	<10	20	19	13	26	120	100
Co	ND	<0.13	<0.13	7.5	<0.13	<0.13	ND	<0.13	2.3	<0.13	ND	<0.13	0.57	0.55
Ni	<1	<1	<1	<1	1.3	1.1	<1	<1	<1	<1	<1	<1	<1	<1
Cu	0.66	0.26	0.68	0.63	37	11	6.0	0.27	23	3.9	42	3.3	14	7.5
Zn	25	0.24	1.1	1.2	150	29	100	29	76	3.7	230	5.4	140	42
As	0.89	0.28	0.41	0.17	ND	ND	ND	0.18	<0.13	0.68	ND	ND	0.93	1.1
Se	1.8	ND	ND	1.8	2.0	2.3	0.99	0.32	ND	ND	<0.13	ND	3.3	7.1
Rb	0.14	<0.13	<0.13	<0.13	2.7	2.7	20	19	10	10	9.7	10	3.2	3.1
Sr	7.6	7.9	7.9	7.7	29	29	2.0	1.9	2.0	1.9	1.9	1.8	21	20
Mo	0.41	0.51	0.35	0.53	0.22	0.18	0.40	0.16	5.4	0.35	<0.13	<0.13	0.47	0.32
Cd	<0.13	<0.13	ND	ND	<0.13	<0.13	0.13	ND	0.19	0.17	0.19	ND	<0.13	<0.13
Sn	<0.13	0.26	0.22	0.31	0.36	0.31	0.13	<0.13	3.8	3.0	2.5	2.6	0.24	<0.13
Ba	2.3	2.4	3.0	2.3	1.9	1.2	0.32	0.42	0.99	0.91	0.84	0.95	3.4	3.5
Hg	0.32	0.29	0.54	ND	0.98	2.1	0.73	ND	0.95	0.94	2.4	0.44	1.5	<0.13
Pb	3.3	0.39	0.84	0.59	30	19	14	ND	18	0.94	38	0.26	14	0.37
W	<0.13	<0.13	0.14	<0.13	ND	<0.13	ND	ND	38	11	ND	ND	<0.13	<0.13

Notes:

Results are expressed in $\mu g\ g^{-1}$ of sample "as received" (i.e. corrected for dilution factor of 1250) and have been subtracted with the relevant blank.

Reported accuracy is to within a factor of 2.

ND = not detected, i.e. <0.1 ng ml^{-1} difference between sample and blank.

To test this theory, six blank acid samples were heated in pairs, with and without connection to the pressure line, and with no cleaning of the pressure line tubing between runs. Microwave power was applied until the pressure in the vessels reached 90 psi (620 kPa), the usual maximum pressure for a sample digestion. The resulting "digests" were then analysed for copper by FAAS. However, no elevation of copper levels was observed for those connected to the pressure line, all six solutions giving copper levels below the detection limit. Tomato puree digests P1 and P2 were also analysed for copper by FAAS and the results agreed with the ICP-MS results, showing a much higher level for P1 than P2 (17 $\mu g\ g^{-1}$ and 2.9 $\mu g\ g^{-1}$ respectively).

The failure to reproduce the Cu contamination with acid blanks may be due to differences in the way the sample digests behaved. During pressure digestion of the samples, high pressures were reached at relatively low temperatures, through the formation of carbon dioxide, and part of the digest could be seen, as a dark blue/green liquid, in the pressure line tubing. This was then sucked back into the sample vessel on cooling in liquid nitrogen. The blanks, however, would only generate pressure at relatively high temperatures, through generation of NO_2 and boiling of the acid, and while it was difficult to monitor (as it does not turn a blue-green colour under pressure), it may well be that very little or no liquid acid entered the pressure line tubing to be contaminated with copper, zinc and lead.

As a result of this contamination apparently from the pressure line, it was decided not to pressure-monitor "acid blank" runs and to flush out the pressure line tubing immediately before each digestion with deionized water. Even then, Cu, Zn and Pb results from pressure-monitored digests should be treated with caution, and a duplicate digest with a standard cap should be prepared simultaneously for analysis.

It can be seen from Table 5 that the more rigorous two-stage digestion of sodium alginate (T3, T4), which gave completely clear solutions, produced higher recoveries for some elements, notably chromium, by comparison with the single stage digests (T1, T2), indicating possible losses of analyte in the precipitate left in the latter. The second milk powder digest gave low results for a few elements, notably chromium and aluminium. Whilst M2 did not appear to vent, there was much more undigested fatty residue in M2 than M1, indicating incomplete decomposition which could explain these low values. As also seen in the sweet bay values, the chromium results seem particularly sensitive to completeness of digestion. This could be due to interferences, caused by organic residues forming such species as $^{40}Ar^{12}C^+$ in the plasma, which interfere with the determination of Cr.[80]

6.7 Comparison with AMC Results

A summary of the Al and Cr results from Table 5, using the mean values for each sample - or the values from the more completely digested sample where there are discrepancies between pairs of results - is shown in Table 6. The results are compared with those from an inter-laboratory study (8 laboratories) involving digestions using conventional wet-ashing procedures.

As can be seen from Table 6, the ICP-MS results of analysis of digests prepared in this project and the results from the AMC interlaboratory study of the same samples is generally fairly good (although the spread of AMC results and the uncertainty in the semi-quantitative ICP-MS results make detailed comparison difficult). The only ICP-MS results falling outside the AMC range are those for Al in sample Q (spiked tomato puree), which is a little high, and Al in sample K (pet food), which is slightly low. However, if the 50% uncertainty factor in the semi-quantitative analysis is taken into account, these results are also in agreement.

Table 6. Microwave/ICP-MS v Hotplate/AAS&AES

Sample	Cr $\mu g\ g^{-1}$		Al $\mu g\ g^{-1}$	
	MW	HP	MW	HP
Milk Powder	0.5	0.5 - 2.8	2.8	0.5 - 6.5
Tomato Puree	0.8	0.1 - 1.9	6.2	1.5 - 8.0
Spiked Tomato Puree	13	8.1 - 14.3	82	36 - 60
Pet Food	1.4	0.9 - 2.5	25	31 - 56
Sodium Alginate	1.2	0.08 - 4.2	12	3.0 - 12.0
Sweet Bay	1.0	0.2 - 2.0	51	35 - 67

Notes to Table 6:

MW: Data obtained from microwave digestion/ICP-MS semiquantitative analysis, subject to a 50% uncertainty factor.

HP: Data obtained from hotplate digestion/AAS (flame & ETA) or ICP-AES (interlaboratory study); data supplied by the Metallic Impurities in Organic Matter Sub-Committee of the Analytical Methods Committee of the Royal Society of Chemistry (Analytical Division).

6.8 Conclusions

The all-Teflon PFA, 120 psi (830 kPa) vessels used in this work have proved generally unsuitable for single-stage digestion of 1 g samples of high organic content. If these vessels are used, excess pressure must be vented several times (cooling to room temperature before venting) before dissolution is reasonably complete. Even then, digestion with nitric acid alone is insufficient for complete decomposition of the samples investigated. Problems caused by unreliability of the vessel sealing and venting mechanisms have further added to the difficulties.

Analysis of digests of the AMC samples by ICP-MS has shown acceptable recoveries of Cr and Al, allowing for the uncertainties in the semi-quantitative analysis. The Cu and Zn results in the pressure-monitored digests indicated a problem of contamination from brass components in the pressure line. The premature venting of some samples caused them to be incompletely digested and this was reflected in some of the results.

It was concluded that the above methods (as detailed in Section 6.3) could be used for the digestion of 1 g organic samples for ICP-MS (or ICP-AES) analysis of a wide range of elements, providing certain precautions are taken:

(a) If pressure monitoring is employed (which whilst not essential is highly recommended, to prevent premature venting), metal components (relief valves/connections) should be excluded from the pressure line.

(b) Fatty residues/precipitates in the digests should be minimized, by carrying out further heating cycles if necessary. This is illustrated by the cases of sodium alginate, where low recoveries of some elements were obtained for the single-stage program, and milk powder, where the digest showing significant fatty residues gave lower results than the more completely digested sample. With hindsight, it would probably have been better to use a 3-stage digestion for milk powder, as for the sweet bay.

For analytical techniques more susceptible to organic interference, however, it is likely that more complete decomposition of the organic matrix would be required, possibly by the use of higher pressure vessels or a more powerful oxidising agent, or both.

CHAPTER 7
INVESTIGATION OF REFLUX PRE-DIGESTION

7.1 Introduction

It was shown during the development of a digestion method for the sweet bay (see Section 6.4.3 (e)) that an open pre-digestion step was useful in alleviating the problem of excessive pressure build-up during the first closed digestion stage. Such pre-digestions have also been reported in the literature, having been carried out either on a hot-plate[53,101,119] or, more quickly, by microwave heating.[89,112] This allows the escape of gaseous reaction products, notably carbon dioxide produced from the decomposition of easily-oxidised material, before the vessels are sealed. However, unless the temperature is carefully controlled (which is difficult when heating by microwaves, particularly where exothermic reactions are involved), this leads to loss of acid through evaporation and the potential loss of volatile analytes. A lengthy pre-digestion can negate one of the main advantages of microwave digestion, namely its speed, and the addition of further acid to compensate for evaporation losses is wasteful of expensive reagent and introduces higher impurity levels in the digests, of importance in ultra-trace analysis.

To benefit from the advantages of open-vessel microwave pre-digestion without suffering its drawbacks, it was decided to design a reflux system, using a coolant transparent to microwave radiation, whereby excess carbon dioxide could escape, but acid vapour would be condensed and returned to the digestion mixture.

Whilst the idea of a non-polar coolant liquid for open-vessel reflux in the microwave unit had been mentioned as a possibility,[8] it had apparently not previously been tried. Reactions under reflux using microwave heating have been carried out using a water-cooled reflux condenser outside the microwave cavity,[12,171] or by the rapid pumping of chilled water through a small reflux condenser in the microwave cavity.[172] The latter method results in a large proportion of the microwave energy being absorbed by the water rather than the reaction mixture, which proved useful for controlling temperature in studies of specific reactions, but is not very desirable for acid digestion where high temperatures are required, as well as being wasteful of energy. A semi-closed Teflon reflux vessel has been designed,[1] but this only allows for gentle heating and is not suitable for subsequent pressure digestion.

Possible coolant liquids were investigated; the one chosen had to be microwave

transparent (i.e. non-polar), have a low freezing point and (relatively) high boiling point, have low viscosity and preferably be non-flammable. Liquid nitrogen was a possibility, if the drawback of its low boiling point could be overcome. Heptane met all the requirements except for its flammability, being essentially microwave transparent (tan $\delta = 1 \times 10^{-4}$ at 3000 MHz and 25°C [7]) and having a useful liquid range (–90°C to +98°C).

7.2 Design of Reflux Apparatus

7.2.1 Simple Cold Finger

As an initial attempt, a "cold finger" was designed and constructed from borosilicate glass (which has a reasonably low dissipation factor, tan $\delta \approx 10.6 \times 10^{-4}$), to fit onto a digestion vessel. This consisted of an open cup which sat on the rim of an uncapped vessel, leading into a finger-shaped tube protruding downwards into the vessel, as shown in Fig. 9. The open top allowed it to be filled with liquid nitrogen for cooling during a pre-digestion.

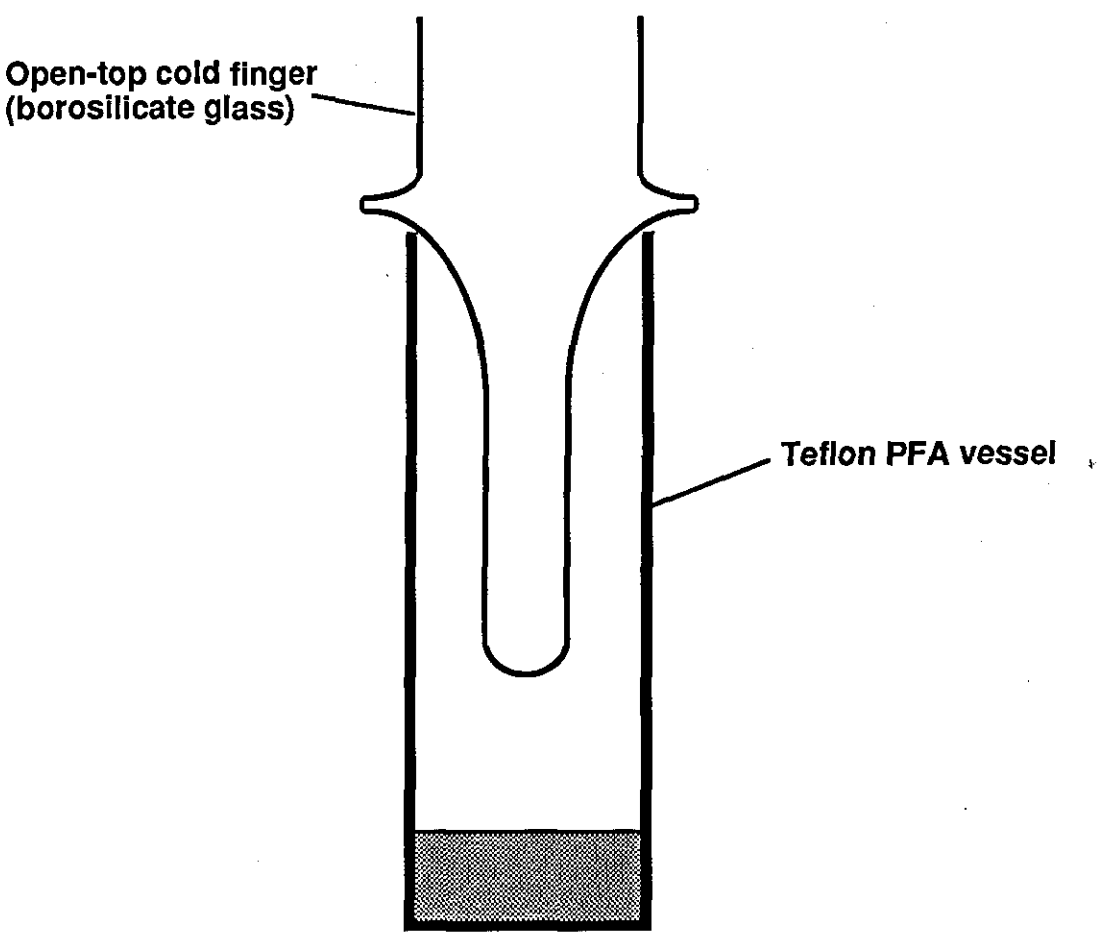

Fig. 9 Simple cold finger

This simple glass finger was tested for its usefulness in preventing sample loss in an unsealed pre-digestion. Water was heated in the Teflon vessel, with the finger - and cup above it - filled with liquid nitrogen. As soon as the water boiled, the liquid nitrogen very rapidly boiled away, the finger became hot, and water spat out from the rim of the vessel. This form of reflux cooling was therefore insufficiently controllable, so it was decided to try pumping a coolant liquid through the finger. For the reasons stated above, heptane was felt to be a suitable coolant, but due to its highly flammable nature, the possibility of ignition from a spark from the electrics could not be excluded, so a completely enclosed system was needed.

7.2.2 Reflux Condenser

A completely enclosed finger was constructed, with inlet and outlet ports for the coolant. However, initial difficulties with the interface between the finger and the vessel led to an alternative design of a small reflux condenser, as shown in Fig. 10, which is used in conjunction with the vessel's own cap, making the vessel/condenser interface more stable. It also has a smaller capacity than the finger design (35 ml as opposed to 60 ml) and hence can be more rapidly replenished with coolant.

Experiments with this condenser proved the viability of reflux pre-digestion in the microwave oven using a microwave transparent coolant (see Section 7.7), but the condenser suffers from several disadvantages. As it has to be fairly short to fit into the microwave cavity, it has a smaller surface area in contact with the digestion vapour and hence a potentially less efficient condenser effect, and it was found necessary to use a glass stopper to prevent escape of acid vapour. It is also fairly cumbersome and relies on an efficient seal between the vessel cap and the condenser assembly. Hence the cold finger idea was re-investigated.

7.2.3 Enclosed Cold Finger Assembly

After several design modifications, the assembly shown in Fig. 11 was made. This includes symmetrical inlet/outlet ports, and a broad rim to enable it to seat more firmly in the vessel. These features are necessary to enable the assembly to remain stable whilst being rotated on the turntable with the tubing attached to the ports via Teflon connectors. Four grooves were made in the rim of the finger assembly to prevent the interface between finger and vessel becoming sealed by freezing of condensed vapours, as happened when cold heptane was pumped through the ungrooved finger while heating nitric acid in the vessel.

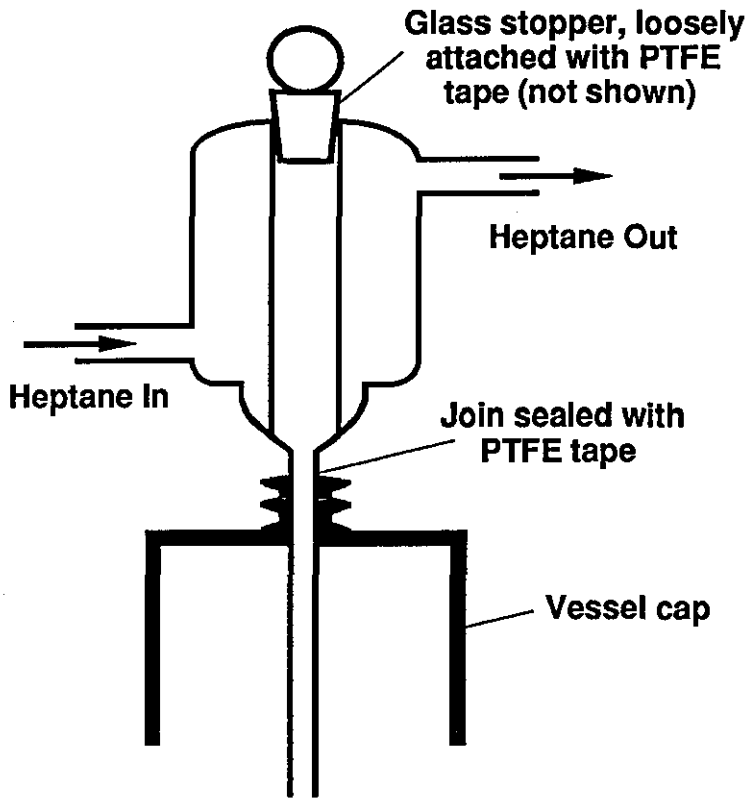

Fig. 10 Borosilicate glass reflux condenser

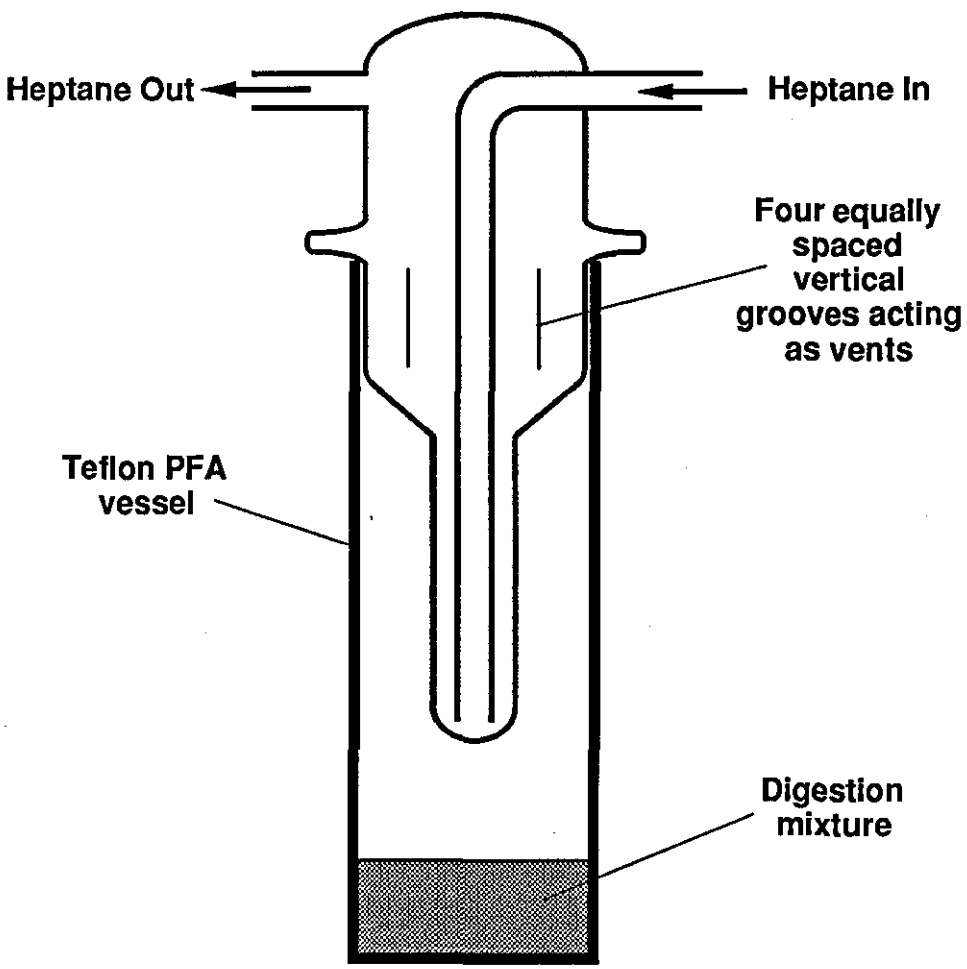

Fig. 11 Borosilicate glass cold finger assembly

Initially, the whole of the assembly above the vessel rim was insulated with a jacket of polystyrene to prevent the outside of the glass from frosting up, with subsequent melting of this ice due to heating by the lamps and the microwaves. However, when the top of the assembly was insulated in this way, the glass heated up significantly when subjected to microwave energy, whilst microwave irradiation of the empty, uncovered glass finger produced negligible heating. A Teflon jacket was constructed out of the screw-threaded section of an old digestion vessel and tied around the reservoir with PTFE tape. This prevented the outside of the reservoir from frosting up, whilst allowing cooling of the top by the fan in the microwave cavity.

7.3 Coolant Delivery System

The coolant, laboratory-grade n-heptane, dried over molecular sieve, was delivered from a 1 litre shielded glass bottle, cooled by immersion in a dry ice-methanol slurry and pressurised using nitrogen to produce a flow rate of approximately 100 ml min^{-1}. It was necessary to use all-Teflon connections to the 3-valve cap (Omnifit) fitted to the bottle, as use of metal (in valves with metal screw threads, or gripper fittings) caused slight leakage when the bottle was cooled, with consequent loss of pressure and lower flow rates. This was presumably due to a difference in contraction of the metal and Teflon in the seals at low temperatures, as there was no leakage at room temperature. (Differences in thermal behaviour of Teflon and metal caused a similar problem with valves in the pressure line; see Section 3.1.3).

The heptane was delivered to the microwave cavity via $^1/_8$ " (3.2 mm) o.d. Teflon tubing which entered and left the cavity through inlet/outlet ports at the rear of the unit. The tubing carrying heptane out of the microwave unit was fed into a 1 litre bottle through a vented stopper. The heptane was then returned to the pressure bottle and cooled for the next pre-digestion cycle. Temperature measurements of the heptane, before and after it flowed through the cavity, were made using a Technosyn temperature probe. The system is shown schematically in Fig. 12.

Whilst the above system produced sufficient cooling for reflux pre-digestion to be carried out, its use was limited by the time taken to exhaust the coolant supply from the 1 litre bottle. A better method would be to circulate the coolant liquid in a closed, continuous loop system where the heptane could be circulated for an indefinite period. A refrigerated circulator (Model F81-VC, Julabo Labortechnik GmbH, Seelbach/Schwarzwald), having a pumping system rated at 10 l min^{-1} with no back pressure, was available for use in the laboratory. This would be

ideal if a sufficient flow rate could be achieved, so the possibility of its use was investigated. Methylcyclohexane (recommended by the manufacturers as suitable for use in the circulator) could be used as a microwave transparent coolant. The problem was the reduced flow caused by the narrow (1.5 mm i.d.) Teflon tubing used for delivery into the microwave cavity. Wide bore (10 mm i.d.) tubing was used to carry the coolant to and from the oven port to the circulator, but this still left some 2 m of narrow tubing (the minimum length required for delivery through the transfer port, and to and from the reflux assembly in the microwave cavity), and the flow rate was only 50 ml min^{-1}, insufficient for adequate cooling. Hence the circulator could not be used effectively.

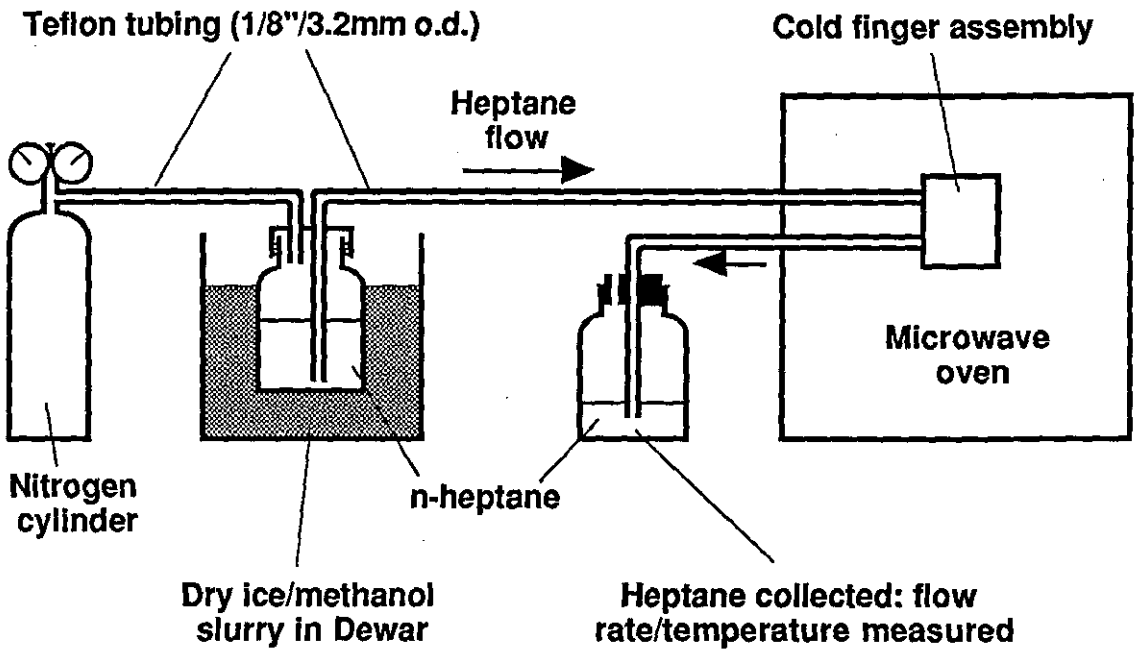

Fig. 12 Schematic diagram showing flow of heptane coolant

7.4 Measurement of Evaporation Losses

Suitable conditions for reflux pre-digestion, in terms of time, microwave power and flow rates, were found through several heating cycles with the vessels containing nitric acid alone. Heating times and applied pressure were adjusted so that the coolant could be pumped through at a sufficient rate to keep the finger cold, but not so fast that the limited volume of cooled heptane was depleted too quickly. The temperature of the heptane emerging from the microwave unit was monitored, so that it was kept well below room temperature (<10°C), and heating was timed to allow a further 2 min of heptane circulation after the end of the microwave program. Suitable conditions for two vessels (one with reflux condenser, one unsealed), each containing 10 ml conc. nitric acid, were found to be:

Coolant: 1 litre of heptane, pre-cooled to *ca.* –50°C in dry ice-methanol slurry

Applied pressure: 12 psi (83 kPa)

Flow rate: 100 ml min^{-1}

Microwave program; 50% power for 7 min

The efficiency of the reflux was tested by comparing the mass loss from 10 ml acid in a vessel using the cold finger, to that from 10 ml acid in an unsealed vessel (with its cap loosely fitted) heated simultaneously at 50% power for 7 minutes, measurements being made for six replicate runs. These showed consistently low mass losses of 0 - 0.3 g from the reflux vessels, whilst losses from the unsealed vessels varied from 0.5 - 4.6 g (mean 2.1 g) for different vessels. This variation could be partially due to the fact that some caps fitted more tightly than others when partly unscrewed.

It was also noticed that boiling was instigated more quickly in some vessels than others when heated simultaneously, and this resulted in higher evaporation losses when it occurred in the unsealed (non-reflux) vessels. As the samples were continuously rotated, this could not be due to inhomogeneity of the microwave field, or variation in the cooling effect of the fan in different parts of the cavity. A possible explanation is found by considering differences in superheating above normal boiling point due to differences in the condition of the internal vessel walls.[13] Fine scratches and abrasions on the walls of some vessels may discourage superheating by providing active sites for boiling, thus resulting in the acid boiling at a lower temperature than in vessels having very smooth internal surfaces (see Section 1.3.2). It was also found that the reflux vessel cooled much quicker than the open vessel, enabling easier handling.

7.5 Comparison of Sweet Bay Digestion Regimes

7.5.1 Comparison of Mass Losses

Using conditions described above, two pairs of 1 g samples of sweet bay were pre-digested in the microwave cavity by heating them together at 50% power for 7 min. The cold finger condenser was fitted to one of each pair of vessels, whilst the other had its cap loosely fitted. A pair of blanks were similarly prepared. The efficiency of the reflux was tested by comparing the mass loss from the reflux vessel to that from the unsealed vessel. These were also compared with the mass loss resulting from a completely sealed pressure digestion of a 1 g sample of sweet bay, when microwave power was adjusted to give a pressure of 80 psi

(550 kPa) over three heating, cooling and venting stages.

For each pair of sweet bay pre-digestions, the open vessel almost boiled dry, whilst the acid level in the reflux vessel was only slightly reduced. The mass losses are shown in Table 7.

Table 7. Mass loss during microwave acid pre-digestion of sweet bay; comparison of reflux, unsealed and sealed vessel methods.

	Mass Loss/g				Equivalent Vol. of HNO_3/ml
	Reflux	Unsealed	Sealed	Difference/g	
Run (A)	2.3	10.5	—	8.2	5.8
Run (B)	2.2	11.8	—	9.6	6.8
Run (C)	—	—	2.8	—	—
Blank Acid	0.2	1.0	—	0.8	0.5

Comparison of weight loss from the reflux vessels with that from the sealed vessel strongly indicates that the weight loss in the reflux digestions is due to evolution of gaseous products, particularly carbon dioxide, from the decomposition of the sample, rather than acid loss through evaporation.

As the evaporation losses from the acid blanks were much less than from the sweet bay digestions, more acid had to be added to the latter to replace these losses. Thus the level of impurities in the blank would not reflect that in the samples due to the differing volumes of acid used, and hence would be difficult to allow for in low-level analyses.

7.5.2 Comparison of Manganese Levels

The volume of acid in the unsealed vessels was made up to that in the reflux vessels (i.e. to compensate for different mass losses), before capping and sealing the vessels and carrying out pressure digestions to complete sample dissolution. These required two or three heating, cooling and venting cycles before the digests were clear. In each heating cycle, applied microwave power was adjusted to achieve and maintain an internal pressure of 70 - 80 psi (480 - 550 kPa) for 15 min. Following pressure digestion, the digests were transferred to 25 ml volumetric flasks and made up to volume with water. The solutions were stored in 60 ml

polypropylene screw-capped bottles prior to analysis.

Manganese levels were determined by FAAS, with operating parameters as given in Table 8. Calibration standards were prepared in 10% nitric acid by dilution of a standard solution containing 1000 mg l^{-1} of manganese (Pierce Inorganics, Rotterdam). To check for matrix interferences, an aliquot of each digest was diluted by a factor of two and reanalyzed; the measured manganese concentrations were half those in the original digests, and hence no matrix effects were apparent. For comparison, manganese levels were also measured in digests produced using two completely closed pressure digestion methods.

Table 8. Operating parameters for the determination of Mn by FAAS

Flame	Air/acetylene
Lamp current	9 mA
Wavelength	279.5 nm
Slit width	0.2 nm
Measurement mode	Integrated Absorbance
Integration time	5 s

The results of analysis of the sweet bay samples by FAAS are summarized in Table 9. No significant differences were found between measured manganese concentrations following the various pre-digestion methods. The results also agreed with the Mn concentration determined in the sweet bay after a single stage closed microwave digestion of four 250 mg samples, when the digests were combined for analysis and Mn concentration was measured at 25.5 ± 0.2 μg g^{-1}.

Table 9. Determination of manganese concentration in sweet bay powder

Digestion Method*	Mn conc./μg g^{-1}	No. of determinations
A	25.0 ± 0.6	8
B	24.9 ± 0.6	7
C	24.5	2
D	24.8	2

Notes to Table 9 on next page

Notes to Table 9:

*Samples prepared for FAAS analysis by microwave pressure digestion following four different pre-digestion methods:
A: Microwave pre-digestion under reflux;
B: Microwave pre-digestion in unsealed vessel;
C: Pre-digestion at room temperature for 18 h;
D: No pre-digestion (lengthy multi-stage pressure digestion).
In all cases, 1 g samples were digested with 10 ml conc. HNO_3.
Results are given as mean ± standard deviation.

7.6 Coolant Temperature Measurements

The effectiveness of cooling was monitored by measuring the flow rate and temperature of the coolant at 1 - 2 min intervals after it emerged from the outlet tube from the microwave unit. The initial temperature of heptane at the top of the pressure bottle (representing the warmest part of the vessel and hence the maximum initial coolant temperature) was measured before sealing the bottle prior to each run.

The initial temperature of the heptane at the top of the pressure bottle lay between –25°C and –35°C. The maximum outlet temperature of the heptane coolant was measured at 13°C, during the last minute of heating, rising gradually from 2°C after the first minute of heating. This fell to 0°C 2 min after heating ended. The flow rate remained stable at 100 ml min^{-1}.

7.7 Alternative Reflux Condenser Design

The alternative design of the small reflux condenser, as shown in Fig. 10, was also tested for its efficiency as a pre-digestion aid, and initial experiments were encouraging in terms of reduced evaporation losses of nitric acid, by comparison to heating digests in covered, unsealed vessels (i.e. with their caps loosely fitted).[173] The outside of the condenser was insulated with polystyrene, and the coolant delivery system was as described above. However, because of the reduced surface area for condensation, the microwave power had to be carefully controlled and the top of the condenser loosely plugged with a glass stopper to prevent significant losses of acid vapour, whilst still allowing the escape of carbon dioxide. As with the cold finger design, nitric acid digestions of sweet bay were carried out with (a) reflux pre-digestion and (b) pre-digestion in unsealed vessels, followed by pressure digestion in each case. Determination of manganese by FAAS in these digests showed no significant differences between pre-digestion methods.

7.8 Conclusions

Reflux pre-digestion using a microwave-transparent coolant proved useful in avoiding loss of acid whilst heating the digests in unsealed vessels to liberate evolved carbon dioxide, prior to sealing the vessels for a more rigorous, pressure digestion. No losses of manganese were found, and whilst it is appreciated that the method would need to be evaluated for more volatile elements, initial results on acid loss indicate that volatile elements are more likely to be quantitatively recovered using the reflux method by comparison to an open-vessel pre-digestion. Also, the lower volumes of acid required should lead to lower impurity levels and thus lower limits of detection.

CHAPTER 8

DEVELOPMENT OF DIGESTION METHODS FOR ORGANIC SAMPLES USING MEDIUM PRESSURE VESSELS

8.1 Introduction

Following problems experienced in the digestion of larger organic samples using the all-Teflon PFA (120 psi, 830 kPa) vessels (see Chapter 4), it was hoped that the use of a new set of lined (200 psi, 1380 kPa) vessels would enable complete dissolution of the samples to be effected in a single heating stage. Although cooling of these vessels was slower than for the low pressure vessels (due to their double skin and the inability to use liquid nitrogen), it was anticipated that the higher pressure capabilities and more reliable seal should simplify digestion procedures, and produce a greater degree of decomposition. The manufacturers' recommended maximum sample size of 0.5 g was a problem, although digestions in these vessels using larger sample sizes have been reported,[170] and with careful pressure monitoring and suitably chosen conditions, it may be possible to develop methods for 1 g samples. If not, the problem could be overcome by combining pairs of digests if required.

The use of hydrogen peroxide in a second digestion stage would also be investigated to see if the degree of decomposition was improved. Whilst single stage nitric acid/hydrogen peroxide digestions in these vessels are possible, spontaneous reactions may be instigated,[170] and with vessel cooling more difficult than with the low pressure vessels, this was felt to be too risky, as the pressure rise may be difficult to control. Indeed, the manufacturers recommend that where hydrogen peroxide is used, it is added following a heating cycle with nitric acid alone.

To achieve complete decomposition, it was thought likely that treatment with perchloric acid, following the nitric acid acid digestion, would be required and this would also be investigated.

Of particular interest when considering the degree of decomposition are involatile organic compounds which cannot easily be removed by evaporation without loss of analyte. The percentage of carbon remaining in the involatile residues of the digests would provide a measure of completeness of decomposition, enabling a comparison of decomposition efficiencies of various digestion regimes as applied to different sample types.

8.2 Initial Experiments

8.2.1 Pressure Monitoring

Some difficulties were initially experienced in obtaining a leak-tight seal between the pressure line tubing and the transfer port on the new pressure monitor cap; during tests with water, the tubing tended to blow off at about 150 psi (1030 kPa). The seal was much improved by annealing the new cap with the tubing attached at 120°C, but repeated connect/disconnect operations tended to deform the tubing (by repeated compression and release) and degrade the seal. The use of the two-way Omnifit valve enabled disconnection without disturbing this seal.

Heating four vessels containing 10 ml nitric acid produced smooth pressure rises of 25 psi min^{-1} (170 kPa min^{-1}) at 100% applied power, and 5 psi min^{-1} (34 kPa min^{-1}) at 50% power. When the pressure had reached 100 psi (690 kPa), application of 25% power resulted in a steady decline in pressure of 8 psi min^{-1} (55 kPa min^{-1}). These pressure/time profiles for blank acid were useful for comparison with sample digests, as they showed the background pressure due to acid vapour, enabling evaluation of pressure rises due to evolution of decomposition gases as opposed to evaporation of acid. This would give an indication of digestion completeness (i.e. when the pressure rise matches that due to acid alone, decomposition has gone as far as it can with the acid used).

It was found that, contrary to the manufacturers' instructions, the disposable rupture membranes could be used several times without failure. They would change shape, the pressure causing a circular raised area to appear in the centre of the disc, but this did not cause them to burst prematurely on subsequent runs. However, when high pressures were employed over relatively long periods, it was advisable to change the rupture discs between digestions. This was confirmed when one re-used rupture disc burst at 100 psi (690 kPa), following a gradual pressure rise over a period of 12 min, and the rupture discs were therefore changed prior to each digestion.

8.2.2 Vessel Cooling

Liquid nitrogen (or even dry ice) cooling is not recommended for these vessels as they are not nearly as thermally robust as the all-Teflon PFA vessels (the outer vessel is made of polyetherimide for mechanical rather than thermal strength). CEM recommend ice-water cooling.

Due to the double-walled nature of the vessels it was difficult to tell when the vessel contents had been cooled sufficiently, as the temperature of the outside

walls of the vessels was not a reliable guide to the internal temperature. To discover how long the vessels needed to be immersed in ice-water in order to cool the digests to room temperature, the following experiment was carried out. Four vessels, each containing 10 ml conc. nitric acid, were heated at 100% power for 4 min, then 50% power for 4 min (final pressure 80 psi (550 kPa)), then cooled for varying periods in ice-water before venting, opening and measuring the temperature of the acid. After 10, 15, 20 and 25 min cooling, the temperature was measured at 52°C, 40°C, 26°C and 19°C respectively, the room temperature being 21°C. Hence it was concluded that a cooling time of about 25 min was required.

8.2.3 Acid Resistance of Vessel Materials

Whilst the Teflon PFA parts of the vessel - the liner, cover, rupture disc and vent tube - showed a high degree of chemical inertness, as expected, and were unaffected by contact with hot concentrated nitric acid, it was found that the polyetherimide outer casing was prone to acid attack. It was unaffected by nitric acid at room temperature, but the screw-threaded area of the vessel body became discoloured and sometimes slightly blistered if any traces of acid were trapped there during a heating cycle. To avoid this, it was found necessary to clean (with water) and dry this area of the outer vessels and caps between each heating cycle, even between an acid cleaning cycle and a sample digestion (which had been unnecessary with the low pressure vessels), as small drops of acid were easily transferred from the Teflon cover into the outer cap when opening the vessels.

8.3 Factors Affecting Digestion of AMC Samples

8.3.1 Sample Size

Experiments with milk powder and sodium alginate digestions showed that complete dissolution of 0.5 g samples could be achieved in a single stage, whereas 1 g samples required two heating, cooling and venting cycles to completely dissolve them. Also, pressure rises were much harder to control for the larger milk powder samples, as the pressure would continue to rise after the power was switched off. No such "runaway" pressure rises were encountered with 0.5 g samples, and the internal pressure could be controlled and stabilised on the first heating cycle for all matrices. Hence it was concluded that rapid, reproducible digestions could be achieved much more readily with 0.5 g samples (except for tomato puree, whose high water content meant that 1 g samples could easily be handled). Two digests could be combined to produce digests of 1 g samples and thus minimise errors due to sample inhomogeneity, although clearly weighing errors would be doubled.

8.3.2 Performance under Pressure

In contrast to the all-Teflon PFA vessels, the lined vessels proved very reliable when used for the digestion of the AMC samples. At 150 psi (1030 kPa), no venting problems were encountered with the vessels themselves, providing the disposable rupture discs were changed frequently.

However, as mentioned earlier (see Section 3.1.3), connection of the pressure monitoring vessel to the pressure line via the two-way Omnifit valve, as used previously for the low pressure vessels, resulted in intermittent leakage problems from the valve at higher pressures when digesting samples. Inserting new Teflon cones and re-cutting the ends of the tubes held by the gripper fittings offered temporary cures, but the seals at the valve proved unreliable at high pressures when the valve was warm. The elimination of gripper fittings (with their stainless steel component) and use of Teflon cone seals alone in the Omnifit valve was also considered, but these seals were only rated to 50 psi (340 kPa) and would tend to degrade with frequent connection and disconnection.

The use of the valve was eventually discontinued, somewhat reluctantly, as it had proved useful in enabling the removal of the entire turntable, including the pressure monitoring vessel, from the microwave cavity at the end of heating. Although a microwave transparent valve serving the same purpose was available from CEM, it was felt too expensive (over 15 times the cost of an Omnifit valve). Hence a direct connection of the pressure line tubing to the pressure monitor cap was adopted for later work, with consequent inability to isolate the pressurised monitor vessel for easy removal of the turntable from the oven for cooling. This necessitated cooling of the pressure monitor vessel in a beaker of ice in the microwave cavity before disconnecting from the pressure line; the other vessels could be removed in the normal way for cooling. Also, the end of the tube making the connection to the pressure monitor cap had to be regularly trimmed to produce a fresh end with which to form a good seal (see Section 8.2.1).

8.3.3 Preparation of Digestion Mixture before Heating

Two simple pre-digestion treatments have been reported[116] as improvements to microwave digestion procedures. These are (a) moistening the powdered sample with water before addition of acid; and (b) intensive mixing of the sample and acid before closing the digestion vessel. Both these treatments were tried: (b) was found to be advantageous, and vigorous manual shaking of each vessel for 30 s was incorporated into the procedure. However, (a) appeared to hinder mixing. A small amount of water was added to one of two similar samples of milk powder before adding nitric acid to each, shaking and leaving to stand for 5 min. The

wetted sample was poorly mixed by comparison to the unwetted sample, the former containing several clumps whilst the latter was mixed to give a fine suspension. Therefore, the moistening step was not adopted in this work.

8.4 Digestion Methods

The AMC samples were all digested using each of the following three methods:

A. Single Stage Pressure Digestion with Nitric acid

The following procedure resulted in complete dissolution of all the matrices, producing clear, pale yellow solutions in all cases, except for sweet bay, where a small amount of a fine, grey precipitate remained (similar to that previously encountered after digestions using the low pressure vessels). Up to 12 vessels (including blanks) could be heated simultaneously.

i) New rupture membranes were fitted into vessel covers of up to 12 acid-cleaned vessels, including one designed for pressure-monitoring.

ii) Up to 10 vessels were each loaded with 0.5 g sample, or 1.0 g for tomato puree, and 10 ml conc. HNO_3, followed by manual shaking of the vessel for 30 s to mix.

iii) 1 - 4 empty vessels were loaded with 10 ml HNO_3 to act as blank(s).

iv) All vessel caps were secured hand-tight (taking care **not** to overtighten) and the vent stems screwed down.

v) The turntable was loaded with vessels (2 - 12) evenly spaced around the circumference; a vent tube was attached from each vessel cover to the central trap. The turntable holding the sealed vessels was placed in the microwave cavity and one of the vessels attached to the pressure-monitoring line. (if dissimilar samples were being digested, this would be the vessel containing the largest or most reactive sample).

vi) The oven fan was set to maximum and the turntable activated.

vii) Microwave power was applied to give a smooth pressure rise to 150 psi (1030 kPa) over about 10 min. (*See Note below*)*

viii) Applied power was adjusted to maintain a pressure of 150 psi for a further 15 min.

ix) After switching off microwave power, the vessels were transferred to an ice-

water bath and allowed to cool to room temperature (25 - 30 min).

x) The vessels were vented and opened in a fume cupboard; the digest solutions were then quantitatively transferred to volumetric flasks and made up to volume, or to beakers for further treatment if required. Digests from two (or more) vessels could be combined at this stage if sample weights of 1 g or more were required to overcome inhomogeneity problems.

Note: The applied power level varied according to the number of vessels and the matrix being digested and was adjusted during the heating cycle according to the observed pressure rise. For example, for digestion of milk powder using six vessels, applied power of 50% was normally appropriate. The following rough rule of thumb (as recommended by CEM) was found to be useful: for each extra vessel increase applied power by 5%; for fewer vessels, decrease applied power by 5% per vessel. 100% power was normally only needed when heating a full turntable (12 vessels) and was used with caution.

B. Post-Digestion Treatment with Hydrogen Peroxide (1st Method)

The above procedure (Method A) was carried out for all the AMC samples; eight 0.5 g samples of each matrix together with four blanks were heated at one time (i.e. a full turntable of 12 vessels, except in the case of tomato puree where four 1 g samples and two blanks were prepared). The digests (except for tomato puree) were then combined in pairs to give four digests and two blanks. Two of the digests and one blank were diluted to 50 ml with water and transferred to plastic bottles for storage. The other two digests and blank were transferred to beakers (with washing of the digestion vessels and caps) and heated to boiling on a halogen hot plate. 30% hydrogen peroxide was then added dropwise until the digests went virtually colourless (after this stage was reached, addition of further peroxide did not turn them completely colourless). This took varying amounts of time and added H_2O_2, depending on the matrix. The sodium alginate went colourless almost immediately when only a few drops of peroxide had been added, whilst the other matrices needed between 2 ml and 8 ml of H_2O_2. On average, this stage took 20 - 30 min, after which the digests were cooled and made up to 50 ml with water and the addition of 2 ml conc. nitric acid (to maintain acid strength and prevent precipitation of water-insoluble salts).

After this peroxide treatment all the matrices gave clear, virtually colourless solutions, except the fine, grey precipitate was still present in the sweet bay digests. On the two occasions where the pressure tube blew off, as mentioned earlier, the digestions were repeated, and their residues compared with the

prematurely vented digests.

C. Post-Digestion Treatment with Hydrogen Peroxide (2nd Method)

To try and achieve an improvement in decomposition, a modified version of the procedure described by Friel et al.[80] was carried out, as follows:

a) A 5 ml aliquot of "nitric acid only" digest (prepared as described above, Method A) was evaporated to near dryness in an evaporating dish on a steam bath.

b) 2 ml conc. nitric acid were added, followed by the slow, dropwise addition of 1 ml H_2O_2, to minimise foaming.

c) The solution was evaporated to near dryness.

d) Steps a) to c) were repeated until the solution became colourless, and foaming was reduced to that of a blank treated in parallel (Three to four repetitions were normally required).

e) The treated digest was then evaporated to dryness and constant weight in an oven at 90°C. The dried residues were retained for further analysis.

The modified hydrogen peroxide post-digestion treatment described above was carried out several times on all the AMC sample matrices except for sodium alginate. Initially this treatment was done in evaporating dishes, but inconsistencies in the residue weights suggested that sample losses may have been occurring over the edges of the dish, so beakers were used instead to eliminate this risk, though the treatment took longer due to increased evaporation time (about 1 h), and the consistency of residue weights was not significantly improved.

In addition, samples of the milk powder were digested using the following two methods:

D. 2nd Stage Microwave Pressure Digestion with Hydrogen Peroxide

The effectiveness of a second stage microwave pressure digestion using hydrogen peroxide was investigated. Methods supplied by CEM for for the digestion of food samples using lined vessels in their MDS-2000 system (which has a built-in pressure feedback control system), were used as a starting point and adapted for use with the MDS-81D.

Following the nitric acid digestion described above (Method A), hydrogen peroxide (2 ml) was added to each vessel, the vessels sealed and the turntable returned to the microwave cavity. Microwave power (55% - 75%) was applied, gradually increasing the pressure to a maximum of 100 psi (690 kPa) over a 15 min period. The applied power was then reduced to maintain pressure for up to 15 min. After cooling, the digests were transferred to volumetric flasks and diluted to 25 ml with water.

It should be mentioned that pressure digestion with hydrogen peroxide resulted in "stress marks" appearing on the inside of some of the vessel caps. The manufacturers could offer no definite explanation for this other than some sort of visualisation/etching of machining marks due to contact with condensed acid/peroxide vapours at high pressure (although the measured pressure never went above recommendations and none of the rupture discs vented, some over-pressurisation may have occurred). They suggested a less rigorous heating regime and a change to their new, redesigned cap when possible. (This cap is designed to deal much better with very rapid pressure rises, as the rupture discs are in closer contact with the vapour and therefore vent more reliably). However, the stress marks gradually disappeared with time and did not appear to adversely affect the operation of the vessels.

E. Post-Digestion Treatment with Perchloric Acid

Following the nitric acid digestion described above, the contents of each vessel were transferred (with washing of caps and vessels) to acid-washed glass beakers on a hot-plate. 2 ml of 71% perchloric acid were added to each digest. The beakers were covered with watch glasses and heated until dense white fumes of perchloric acid were evolved. After simmering for a further 15 min - maintaining fumes but taking care that the beakers did not boil dry - the digests were cooled, transferred to volumetric flasks and diluted to 25 ml with water.

8.5 Evaluation of Digestion Methods

8.5.1 Speed of Sample Preparation

To measure the overall preparation time per sample, for the nitric acid pressure digestion, the following factors were taken into account:

(a) number of samples = 12
(b) time taken to weigh samples/load vessels = 40 min
(c) heating time = 25 min
(d) cooling time = 25 min

(e) time for transfer/dilution of digests = 30 min

This gives an average preparation time per sample - including blanks - ((b+c+d+e)/a) of 10 min. This compares favourably with the average times using the low pressure vessels (see Section 6.4.1), and the digest quality was visibly much better and more reproducible. However, it should be borne in mind that, except for tomato puree, the sample size was half that used in the method for the low pressure vessels (0.5 g compared with 1.0 g), thus if the digests are combined, the preparation time per sample would be doubled. However, the improvement in digest quality could be worth the extra time taken. Also, as the above method was designed to dissolve the more difficult matrices (milk powder, pet food and sweet bay), the heating times for the more easily decomposed matrices (tomato puree and sodium alginate) could be reduced significantly and still produce complete dissolution. Again, however, the improvement in decomposition could be worth the extra heating time (given that the other factors - including cooling time - could not be reduced).

For the digests subjected to post-digestion treatments with hydrogen peroxide or perchloric acid, a further 40 min has to be added to the overall preparation time (this assumes sufficient hotplate/steambath capacity to heat up to 12 beakers simultaneously for the post-digestion treatment). Although the use of the hydrogen peroxide in the second microwave pressure digestion requires less heating time, when cooling is taken into account the additional time is similar. Thus the preparation time per sample is increased to 13 min.

8.5.2 Comparison of Involatile Residues from Digests

A 5 ml aliquot of each digest prepared as described in Section 8.4 was evaporated to dryness in an evaporating dish on a steam bath and dried to constant weight in an oven at 90°C. It was important that the oven was not too hot: at a temperature of 105°C some of the residues began to char, whereas at 90°C these residues remained waxy, but consistent weights were obtained.

The colour, texture and weights of the dried residues varied considerably with digestion method and sample matrix, although replicates of the same sample/method combination were generally reproducible. The nitric acid only method (A) produced yellow, waxy residues in the cases of milk powder, sweet bay and pet food, pale yellow powder for tomato puree and white powder for sodium alginate. The peroxide treated digest residues were paler, less waxy and of lower weight than those subjected only to nitric acid digestion, except for the sodium alginate digests where all the residues were white powders of similar weight.

Reproducibility of residue weights was good between replicate digests (e.g. 11.8, 13.0, 12.4 & 12.3 mg for four sweet bay digests prepared by the first H_2O_2 method) and between replicate evaporations of the same digest carried out on different days (e.g. 39.0, 39.6 & 38.3 mg for three aliquots of one HNO_3 milk powder digest), except for residues from method D (see below). It was found that the amount of involatile residue in those digests affected by premature venting was reduced by about 10%, indicating that a small proportion of the digest had been lost as an aerosol.

Whilst the peroxide-treated blanks showed a small amount of visible residue (the nitric acid blanks showed none), this had no significant weight (i.e. was within weighing error), consistent with the involatile impurity specification of the peroxide (<50 ppm).

Residues from the modified peroxide treatment (method C) were paler and drier (less waxy) than for the original peroxide treatment (method B), but the milk powder and pet food digest residues were less reproducible in appearance and weight than previously. However, the residue weights were consistently lower than for the original peroxide treatment. Residues from the two stage pressure digestion (method D) were very pale, almost white, but waxier in appearance than those from the modified peroxide treatment, and of reproducible weight (8 determinations in range 19.1 - 24.1 mg).

The weights of some of the dried digest residues (W2) are shown in Table 10.

8.5.3 Comparison of Residual Carbon Content

The percentage carbon contents of the original undigested samples (C1) and of the dried digest residues (C2), were determined by elemental analysis. The residual carbon content (RC%) as a percentage of the carbon content of the original sample was then calculated from the formula

$$RC\% = \frac{C2 \times W2 \times 100}{C1 \times W1 \times F} \%$$

where W1 and W2 are the weights of the undigested sample and the digest residues respectively and F is the fraction of digest evaporated to dryness. ($F = 1/5$ for tomato puree digests, and milk powder digested by methods D and E; $F = 1/10$ for the other digests).

The % carbon contents of the original undigested samples (C1) were determined

in duplicate for each matrix as follows:

Milk Powder:	49.19% ; 49.06%.	Mean = 49.13%
Pet Food:	41.67%; 41.43%.	Mean = 41.55%
Sodium Alginate:	30.41%; 30.33%.	Mean = 30.37%
Tomato Puree:	12.01%; 11.56%.	Mean = 11.79%
Sweet Bay:	48.40%; 48.30%.	Mean = 48.35%

The carbon content results for each digest analysed are detailed in Table 10. The digestion methods are refererred to by letter as listed in Section 8.4. The results for method C from the same subsample number (e.g. M1) are from separate aliquots of the same digest prepared by method A. The values for W1 are the total weights of pairs of 0.5 g samples, where the digests were combined after the nitric acid digestion (except tomato puree where 1 g samples were digested).

The mean residual carbon contents for each matrix/digestion method combination (Mean RC% in Table 10) are compared three-dimensionally for methods A - C in Fig. 13. As can be seen, the milk powder proved the least well digested sample for each method. This is almost certainly due to its high protein and fat content, as it has been shown[156] that these compounds require higher temperatures for decomposition than do carbohydrates, which make up a greater proportion of the other samples.

The trace of carbon recorded in the case of sodium alginate was negligible (below the standard error of the elemental analysis of 0.3%), indicating essentially complete decomposition with nitric acid alone. The carbon remaining in the tomato puree, although detectable, was also very low indicating almost complete decomposition. Significant carbon remained in the other digests, except for the milk powder subjected to perchloric acid treatment, where the residues consisted essentially of inorganic perchlorates. Solutions of these residues were tested with Brilliant Green - a triphenylamine dye - where the formation of a blue-green complex extractable into toluene confirmed the presence of perchlorate;[174] a chloride test with silver nitrate was negative. As can be seen from Table 10, milk powder digested by method D (nitric acid/hydrogen peroxide with microwaves under pressure) resulted in an RC% value of 11%, slightly higher than the mean level from method C (nitric acid/hydrogen peroxide dropwise on steam bath).

Table 10. Weights of Dried Residues and Residual Carbon Measurements

Sample & Subsample	Digestn Method	W1 mg	W2 mg	C2 %	RC% %	Mean RC%
Milk Powder, M1	A	1000.6	39.6	34.28	27.61	
Milk Powder, M2	A	1000.7	39.8	33.69	27.27	27.44
Pet Food, K1	A	999.8	25.9	24.26	15.13	
Pet Food, K2	A	999.7	24.8	23.16	13.83	14.48
Sweet Bay, S10	A	1000.2	21.9	33.43	15.14	
Sweet Bay, S11	A	999.8	22.1	33.15	15.16	15.15
Tomato Puree, P1	A	1008.7	10.3	7.79	3.37	
Tomato Puree, P2	A	1008.4	9.9	4.84	2.02	2.69
Sodium Alginate, T1	A	1000.0	33.4	0.24	0.26	
Sodium Alginate, T2	A	1000.2	33.3	0.15	0.16	0.21
Milk Powder, M4	B	1000.6	33.9	32.01	22.07	
Milk Powder, M7	B	1000.3	32.7	31.33	20.85	21.46
Pet Food, K4	B	999.7	23.3	20.16	11.31	
Pet Food, K5	B	999.7	22.8	19.86	10.90	11.10
Sweet Bay, S7	B	1000.1	12.4	19.94	5.11	
Sweet Bay, S8	B	999.6	12.3	20.70	5.27	5.19
Tomato Puree, P4	B	1002.7	9.7	6.05	2.48	
Tomato Puree, P5	B	999.9	8.8	3.28	1.22	1.85
Sodium Alginate, T4	B	1000.2	31.8	0.16	0.17	
Sodium Alginate, T5	B	1000.2	32.3	0.16	0.17	0.17
Milk Powder, M1	C	1000.6	23.9	22.88	11.12	
Milk Powder, M2	C	1000.7	24.6	23.65	11.83	
Milk Powder, M1	C	1000.6	14.6	17.15	5.09	8.11
Milk Powder, M2	C	1000.7	14.8	18.14	5.46	
Milk Powder, M1	C	1000.6	17.7	18.75	6.75	
Milk Powder, M2	C	1000.7	19.5	21.13	8.38	
Pet Food, K1	C	999.8	17.5	13.8	5.81	
Pet Food, K2	C	999.7	15.8	10.49	3.99	
Pet Food, K1	C	999.8	13.3	11.68	3.74	4.25
Pet Food, K2	C	999.7	15.1	13.86	5.04	
Pet Food, K2	C	999.7	13.8	8.07	2.68	
Sweet bay, S10	C	1000.2	10.0	20.32	4.20	
Sweet Bay, S11	C	999.8	9.9	20.77	4.25	4.23
Tomato Puree, P1	C	1008.7	10.7	6.24	2.81	
Tomato Puree, P2	C	1008.4	9.8	3.27	1.35	2.08
Milk Powder, M22	D	500.4	20.9	26.98	11.47	
Milk Powder, M23	D	500.2	19.7	25.82	10.35	11.03
Milk Powder, M18	D	499.5	19.4	28.51	11.26	
Milk Powder, M26	E	501.2	15.7	0.36	0.11	
Milk Powder, M27	E	500.3	15.2	0.15	0.05	0.08

Note: Digestion methods A - E were applied as described in Section 8.4.

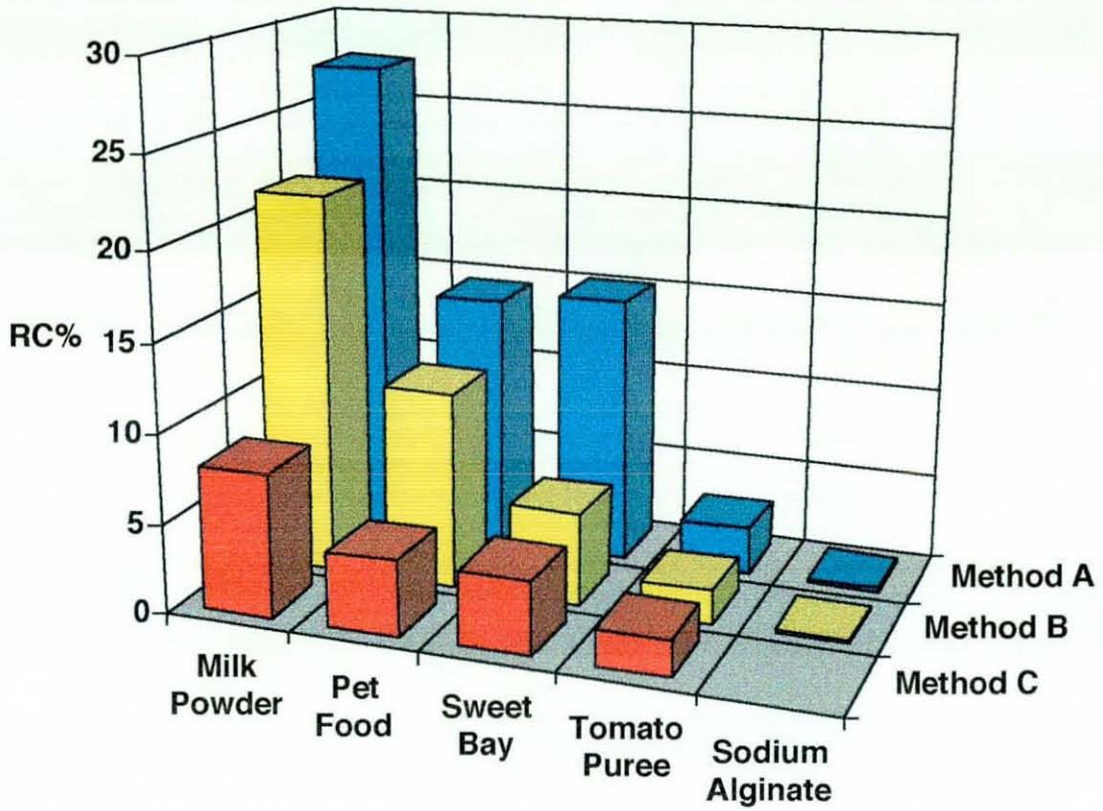

Fig. 13 Comparison of residual carbon content after digestion of different matrices by various methods: A:- HNO_3 only, B:- HNO_3/H_2O_2 (1st method), C:- HNO_3/H_2O_2 (2nd method)

As can be seen from Table 10, agreement between duplicates was generally very good. However, in the case of the 2nd peroxide treatment (method C), the appearance and weights of the digest residues were found to be more variable than with the other methods, and replicate carbon contents determinations confirmed this variability with a range of 5.1 - 11.8 % for milk powder (6 determinations) and 2.7 - 5.8 % for pet food (5 determinations). However, it can be seen that the carbon contents of the residues from this method were consistently lower than those from the 1st peroxide treatment method (B) which in turn were lower than those from the nitric acid only digests.

The results indicate that for sodium alginate or tomato puree, digestion method A (nitric acid only) produced complete or almost complete decomposition of the sample matrix. For milk powder, sweet bay and pet food the degree of decomposition was improved substantially with post-digestion peroxide treatment, especially the more intensive (albeit slower) 2nd method (C). It was shown that complete decomposition could be achieved, if required, by treatment with perchloric acid (method E).

To investigate the products of decomposition, the dried residues were subjected to further chemical analysis, as detailed in Chapter 9.

8.6 ICP-MS Semi-Quantitative Analysis

In an attempt to compare analytical results from the various digestion regimes, suitably diluted digests of each sample matrix, prepared by different digestion methods, together with appropriate blanks, were analysed by semi-quantitative ICP-MS using indium as internal standard. This was carried out using the VG Isotopes instrument in the Chemistry Department at Loughborough. Solutions for analysis were made up by diluting a 2 ml aliquot of each digest or blank to 50 ml with the addition of 250 µl of a 10 ppm In standard solution, to give an In concentration of 50 ppb in the solution and a total dilution factor for the sample of 1250. A total of 18 samples and 9 blanks were analysed, i.e. two digests plus one blank for each of the following sample/method combinations: all five matrices digested by method A; pet food and sweet bay digested by method B; and milk powder digested by methods D and E.

The results of the ICP-MS analysis were disappointing, and did not allow a valid comparison between digestion methods. There was a progressive loss of instrument sensitivity throughout the (ca. 2 h) duration of the analysis, and although it was felt this problem would be compensated for by the internal standard, there was obviously some fault with the system. There was a problem with the copper results, which were all very high, including the blanks, the readings gradually decreasing with successive samples. The aluminium results were very high and inconsistent; the chromium results were generally reproducible and within the AMC range but towards the high end. For the perchloric acid digests, very high blanks led to poor results. For most of the lower mass elements - in all of the matrices - the results showed consistent levels about 3 times higher than previous analyses (ICP-MS at ICI, DPASV and AAS analysis by the author); elements of higher mass (e.g. Ba) showed better agreement with the previous ICP-MS results.

The integrity of the 10 ppm indium standard solution used as the internal standard spike was confirmed by DPASV analysis (its concentration was measured at 10.0 ppm), so this was ruled out as a cause of the problem. A possible explanation of the high values could lie in the calibration of the ICP-MS instrument whereby a response curve is applied to quantify elements of different masses. If this were incorrect, it could lead to significant errors at the low mass end. The only real solution appeared to be fully quantitative analysis, using a mixed standard containing the elements of interest, with the analysis of standard

reference material to check accuracy. Unfortunately, due to the complete breakdown of the instrument shortly after the above analyses, further work in this area has not been possible.

CHAPTER 9
ANALYSIS OF DECOMPOSITION PRODUCTS OF MICROWAVE DIGESTION

9.1 *Introduction*

As discussed in Chapter 2 (see Section 2.6), studies of the decomposition products of nitric acid pressure digestion have been reported for microwave[140] and conventional[153] heating. Of particular interest are involatile compounds which cannot easily be removed by evaporation without loss of analyte. These include aromatic acids particularly resistant to oxidation. Nitrobenzoic acid, especially the para-isomer, has been reported as a major interferent in electrochemical analysis of these digests.[140,154] However, the study of decomposition products of microwave digestion has been somewhat patchy, and the processes are still not very well understood. Studies of specific decomposition temperatures for the digestion of biological matrices[156] have thrown some light on the subject by identifying temperatures required for decomposition of different classes of compounds, as dicussed earlier (see Section 2.7), but information on residual materials after a typical microwave digestion, where temperature monitoring is not available, is still lacking. Decomposition products of microwave digestion have not been identified in detail and have not been related to residual carbon content or digestion methods.

In this work, the involatile residues of microwave digestion were studied, to compare the effect of different digestion regimes on the quality and quantity of residual material, in an attempt to better understand the processes involved. The aim was to identify the broad groups of compounds produced, to investigate their relative resistance to oxidation and to gauge their significance for analytical techniques particularly sensitive to organic interference.

9.2 Initial Studies of Decomposition using Low Pressure Vessels

The digests which had been prepared for ICP-MS analysis (see Section 6.5) were subjected to chemical analysis as described below.

9.2.1 Infrared Analysis

Where insoluble particles remained in the digests (sweet bay, pet food, sodium alginate short digest), they were centrifuged to separate out the small amounts of insoluble residue. Then a few ml of each clear digest were evaporated to dryness (on a water bath, then in the oven at 90 - 100°C), and the residues analysed by

Fourier Transform Infrared Spectrometry (FTIR) in the form of KBr discs.

The infrared spectra indicated the presence of carboxylic acids and (probably) carbohydrate residues, but the complex mixtures needed further separation. Various solvent extractions were tried with limited success, though addition of methanol to the evaporated digests in each case produced a yellow solution and a white or off-white precipitate.

Analysis of the methanol insoluble fractions of digests of three of the matrices (pet food, milk powder and sweet bay) produced simple, almost identical spectra, which appeared to be due to oxalates (these, unlike oxalic acid itself, are insoluble in methanol, the spectra showed characteristic carboxylate ion stretching bands and the substance, when acidified, reduced potassium permanganate). This was confirmed by infrared analysis of a sample of calcium oxalate which gave a spectrum matching those from the digest residues. The methanol insoluble fractions of the sodium alginate and tomato puree digests produced more complex spectra; the former could well contain undigested mannuronic acid, which is insoluble in methanol, from fragmentation of the alginic acid chain, which consists of copolymeric D-mannuronic and L-guluronic acids.[175]

The methanol soluble fractions of the digests showed spectra indicating the presence of carboxylic acids (strong C=O stretching band at ca. 1700 - 1740 cm^{-1}, and hydrogen bonded O-H stretching bands at 3000 - 2500 cm^{-1}) and probably carbohydrate residues (multiple bands at 1200 - 1000 cm^{-1} indicating C-O-C stretching). Perhaps surprisingly, in view of previous work in which nitrobenzoic acids have been identified as end-products of protein digestions,[140,153] there seemed to be no definite evidence of aromatic residues (the aromatic bands at 1600 - 1450 cm^{-1} and 900 - 700 cm^{-1} were absent or weak), and while strong bands due to nitrate ions (1380 and 830 cm^{-1}) occurred in a few of the spectra (notably sodium alginate), organic nitro stretching bands at 1550 and 1350 cm^{-1} could not be readily seen (though may have been masked).

9.2.2 Thin Layer Chromatography

The methanol extracts of the digests were examined by thin layer chromatography (TLC) using silica and cellulose plates (with and without fluorescent indicator) and various solvent systems. Residual amino acids were sought using 2 solvent systems: isobutanol:acetone:ammonia:water (10:10:5:2), and isopropanol: formic acid:water (20:1:5) and spraying with ninhydrin.[176] Several UV absorbant TLC spots were observed for all the samples, with much variation between the matrices. No undigested amino acids were detected (all

gave negative ninhydrin tests), though some were shown to be acidic, turning purple with ninhydrin after conversion to their diethylamine salts, using n-butanol:diethylamine:water (85:1:14) as eluent.[177]. All except the sodium alginate produced a bright yellow spot, especially strong in the sweet bay digest, showing no acidic character.

The methanol extracts of the digests were compared with control samples of 2-, 3- and 4-nitrobenzoic acids (NBAs), on silica plates using isopropanol:formic acid:water (20:1:5) as the eluting solvent system. The pet food and milk powder samples gave strong spots corresponding to 4-NBA and very much weaker spots corresponding to the 2- and 3- isomers. The sweet bay gave weak, streaky spots corresponding to all 3 isomers, and the tomato puree showed a possible trace of 4-NBA (the sodium alginate showed none). This was in addition to the strong yellow spots mentioned above.

Samples of the sweet bay digest (with and without prior TLC separation) were submitted for mass spectrometric analysis, but the results were inconclusive.

9.2.3 TLC-FTIR Analysis

In an attempt to identify the organic residues in the methanol extracts, TLC was used to separate out the fractions for FTIR analysis. TLC bands were removed from the plates by suction into a glass tube (See Fig. 14), eluted from the adsorbant with methanol into an agate mortar, ground with KBr and discs pressed.

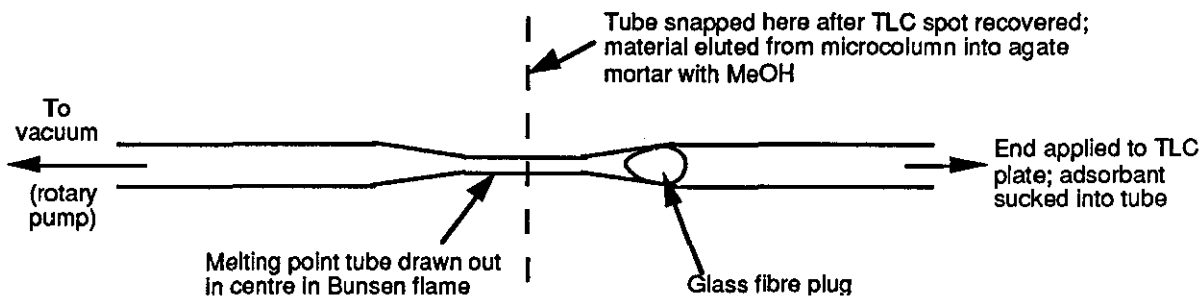

Fig. 14 Modified melting point tube used to transfer TLC spots to KBr discs

The FTIR spectra obtained from recovered TLC spots were disappointing, being rather weak and suffering badly from interferences from substances co-extracted from the silica. Various eluting solvents were tried, but those giving the best separation suffered the highest background (despite pre-cleaning the plates with solvent), and sensitivity was a problem. Attempts were made to produce a "home

made" micro-disc, using a cardboard insert in a standard size disc press, but it proved difficult to get the micro-disc flat and the resulting light scatter led to very low transmittance values and very little improvement in sensitivity. Also the light beam in the FTIR instrument was too large for these microdiscs to offer any real advantage.

The bright yellow spot from the sweet bay extract (mentioned in 9.2.2 above) gave strong IR bands corresponding to C-NO_2 stretching, indicating an organic nitro compound (as might be expected), but it could not be identified precisely, though it was not a carboxylic acid.

9.2.4 UV/Visible Spectroscopy

The UV/visible spectra of methanol extracts of the digests were recorded for each of the AMC sample types, as a qualitative measure of the degree of decomposition achieved in the digestion process. All the digests gave a very strong absorbance in the 230 - 250 nm region and all except the sweet bay showed a weaker, broad shoulder at longer wavelength, ranging from 320 - 380 nm. The sweet bay extract showed three further fairly sharp absorbances at 330 nm (strong), 370 nm (medium) and 390 nm (medium). The peak at ca. 240 nm could be due to aromatic residues and corresponded to the strong absorbance of a methanol solution of nitrobenzoic acid. With the exception of the sweet bay digest, the peaks in the 320 - 380 nm region broadly corresponded to peaks recorded earlier (see Sections 4.2 and 5.4.4) when monitoring digests of tomato puree at various stages. These peaks were found to diminish as digestion progressed indicating progressive breakdown of organic residues. The sweet bay digest showed a more complex absorbance pattern, possibly due to a variety of aromatic or conjugated nitro compounds, but the bands were not identified.

9.3 Decomposition using Medium Pressure Vessels

The digest residues prepared as described in the previous chapter (Sections 8.4 and 8.5.2) having been compared by appearance, weight and carbon content, as described in Sections 8.5.2 and 8.5.3, were then analysed by infrared spectrometry and thin layer chromatography, with the following results.

9.3.1 Infrared Analysis

i) Sodium Alginate

Infrared analysis of the dried residues from the sodium alginate digests showed that oxidation to sodium nitrate was essentially complete, as indicated by the elemental analysis results (15% nitrogen, negligible carbon and hydrogen). This

was confirmed by melting point determination. This was in contrast to sodium alginate digestion using the lower pressure vessels, when infrared analysis showed that significant organic residues remained (see Section 9.2.1). In addition, the weight of residue relative to the weight of sample digested (334 mg g^{-1}) was in good agreement with the theoretical yield (373 mg g^{-1}) for complete conversion of sodium alginate to sodium nitrate. This calculated figure takes account of the sample's measured moisture content of 13.2%, and assumes the sodium content is unaffected by any cross-linkage of the alginate chains.

ii) Tomato Puree

Infrared spectra of the tomato puree digests showed predominantly inorganic nitrate (band at 1384 cm^{-1}), with a small carbonyl band at 1700 cm^{-1}, with virtually no difference between the nitric acid only and peroxide treated digests, as expected from their similar, low carbon contents. Again, the amount of organic residue was considerably reduced in comparison to the low pressure vessel digestions.

iii) Milk Powder, Pet Food and Sweet Bay

The infrared spectra of these residues after digestion by methods A - C showed a mixture of organic material. The predominant bands in spectra of residues from method A (nitric acid only) are attributable to carboxylic acids, with characteristic bands in the 3000 - 2500 cm^{-1} region (O-H and C-H stretching), C=O stretch at 1700 cm^{-1}, together with several bands in the 1450 - 1200 cm^{-1} region (C-O stretching, C-O-H in plane bending) and a band at 940 cm^{-1} (O-H out of plane bend). These would include fatty acid residues ($CH_3(CH_2)_nCOOH$) from the decomposition of glycerides, and glycaric acids ($HOOC(CHOH)_nCOOH$) from the breakdown of carbohydrates, as well as other involatile mono- and dicarboxylic acids, left after nitric acid oxidation of organic material. There was also a sharp band at 1550 cm^{-1} which could be due to salts of these acids. In the case of sweet bay, the spectra were virtually identical to those from the low pressure vessels (see Section 9.2.1), but those of the milk powder and pet food residues showed significantly less absorption in the 1200 - 1000 cm^{-1} region than previously, indicating that the carbohydrates had been more effectively broken down. (In the case of sweet bay these bands were already weak, probably as a result of the more rigorous, multi-stage digestion needed to dissolve the sample).

For the peroxide treated digests (method C), the carboxylic acid bands were much weaker, and the spectra were dominated by bands due to inorganic nitrate (1384 cm^{-1}) and calcium oxalate (1620, 1320, 780, and 520 cm^{-1}), with an indication of inorganic phosphate (weak, broad absorbance at 1000 - 1100 cm^{-1}).

A typical variation in the FTIR spectra between digestion methods is shown in Fig. 15, which shows the differences between sweet bay digests with and without peroxide treatment. Similar results were obtained for the milk powder and pet food digests. It can be seen that the peroxide treated digest shows a higher proportion of calcium oxalate and inorganic nitrate relative to the organic acid residues than the nitric acid only digest.

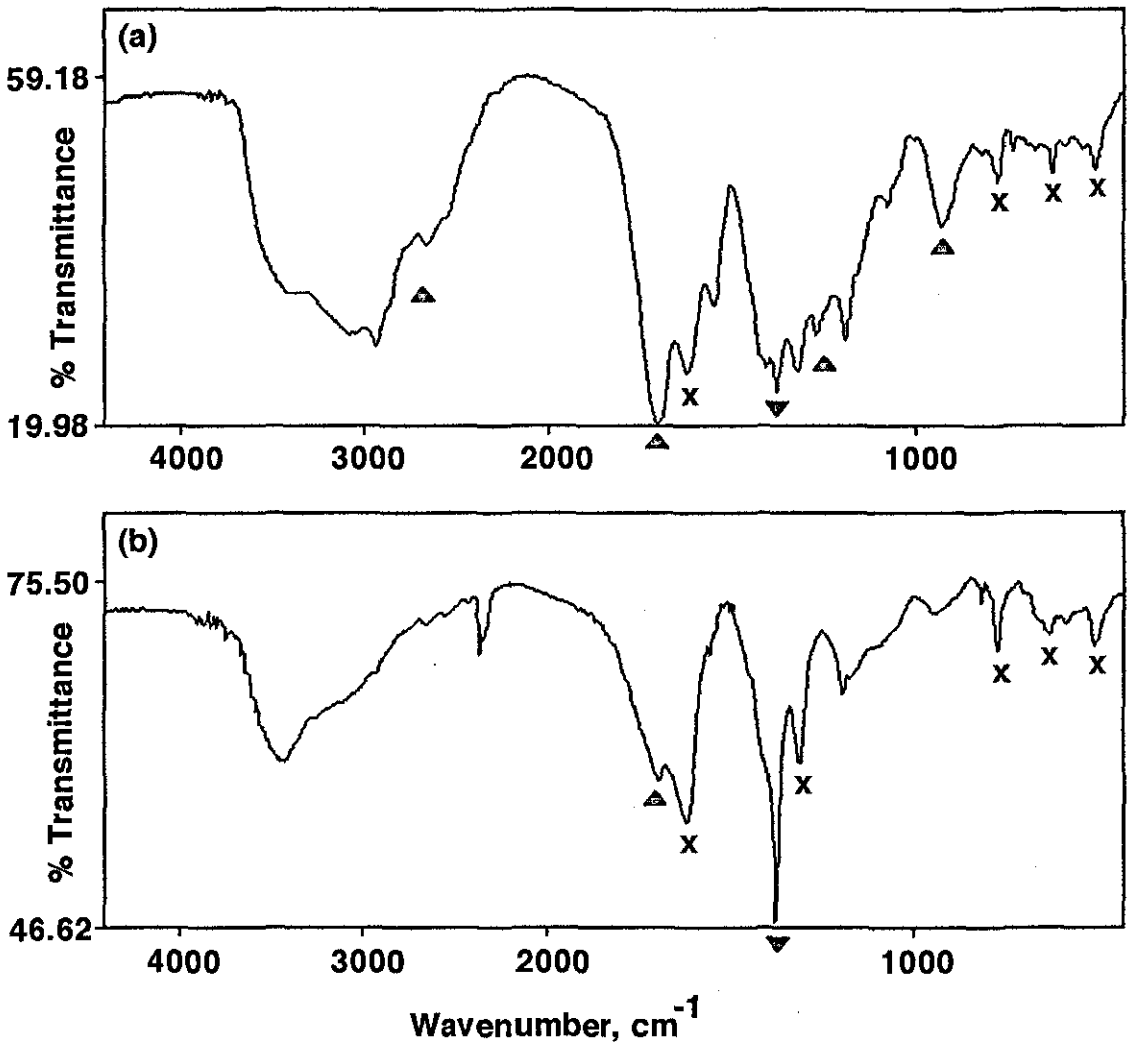

Fig. 15 Infrared spectra (KBr discs) of involatile residues of sweet bay digests after (a) microwave digestion with nitric acid only (Method A), and (b) further treatment with hydrogen peroxide (Method C)

These identifications were substantiated by extraction of the residues with methanol to give a white precipitate - also insoluble in water but soluble in dilute nitric acid - identified as mainly calcium oxalate with a small amount of phosphate, and a yellow solution containing carboxylic acids and nitrate. Superimposition of the infrared spectra of the methanol soluble and insoluble fractions gave patterns matching those of the total residue, indicating that no

significant chemical change had resulted from the extraction procedure. In particular, there was no apparent shift in the carbonyl absorption to higher wavenumber to indicate esterification of the acids in the presence of methanol.

For milk powder digested by method D, the methanol soluble extract showed a similar infrared spectrum to those from methods A - C. Two typical spectra of such extracts from methods C and D are shown in Fig. 16. It can be seen that the proportion of carboxylic acid is somewhat higher for D than C (see later discussion, Section 9.3.2 (ii)).

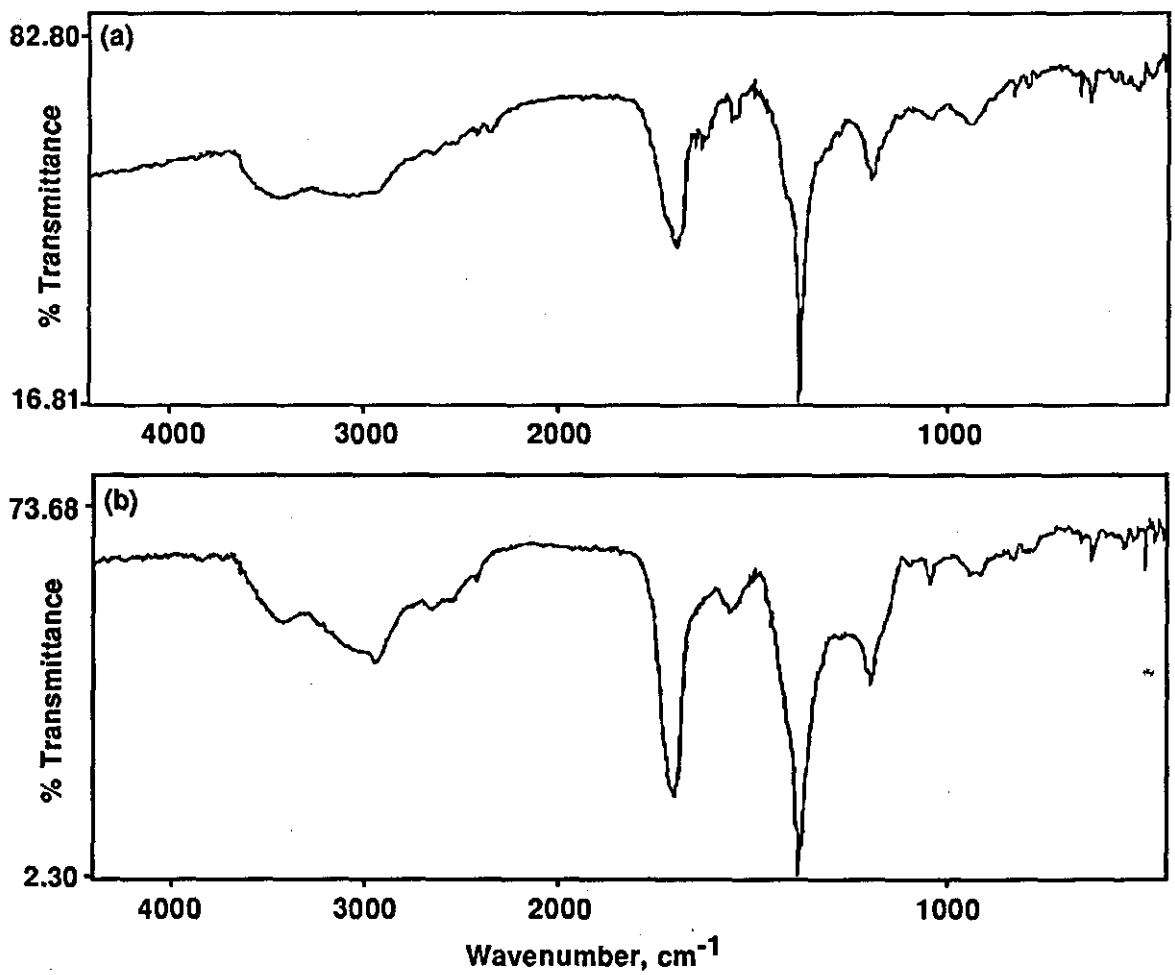

Fig. 16 Infrared spectra (KBr discs) of methanol soluble extracts of milk powder digest residues after (a) microwave digestion with HNO_3 followed by H_2O_2 treatment with conventional heating (Method C), and (b) two stage microwave digestion with HNO_3 + H_2O_2 in 2nd stage (Method D)

However, the precipitate from the methanol extraction of method D residues was found to differ from that from methods A - C, being largely due to phosphate residues, the FTIR spectrum closely resembling that of dibasic calcium phosphate ($CaHPO_4$), which is insoluble in methanol. The presence of orthophosphate was confirmed by treatment with acidified ammonium molybdate to form bright

yellow ammonium molybdophosphate with addition of Crystal Violet to form a deep blue complex.[174] The substance burned orange-red in a Bunsen flame, indicating the presence of calcium. The infrared spectrum also showed a small amount of calcium oxalate.

The dried weight of the methanol-insoluble residue from a 5 ml aliquot of digest solution from method D - corresponding to 0.1 g of original sample - was 6.2 ± 0.3 mg (4 determinations from 4 different digests). Its carbon content was measured (by elemental analysis, as before) at 6.0%, compared to 18.8% if the residue had been entirely calcium oxalate, indicating 68% phosphate and 32% oxalate in the residue. These findings translate to a calcium phosphate yield of 41 mg g^{-1} of sample digested, which compares with a theoretical yield of 37 mg g^{-1}, calculated from literature data for typical phosphorus concentration in milk powder.[178]

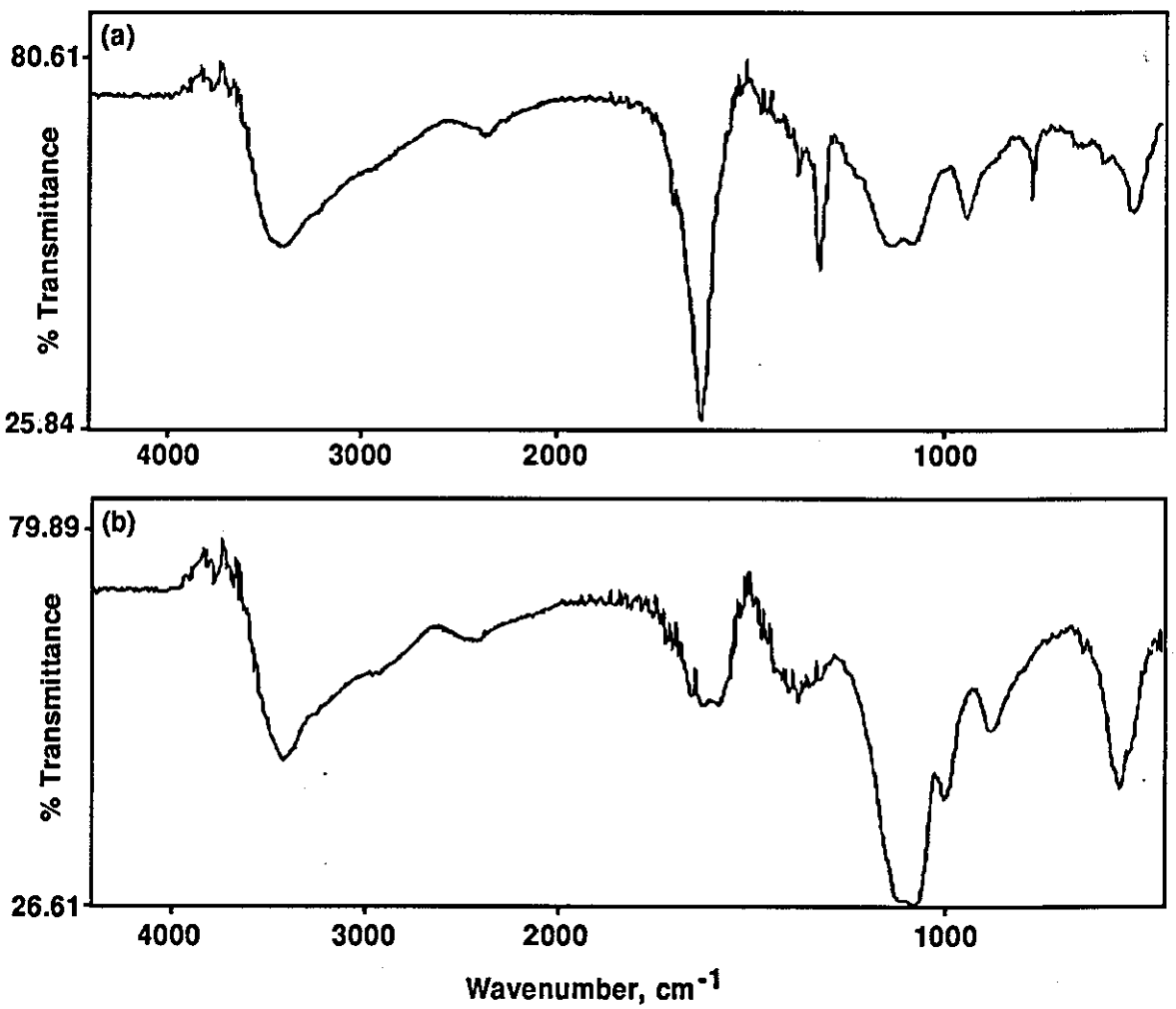

Fig. 17 Infrared spectra (KBr discs) of methanol insoluble precipitates of milk powder digest residues after (a) microwave digestion with HNO_3 followed by H_2O_2 treatment with conventional heating (Method C), and (b) two stage microwave digestion with HNO_3 + H_2O_2 in 2nd stage (Method D)

Hence it was shown that although method D produced a similar residual carbon level to method C, the use of microwaves under pressure for the second stage produced a different decomposition profile, yielding far more phosphate and much less oxalate than peroxide treatment in open vessels using conventional heating. These differences are shown in Fig. 17.

9.3.2 Correlation of Infrared and Carbon Content Results

i) Choice of parameters for comparison

It was observed that, for residues from digestion methods A - C at least, the higher the residual carbon content the stronger the carbonyl peak in the infrared spectrum appeared, and it was felt it might be useful to quantify this correlation, thus providing another measure of degree of decomposition to complement the residual carbon measurements. Clearly, due to differences in sample loading, thickness and clarity of the KBr discs, the absolute peak heights would vary between similar samples, but if the relative peak heights of the carbonyl (1700 cm^{-1}) and inorganic nitrate (1384 cm^{-1}) bands were considered, a meaningful parameter could be obtained. It would probably be necessary to calculate the absorbance values for each peak from the measured transmittance values, to provide a useful linear parameter, and in fact preliminary plots of relative transmittance versus carbon content showed this to be the case.

The relative absorbances were calculated from the infrared spectra using the formula

$$\text{Relative Absorbance (X/Y cm}^{-1}) = \frac{\log T(\text{baseline}) - \log T(X)}{\log T(\text{baseline}) - \log T(Y)}$$

where T(baseline) is % transmittance at baseline in the spectral region of interest and T(X) and T(Y) are % transmittances at X cm^{-1} and Y cm^{-1} respectively.

ii) Correlation of relative absorbance and residual carbon

For each of the residues digested by methods A - C, plots of absorbance at ca. 1700 cm^{-1} relative to that at 1384 cm^{-1} versus residual carbon content proved to be linear, indicating that residual carbon levels varied linearly with organic acid concentration (relative to inorganic nitrate as reference). When the residual carbon content as a percentage of the weight of residue (C2) was plotted, straight lines were obtained for each sample type, but these did not coincide with each other. However, when the residual carbon content expressed as a percentage of the original carbon content of the undigested sample (RC%) was plotted, the lines coincided (see Fig. 18). This is apparently due to the elimination of differences due to differing carbon contents of the original samples and differing weights of residual material remaining after digestion.

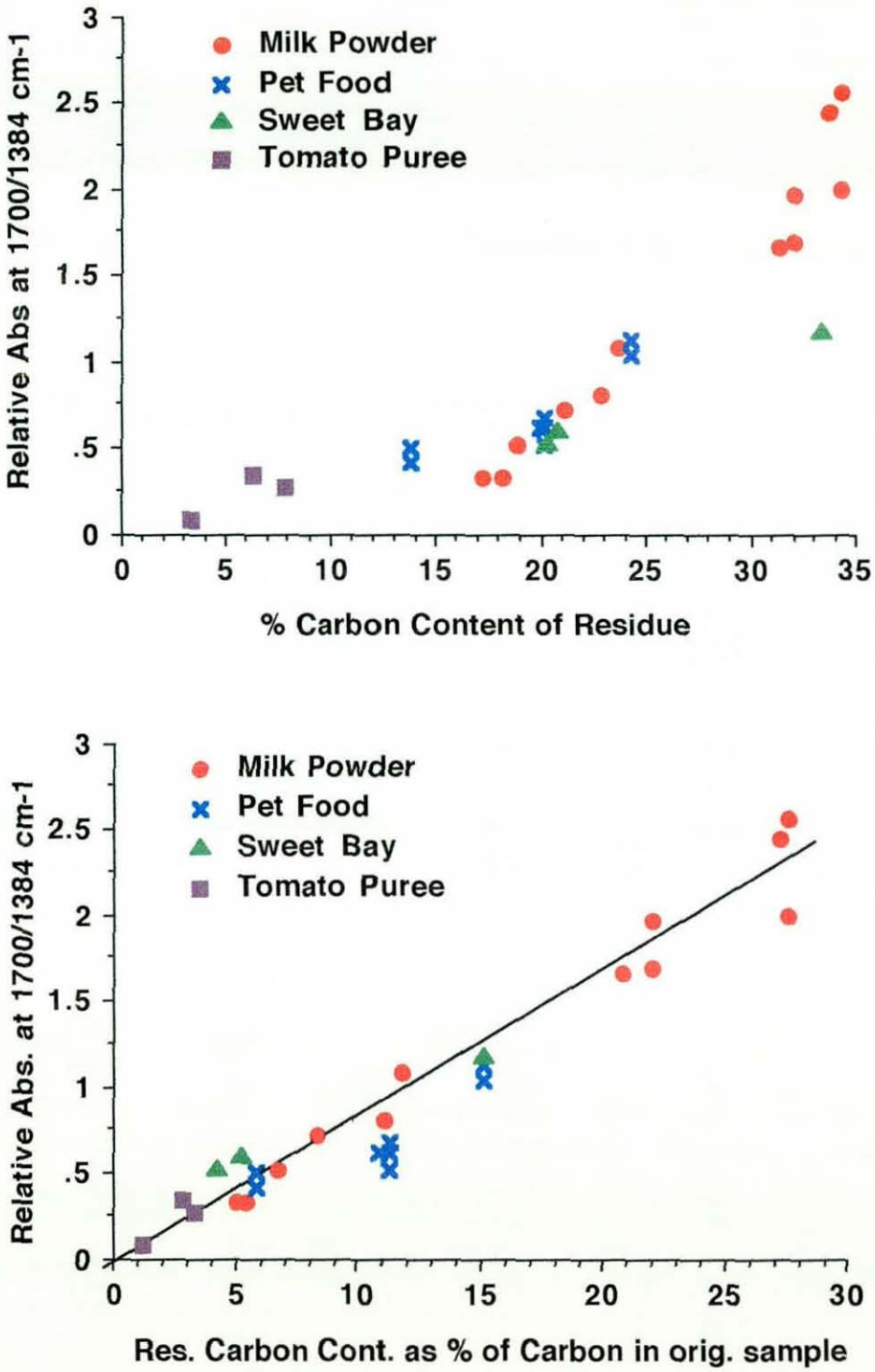

Fig.18 Correlation of infrared spectra and residual carbon content of microwave digest residues for digestion methods A - C: Ratio of absorbance at 1700/1384 cm^{-1} v. residual carbon expressed as a percentage of (*top*) the digest residue, and (*bottom*) carbon in the original sample

For milk powder digested by method D, the results did not fall on this straight line, as the absorbance at 1700 cm^{-1} relative to that at 1384 cm^{-1} was higher for a given RC% value than for the other digests. This is presumably due to the much lower proportion of calcium oxalate, which was a major contributor to the carbon content (but not to the 1700 cm^{-1} absorption) for the residues from methods A - C. This is reflected in the finding reported earlier (see Figs. 16 and 17) that, although the residues from methods C and D showed similar residual carbon contents, those from method D showed higher absorbances at 1700 cm^{-1} and lower absorbances at 1620 cm^{-1} (oxalate) than for method C, which, on balance, produced similar carbon contents but very different 1700/1384 cm^{-1} absorbance ratios.

The relative absorbance at 1700 cm^{-1} /1620 cm^{-1} (organic acid/oxalate) was also examined for its variation with residual carbon content, for all matrices where oxalate was produced, i.e. milk powder, sweet bay and pet food, digested by methods A - C. It was found that this absorbance ratio rose with increasing carbon content, although the relationship was non-linear, as the relative absorbance increased very slowly at low RC% and much more rapidly at RC% levels > 15% (such levels only being found in the milk powder digests). When the values were plotted logarithmically, a straight line resulted, as shown in Fig. 19.

An explanation of these findings is that early in the digestion process, the oxalate concentration is small; as the digestion progresses, the residual carbon content and the carboxylic acid concentration both decrease, whereas the oxalate concentration initially increases, reaching a maximum before falling gradually towards the end of the digestion. Evidence for this was provided by plotting the relative absorbance at 1620 cm^{-1} /1384 cm^{-1} (oxalate/nitrate) versus residual carbon content, as shown in Fig. 20, where, in spite of the considerable scatter of results, the initial trend upwards, followed by a decline at RC% > 15%, can be seen. This is, however, a tentative explanation, as these experiments were not designed specifically to address this issue. Further work would need to be done to be certain of the validity of this explanation.

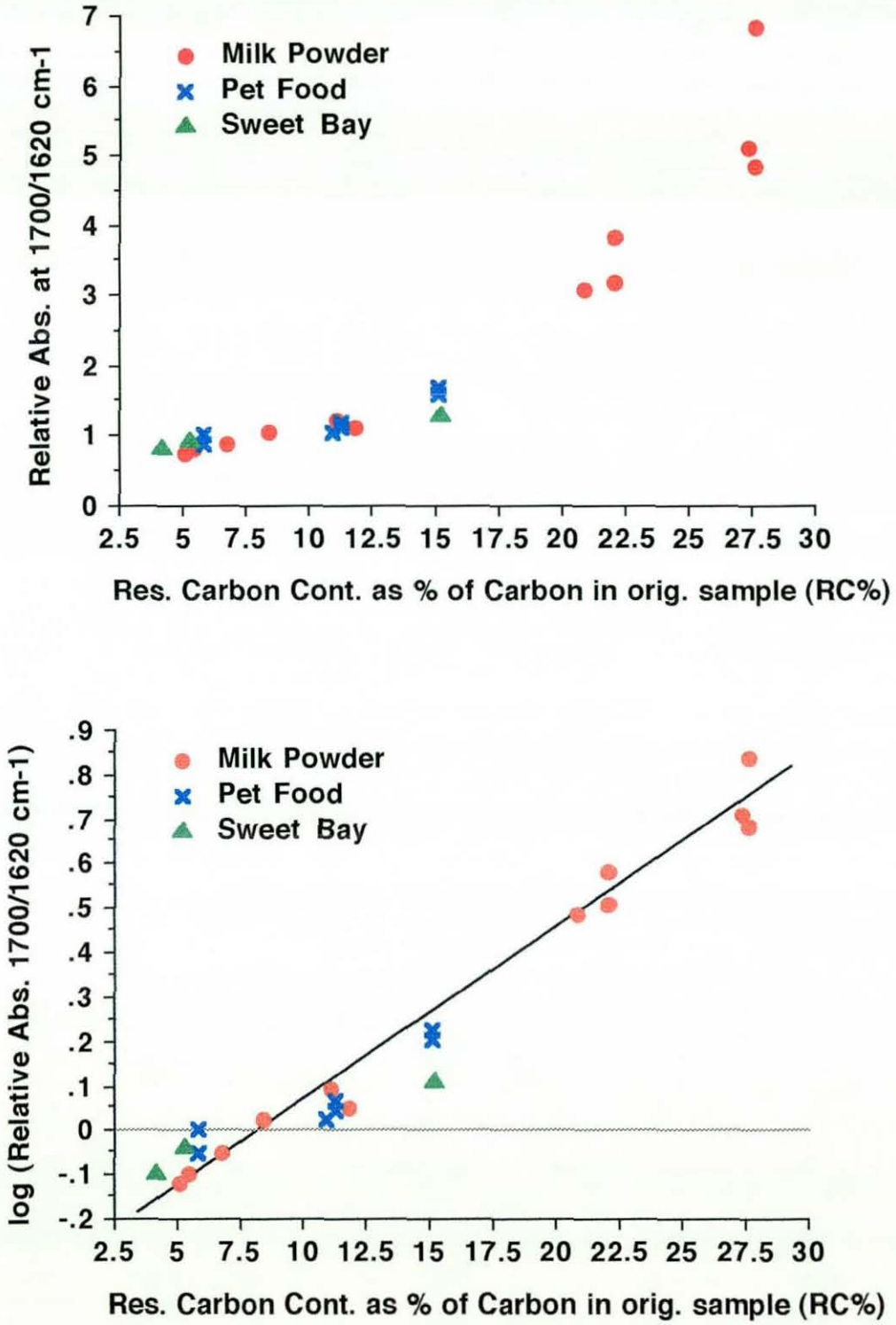

Fig. 19 Correlation of infrared spectra and residual carbon content of microwave digest residues for digestion methods A - C: Relative absorbance at 1700/1620 cm^{-1} v. residual carbon as a percentage of carbon in original sample, (*top*) as a straight plot, and (*bottom*) as a log plot

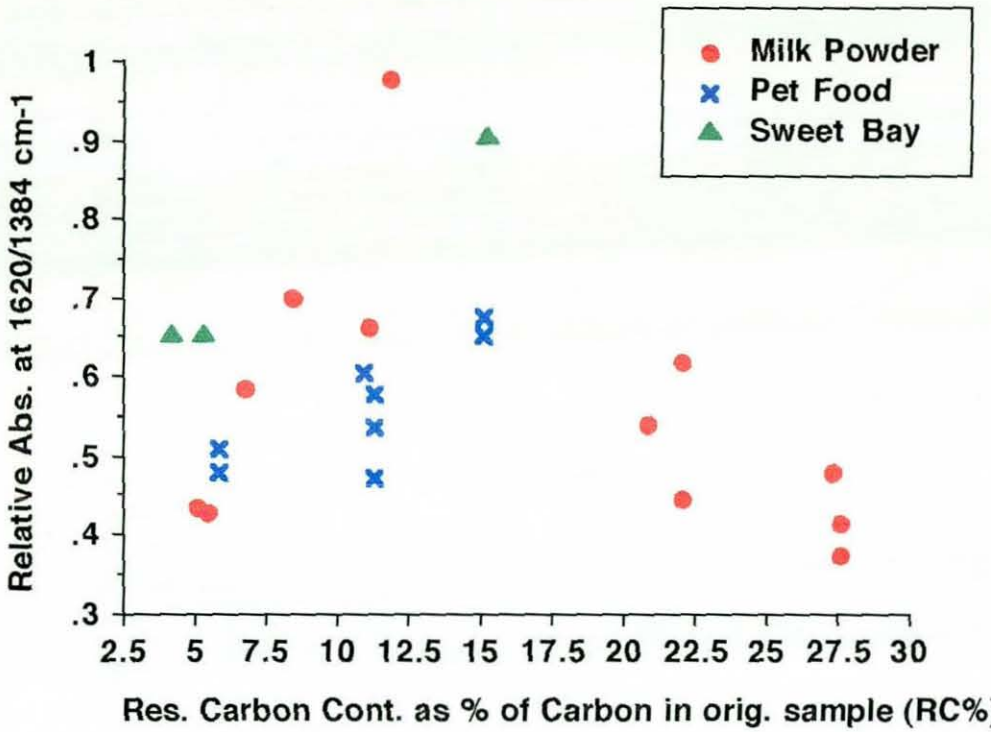

Fig. 20 Correlation of infrared spectra and residual carbon content of microwave digest residues for digestion methods A - C: Relative absorbance at 1620/1384 cm^{-1} v. residual carbon as a percentage of carbon in the original sample

9.3.3 Thin Layer Chromatography

Thin layer chromatography was carried out on the methanol soluble fractions using silica plates with isopropanol:formic acid:water 20:1:5 as eluting solvent. Spots were viewed under UV light (254 nm) and acidic spots were visualised by spraying with bromocresol green (0.3% solution in 1:4 water:methanol containing 4 drops of 30% sodium hydroxide in 50 ml[179]).

Methanol extracts of milk powder, pet food and sweet bay digests all gave strong acidic spots having the same Rf value as 4-NBA, plus weaker spots corresponding to the other NBA isomers.

9.4 Electrochemical Analysis of Medium Pressure Digests

In order to appreciate the problems posed by interference from organic residues, it was decided to try electrochemical analysis of some of the digests prepared without the use of perchloric acid. To this end, the determination of zinc in milk powder digests was attempted by differential pulse polarography using a dropping mercury electrode. Initial attempts in acidic medium (0.1M HNO_3)

were unsuccessful due to interference from the hydrogen wave. To alleviate this problem, Zn determinations are often carried out in acetate buffer at ca. pH 4.5,[180] but it was found that addition of sodium acetate produced an unacceptably high blank due to trace element contamination. The support electrolyte was changed to 1M NH_3/NH_4Cl[181] which gave sufficient sensitivity in aqueous standards, but sample digests (5 ml aliquot neutralised with ammonia and made up to 25 ml with support electrolyte) gave a very high background signal, presumably due to interfering organic species. To avoid signal overload, sensitivity had to be reduced such that Zn was not detectable in the digests.

It was decided that the greater sensitivity of differential pulse anodic stripping voltammetry (DPASV) was needed, whereby preconcentration of the Zn on the hanging mercury drop electrode (HMDE) would enable its signal to be selectively enhanced above the interfering signals. The previous ICP-MS analysis indicated a Zn concentration in the milk powder of 20 - 30 $\mu g\ g^{-1}$, which translates to 0.08 - 0.12 $\mu g\ ml^{-1}$, or 1.2 - 1.8 x 10^{-6} M, in the digest diluted as described above, which should be at a suitable concentration for determination by DPASV.

9.4.1 Determination of Zinc in Milk Powder and Sweet Bay Digests

Several of the milk powder and sweet bay digests were analysed for zinc by DPASV, using the following method: A 5 ml aliquot of digest prepared as described in Section 8.4 was adjusted to pH 9.0 ± 0.2 using 0.88 ammonia (AR grade, Fisons), and then diluted to 25 ml with water before transferring to the electrochemical cell. Initially, a support electrolyte of 0.05M NH_3/NH_4Cl was used,[181] but this was found to be superfluous given the ionic strength of the digest, and led to the unnecessary introduction of low level metal contamination, insignificant in the zinc analysis, but of significance in the later determination of copper at the ng ml^{-1} level. DPASV was carried out at room temperature under oxygen-free nitrogen after purging with nitrogen for 5 min. Measuring conditions for the analysis were as follows: Deposition potential –1.30 V, deposition time 2 min with stirring (plus 20 s settling time), potential scan rate 5 mV s^{-1}, differential pulse 50 mV. The zinc peak occurred at a potential of –1.15 V.

Zinc was successfully determined in milk powder samples digested by method D, neutralised with ammonia and diluted as described above, using the standard additions method. An aqueous stock solution containing 100 $\mu g\ ml^{-1}$ Zn was prepared by dilution of a standard solution of $ZnNO_3$ containing 1000 $\mu g\ ml^{-1}$ of zinc (BDH), and 25 µl aliquots of this were sequentially added (giving added Zn concentrations of 0.1, 0.2, 0.3, 0.4 $\mu g\ ml^{-1}$ in the analysed solution). Plots of peak height versus added zinc were linear, and sensitivity was good, limited only by a

level of 0.01 μg ml^{-1} in the blank digests (probably due to Zn in the peroxide and ammonia). 3 determinations gave results of 24.0, 23.2 and 21.6 μg g^{-1} in the milk powder (0.09 μg ml^{-1} in the analysed solution; all results blank subtracted).

However, interfering peaks in the voltammogram led to inconsistent Zn results for samples digested with nitric acid only (method A) and precluded any determination under these conditions of other trace elements (e.g. Cu, Pd, Cd) in any of the milk powder digest solutions from methods A - D. Zn was also determined at 32.9 μg g^{-1} in sweet bay digested by method B (cf. ICP-MS analysis of 29 μg g^{-1} reported in Chapter 6, Table 5), but even though the Cu level in this sample was high enough to detect, interfering peaks rendered its accurate determination impossible.

9.4.2 Identification of Interferences

Organic interference in DPASV is of three main types:

a) *Complexation of trace metals by organic complexing agents.* This reduces the trace metal response, as only the free metal ions are measured. Such complexes should be destroyed by the microwave digestion, although a reduced metal signal may indicate incomplete release of bound metals.

b) *Surfactant effects.* i.e. competitive adsorption onto the electrode by surface-active organic molecules, inhibiting the deposition of trace metals, thus reducing the analyte signal. The standard additions method employed here compensates for a modest surfactant effect, providing sensitivity is not reduced too severely.

c) *Voltammetrically active interferents.* Organic compounds may give rise to signals which mask the analyte response. This is a particular problem for samples digested with nitric acid, due to the voltammetric activity of the nitro group.

Several large peaks other than Zn were observed, as shown in Fig. 21 (a), indicating interference of type (c) above. One of these peaks was immediately adjacent to the Zn peak and sometimes incompletely resolved from it. This was the case when two "HNO_3 only" digests were analysed, and inconsistent results were obtained. This interferent peak varied in size, and was considerably reduced on leaving the neutralised, diluted digest in a sealed plastic bottle overnight. It was thought to be due to dissolved oxide(s) of nitrogen (NO_x), which - as mentioned earlier (Section 6.2) - are found in nitric acid pressure digests,[15] and have been reported to produce strong interference signals in voltammetric analysis.[154] It was found that the peak was much smaller in the

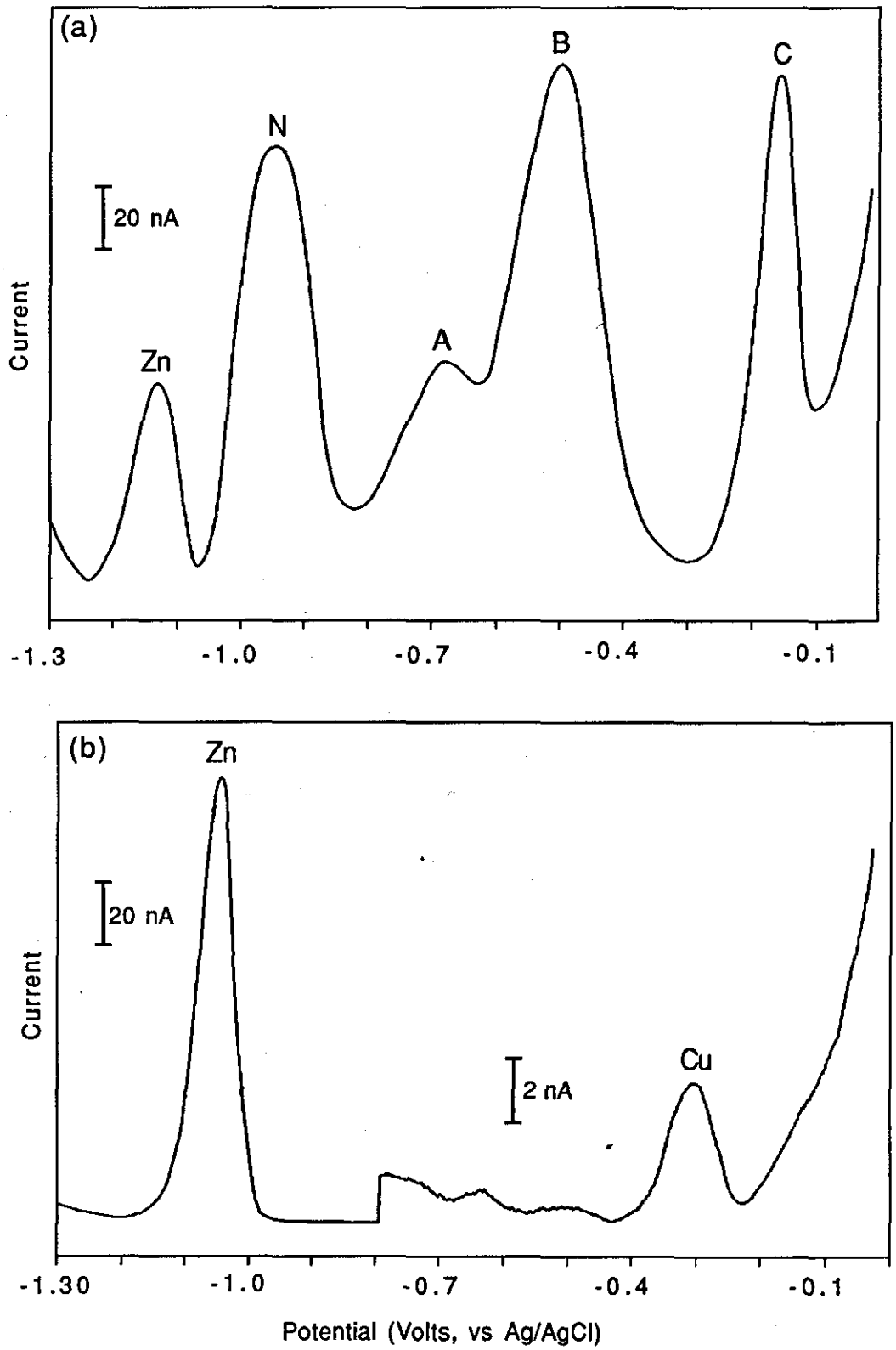

Fig. 21 Voltammograms of milk powder following digestion with (a) HNO_3/H_2O_2 (two stage microwave, Method D) and (b) $HNO_3/HClO_4$ (microwave + hot-plate, Method E), and adjustment to pH 9 with ammonia, showing signals due to Zn, Cu, nitrobenzoic acids (A,B & C) and probably nitrogen oxides (N)

voltammogram of a sweet bay digest which had been treated with peroxide on a hotplate. However, the blank Zn level for this latter digest was higher than for the other (closed) digests, possibly due to Zn impurity in the hydrogen peroxide (as more had been added) or contamination during post-digestion treatment.

In addition to the NO_x peak which gradually diminished on standing, three other prominent, stable peaks were seen in the milk powder digests. Previous workers[140,154] have shown the major interference in voltammetric analysis of nitric acid digests of bovine liver to be due to the 3 isomers of nitrobenzoic acid (NBAs). NBAs have been indicated in the digests of milk powder, pet food and sweet bay in the present work by previous TLC analysis (see Section 9.2.2) and UV/visible spectrometry (Section 9.2.4), although IR analysis of the residues failed to show them up.

Voltammograms were recorded of control samples of NBAs in acid and basic media. These were prepared by dissolution of solid samples of 2-, 3- and 4-nitrobenzoic acids (BDH and Fisons). The resulting signals corresponded to the peaks observed in the digests. In acid solution (1% HNO_3), the digests and control NBAs each gave one major signal at –0.1 V (again masking any copper peak) and a much broader, flatter signal at around –0.6V . These are reportedly due to the reduction of NBAs to amines via hydroxylamines,[154] the two-electron reduction of hydroxylamine to amine taking place at a more negative potential than the four-electron reduction from NBA to hyroxylamine.

After adjustment of the above solutions to pH 9 by the addition of ammonia, each isomer gave 2 peaks, one at –0.15V and the second at –0.50V for 4-NBA, –0.56 V for 3-NBA and –0.72 V for 2-NBA. These are shown in Fig. 22. Comparing with the voltammogram of the milk powder digest shown in Fig. 21 (a), 2-NBA and 4-NBA gave signals corresponding to A + C and B + C respectively, while 3-NBA gave a signal between A and B and one at C. To confirm the identification, measured aliquots of the 3 NBA isomers were added in turn to a digest of milk powder prepared by method D (to give an added 10 $\mu g\ ml^{-1}$ of each isomer in the solution). After addition of each standard, the HMDE potential was held at – 0.8 V for 1 min followed by potential scan to 0.0 V at 5 $mV\ s^{-1}$, with a differential pulse of 50 mV as before. Enhancement of the 4-NBA and 2-NBA peaks was clearly seen, and addition of 3-NBA resulted in a broadening of the 4-NBA peak towards the 2-NBA peak. These voltammograms, shown in Fig. 23, strongly indicated the presence of 2- and 4-NBAs (mainly the latter) in the digests, with a smaller amount of 3-NBA, and agreed with the previous TLC findings.

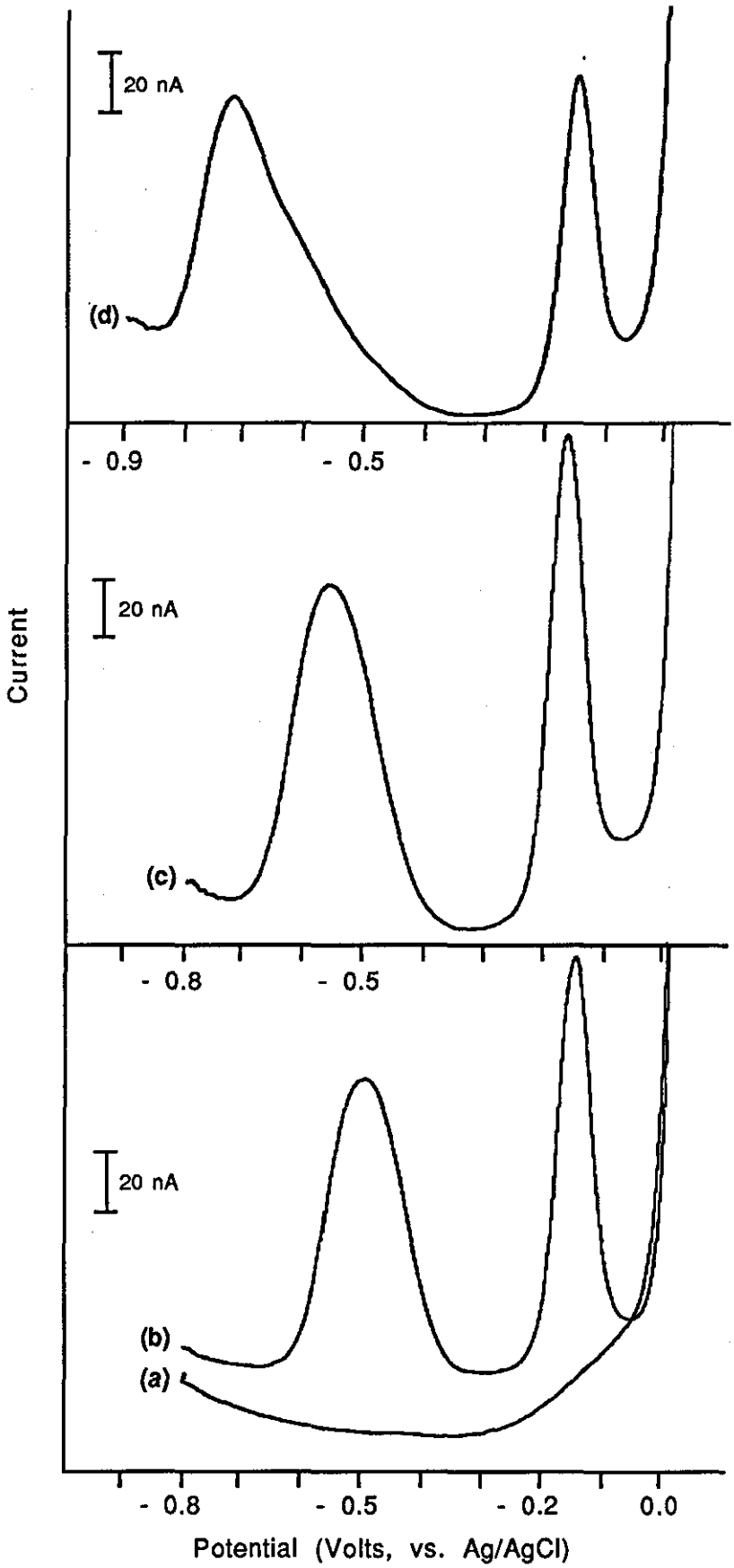

Fig. 22 Voltammograms of nitrobenzoic acids (NBAs): (a) Blank (1% nitric acid + ammonia added to pH 9) (b) 4-NBA; (c) 3-NBA and (d) 2-NBA (40 μg ml^{-1} each)

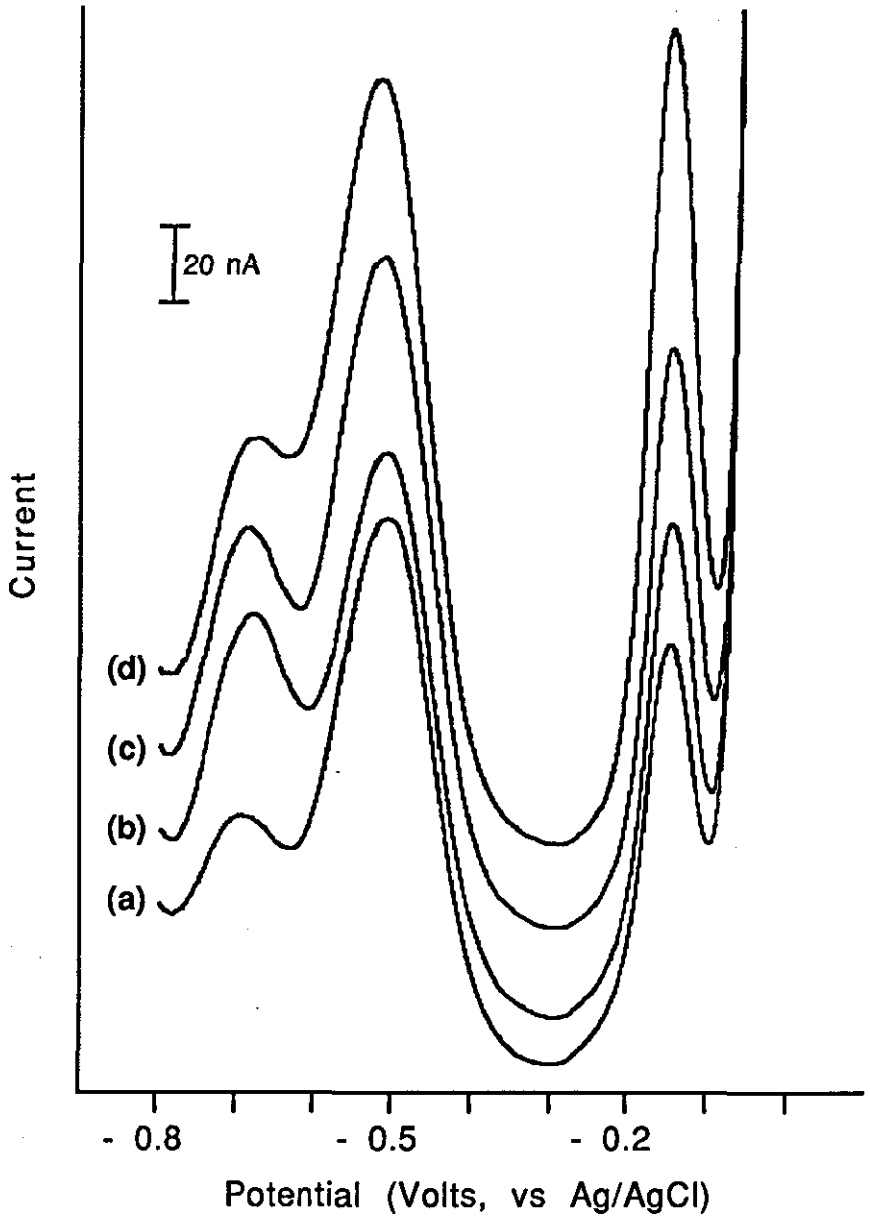

Fig. 23 Voltammograms of diluted milk powder digest with (a) no added NBA, then following addition of 10 μg ml^{-1} each of (b) 2-NBA, (c) 4-NBA , and (d) 3-NBA

By comparing peak heights from the digest and control NBAs at pH 9, the concentrations of 4- and 2-NBAs in the diluted digests were estimated at 40 and 10 μg ml^{-1} respectively (with a smaller amount of 3-NBA at ca. 5 μg ml^{-1}, its concentration being difficult to estimate due to merging with the 4-NBA peak), which translates to a weight of 13 mg NBA per gram of sample digested. This would give 1.3 mg of NBAs in the dried residue of a 5 ml digest aliquot (cf. total residue of 20 mg). Thus the NBAs are a relatively minor constituent (by weight) of the digest residues, which explains why their presence was not apparent from the infrared spectra of the residues, but they constitute the major interferents in DPASV analysis. (Whilst they do not directly interfere with the Zn peak, they

interfere with Pb, Cd and Cu determination).

Assuming the NBA results solely from the decomposition of phenylalanine in the original sample, as previously suggested,[140,153] the corresponding concentration of phenylalanine in the milk powder is 1.3%. This compares with a typical value for phenylalanine in milk powder of 1.40% calculated from the literature.[178] Alternatively, the NBA yield from the milk powder of 13 mg g^{-1} compares with a theoretical yield of 14.2 mg g^{-1}, assuming complete decomposition of the phenylalanine (present at 1.40% in the milk powder) to NBA.

9.4.3 Removal of Interferences

Interference due to dissolved NO_x may be removed by evaporating the digest to dryness.[154] However, the evaporated residues were incompletely soluble in the basic (0.05M NH_3/NH_4Cl) electrolyte, and although a good "interference-free" Zn peak was obtained, the subsequent analysis gave a low value (15 µg g^{-1}). When the residues were dissolved in acid and analysed in 1% HNO_3 the Zn peak disappeared into the hydrogen wave. Dissolving the residues in HNO_3 and taking to pH 9 with ammonia seemed to offer a solution, but again gave inconsistent Zn values (15 - 25 µg g^{-1}) and did not reliably remove the interfering peak (though it was much reduced), although sensitivity for Zn was increased (by a factor of 2-3), presumably due to decreased ionic strength compared to the neutralised digests.

Three other approaches to the removal of dissolved nitrogen oxides were tried:

i) Lengthening degassing time

Previously 5 min purging with nitrogen preceded analyses. Increasing this time to a total of 35 min only resulted in a small reduction (14%) in the "NO_x" peak. (For comparison, leaving for 20 h in a plastic bottle resulted in a 44% reduction).

ii) Adding sulphamic acid

This had no effect on the NO_x peak.

iii) Heating the digest

Heating the neutralised, diluted digest to boiling in a beaker on a hot-plate and simmering for 5 min also had no effect on the interferent peak, but did result in an enhanced Zn peak.

Hence no satisfactory method for simply and rapidly removing this interferent peak was found.

Methods which were considered for the removal of interference due to the NBAs (and any other organic interferents present) included the following:

a) UV Irradiation

This is a favoured treatment for destruction of organics in water samples, as it involves minimal sample manipulation and reagent addition, leading to low blank values.[182] It has also been used for treatment of blood[180] and hair[183] prior to electrochemical analysis, although in the case of blood, sample size was severely restricted, and perchloric acid and hydrogen peroxide were added to the hair prior to irradiation. The presence of H_2O_2 is advantageous as it promotes the formation of OH radicals needed for the process.[184] However, it is time-consuming (3 - 4 h) and requires high power mercury arc lamps not readily available for this work. It also works better at elevated temperature (90°C +) so care has to be taken to avoid evaporation losses. Another problem is that nitrate ions are converted by UV irradiation to voltammetrically active nitrite ions which interfere with Zn determination, necessitating the addition of sulphamic acid to reduce the nitrite to molecular nitrogen. Finally, it is reportedly not very effective with aromatics.[184]

b) Activated carbon

This has been used for cleaning up sugar solutions for heavy metals analysis,[185] when it was said to absorb the major part of organic impurities, but loss of metals by adsorption[186] and contamination from metals in the carbon is a danger.

c) Fumed silica

This has been used for the removal of sorption interferences by organic surfactants in ASV[187] and has been applied to the direct determination of lead in urine by DPASV.[188] Silica adsorbs large surface active organic molecules, but may not be so effective with relatively small aromatic molecules.

d) Perchloric acid treatment

The use of hot perchloric acid with its powerful oxidising properties would almost certainly enable the complete removal of organic residues from the digests. Providing suitable precautions were taken, the problems of organic interference could be solved by treatment with this acid following microwave digestion with nitric acid.

Hence the post-digestion treatment as detailed earlier (Section 8.4, method E) was carried out on several milk powder digests. Care was taken to remove all easily oxidised material through the nitric acid pressure digestion (method A) before

adding perchloric acid to the cold digest and heating the mixture slowly in a fume hood. Calculations of chemical oxygen demand, using the residual carbon measurements reported in Chapter 8, had indicated that 1 ml of perchloric acid would be sufficient to oxidise the remaining organic material, so initially 1 ml of perchloric acid was used, but this was found to leave some organic residues (small NBA peaks appeared in the DPASV analysis), so the added volume of perchloric acid was increased to 2 ml for the treatment of two further digests.

The perchloric acid stage could not be carried out using microwave heating with the vessels used in this work, as the polyetherimide shell is attacked by perchloric acid. However, the use of all-Teflon PFA pressure vessels in a two-stage nitric/perchloric acid microwave digestion has been reported for the determination of copper in blood plasma by DPASV.[124]

9.4.4 Analysis of Perchloric-Acid Treated Digests

Complete decomposition of the milk powder using perchloric acid enabled copper to be successfully determined in the digests, as all the interfering peaks were removed, as can be seen in Fig. 21 (b), which also shows that zinc sensitivity was improved. This is probably mainly due to the removal of competitive reductions of the NBAs at the surface of the mercury, enabling improved zinc accumulation. Aromatic nitro compounds have been reported to be strongly adsorbed onto mercury[189], although at the deposition potential used here (-1.3V) reduction of the NBAs to amines is likely to predominate. In contrast to the Zn signal, the heights of the NBA peaks were unaffected by varying the deposition time, indicating that there was no accumulation/stripping of the NBAs, but rather a reduction of species in solution reaching the electrode by diffusion. The reduced ionic strength of the perchloric acid treated digests could also contribute to the improved sensitivity.

A 5 ml aliquot of each digest was diluted to 25 ml with addition of ammonia to pH 9 and analysed by DPASV as before, this time determining both Zn and Cu in the same solution, again by standard additions, the Zn standards being added as before. For the Cu determination, an aqueous stock solution containing 10 μg ml^{-1} Cu was prepared by dilution of a standard solution containing 1000 μg ml^{-1} of Cu (BDH), and 10 μl aliquots of this were sequentially added (giving added Cu concentrations of 4, 8, 12 and 16 ng ml^{-1} in the analysed solution). By adjusting instrument sensitivity between the zinc and copper signals, to allow for the 25-fold difference in their concentration, both elements were determined simultaneously. Zn and Cu peaks were easily measurable at appropriate sensitivities, and the peaks due to the NBAs and "NO_x" had

disappeared (except for the first digest treated with only 1 ml of perchloric acid, where the remaining NBA interference precluded Cu determination). This was confirmed by the residual carbon content results - showing negligible residual carbon - reported in Chapter 8.

Plots of peak height versus added Cu were linear, blanks showed negligible copper signals and results were in good agreement (Cu levels of 0.78 and 0.76 $\mu g\ g^{-1}$ in the milk powder).

Two of the three perchloric acid digests analysed gave Zn concentrations in good agreement with previous results (22.6 and 23.2 $\mu g\ g^{-1}$ in milk powder). One sample, however, gave low results for Zn (16.1,15.8 & 14.3 $\mu g\ g^{-1}$), and some drifting upwards of Zn response for replicate measurements was observed during the standard additions analysis. Several experiments to discover the source of this drift led to some insight into its causes, though did not provide the whole solution, as discussed below.

It was thought that the solutions may have deteriorated by being left in the measurement cell during the course of an analysis, either through evaporation or adsorption of the trace metals on the glass walls. However, leaving the solution in the cell overnight led to no change in the response the next day.

It was noticed that the temperature in the laboratory often drifted upwards by a few degrees during the course of an analysis, which may have adversely affected the results, so the effect of these temperature changes were studied. It was found that the temperature rise did indeed affect the Zn response (and to a lesser extent the Cu); a gradual rise in temperature of the solution produced a gradual rise in the voltammetric peaks. This was tested by monitoring the change in temperature of the laboratory over the day, taking voltammetric measurements at frequent intervals, and also by cooling the solution to several degrees below room temperature and taking measurements as it rose to room temperature over a period of about an hour. In both cases, a 1 degree C rise in temperature produced an average increase of 6% and 2% in the heights of the Zn and Cu peaks respectively. This could result in falsely low results in a typical analysis, as gradual enhancement of the signals of added standards would lead to errors in the calibration, with a slight upward curvature in the calibration graph. This was, in fact, observed for the sample giving low results, although it was very doubtful if this effect was of sufficient magnitude to cause the observed reduction in Zn values. However, the importance of temperature stability for accurate work was demonstrated.

It was thought that contamination could have been introduced via diffusion of Zn from analytical solutions into the mercury capillary, followed by stripping of this from subsequently dispensed drops, causing enhancement of the Zn signal. This was tested by varying the number of mercury drops dispensed before the deposition step, but no variation of Zn (or Cu) signal resulted, so this was ruled out as a source of error. Similarly, addition of a small volumes of the reference electrode solution (KCl) produced no enhancement of the Zn (or Cu) peaks, thus ruling out the possibility of Zn contamination through seepage from the reference electrode.

9.4.5 Summary of Voltammetric Analysis

The determination of zinc and copper in the milk powder digests by DPASV served to illustrate the effect of interfering species on this type of analysis. By carrying out the analysis at pH 9 the adverse effect of the hydrogen wave was eliminated, enabling zinc to be determined much more easily than in acid solution, although there was a strong peak probably due to dissolved oxides of nitrogen (NO_x) very close to and partially overlapping the zinc wave. There were also several interfering peaks, identified as being due to isomers of nitrobenzoic acid in the digests, which rendered the determination of copper in the nitric acid and peroxide treated digests impossible. The determination of zinc could be carried out successfully on the peroxide-treated digests, but the NO_x peak interfered with accurate baseline estimation for the "nitric acid only" digests, leading to inconsistent results. Removal of the NO_x peak by evaporation also gave inconsistent Zn results on subsequent analysis. Treatment of the digests with perchloric acid removed both the NBA and NO_x interference, enabling the simultaneous determination of Zn and Cu, the latter at a concentration of 4 ng ml^{-1} in the analytical solution.

9.5 Comparison of Decomposition Product Yields

Where decomposition products have been identified and quantified or estimated, their yields (i.e the weight of product, in mg, produced from the digestion of 1 g of sample "as received") have been compared with theoretical yields calculated using data for sample compositions either from ICP-MS analysis (see Chapter 6, Table 5) or literature values[178]. Agreement has generally been good; values are given in Table 11.

Table 11. Comparison of Theoretical & Actual Yields of Decomposition Products

Sample	Product	Theoretical Yield mg g^{-1}	Measured Yield mg g^{-1}
Sodium Alginate	Sodium Nitrate†	373	334
Milk Powder*	Nitrobenzoic acid††	14.2	13
Milk Powder*	Dibasic Calcium Phosphate ($CaHPO_4$)	37	41
Milk Powder*	Calcium Oxalate	16	20
Milk Powder	Calcium Oxalate	40	38
Sweet Bay	Carbon Dioxide	1770**	2300‡

* In HNO_3/H_2O_2 pressure digests (Method C). Other results from "HNO_3 only" digests (Method A).

† Theoretical yield of sodium nitrate calculated assuming no cross-linkage of alginate chains to affect sodium content, and allowing for moisture content of 13.2%; i.e. assuming 1 g sample contained 868 mg of sodium alginate of formula $\{Na^+(C_6H_7O_6)^-\}_x$ completely converted to $NaNO_3$.

†† Theoretical yield of NBA calculated assuming 100% conversion of phenylalanine to nitrobenzoic acid (using phenylalanine concentration as reported for powdered cows milk.[178])

** Calculated from measured carbon content in original sample of 484 mg g^{-1} completely converted to CO_2.

‡ Measured by weight loss of acid/sample mixture after closed microwave digestion (likely to be overestimate, as some loss due to loss of NO_2).

CHAPTER 10
CONCLUSIONS & FUTURE PROSPECTS

10.1 Significance of Decomposition Studies

The results of analysis of the various digests illustrate the necessity of employing different sample decomposition methods according to the sample matrix and the analytical technique being used. For spectrometric methods tolerant of undigested organic material (such as ICP-AES) or for analysis of relatively easily-decomposed materials, a single stage digestion with nitric acid produces suitable solutions. Further digestion using hydrogen peroxide significantly reduces residual carbon content for samples of high protein and/or fat content, without further complicating the matrix (unlike, for example, the introduction of sulphuric acid), which may be important for some techniques (such as AAS or ICP-MS).

On the other hand, complete decomposition is often needed for electrochemical analysis. It has been shown that this can be readily accomplished using a straightforward post-digestion treatment with perchloric acid. As an alternative to perchloric acid treatment, other techniques such as UV irradiation may be successful in some cases. These would need to be tested for particular digests.

10.2 Vessel Comparison

Of the two types of digestion vessel tested, the lower pressure, all-Teflon PFA vessels proved unreliable for the digestion of larger organic samples of high protein or fat content, as the pressure rises often led to premature venting of the vessels, unless an open pre-digestion stage was incorporated. Even then, multiple heating cycles were often needed to fully dissolve 1 g samples with 10 ml nitric acid. Vessel cooling using liquid nitrogen, was, however, very convenient, enabling rapid cooling between multi-stage digestions, and a simple two-way valve in the pressure line enabled rapid removal of the pressure monitor vessel - and thus the whole turntable - from the microwave cavity at the end of a heating cycle.

The higher pressure, lined vessels offer the advantage of simplified procedures through the use of single-stage digestions, and the lined vessels have the advantage of not requiring a capping station and have a better venting system, both for pressure relief and manual venting. However, overall sample preparation time is not significantly reduced owing to longer cooling times, as neither liquid nitrogen nor dry ice can safely be used, and care has to be taken to avoid acid attack on the outer shells. Also, the two-way valve proved unreliable

at the higher temperatures and pressures reached using these vessels.

10.3 Possibilities for Reflux Cooling

To avoid evaporation losses during an open pre-digestion, a reflux system is desirable. The principle of reflux pre-digestion using a microwave transparent coolant was tested and its feasibility shown, but to be truly practical a simple system capable of pre-digesting a full turntable of samples would need to be developed. This would require a suitable enclosed pumping system together with an appropriate condenser design capable of serving multiple vessels instead of just the single vessel system made in this project. Such a system would need to be tested for volatile analytes to prove its effectiveness.

The use of a microwave transparent coolant to cool the top part of a sealed digestion vessel could also prove useful in reducing the internal pressure by cooling the vapour during a closed digestion, without significantly reducing the temperature of the digestion mixture in the bottom part of the vessel. Such an approach, involving the incorporation of a circulating coolant in the walls of a sealed microwave digestion vessel, has been the subject of recent research in the U.S.[190] If some means were found of cooling the top part of a vessel with liquid nitrogen, say, without having to immerse the lower part of the vessel, with the consequent drastic slowing of the digestion, this could be useful, although care would have to be taken not to inhibit the digestion by freezing the vaporised reactants.

10.4 Analysis of Digests

The determination of a range of trace elements in digests of certified reference materials prepared by proposed microwave methods should ideally be carried out, to assess the comparative suitability of the methods for the matrix and type of analytical technique being used. It was hoped to carry out such analyses using ICP-MS in this project, but as explained in Section 8.6 this did not prove possible. However, future investigations could include such analyses.

10.5 Overall Assessment

Microwave pressure digestion has been shown to be a useful tool in sample preparation for trace element analysis, leading to savings in time, energy consumption and reagents, and reduction of contamination. However, it is not a magical answer to every problem, and it can be an expensive solution. Whilst microwave heating is undoubtedly very rapid, the limitation on vessel numbers, the time taken in assembling, cleaning and cooling vessels, and the operator

attention required to monitor pressure - unless using a system with a pressure feedback loop - mean the overall time savings may not be as great as they at first appear. There are also much severer limitations on sample size than is the case with conventional wet digestion at atmospheric pressure.

Larger samples may be accommodated using an open focused microwave system, which also offers the advantage of a more efficient use of power and no pressure containment problems. However, it is not as suitable for volatile elements as a closed system, and care must be taken to avoid evaporation losses. It is also slower, as only one sample is digested at a time, at the lower temperatures achieved at ambient pressure, although with the newer, automated systems incorporating a turntable and automated reagent addition, operator attention can be minimised.

A modern alternative to the batch microwave method studied in this project is offered by the recently developed flow systems. These are sequential systems offering considerable time savings when linked directly to a suitable analytical instrument, and appear to be very convenient. However, they do not appear as flexible as the batch systems and may suffer more problems due to incomplete decomposition and dissolution, as witnessed by the typical incorporation of a filtration stage in such systems. The development of these systems to handle a wider range of matrices and detection systems may well represent the future direction of microwave sample preparation.

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