Reflux Pre-digestion in Microwave Sample Preparation

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In order to prevent excessive pressure rises in closed microwave acid digestion of fairly large (1 g) samples of high organic content, an open-vessel pre-digestion technique under reflux was designed to allow the escape of oxidation products, such as carbon dioxide, without incurring evaporation losses of acid or analytes. A microwave-transparent coolant liquid was circulated through a specially designed reflux condenser fitted onto an uncapped, commercially available digestion vessel, whilst samples and acid were heated in a microwave oven. Following pre-digestion, the vessels were capped and subjected to microwaves under pressure to complete the digestion. To test the method, 1 g samples of powdered sweet bay were digested with nitric acid using an open pre-digestion with and without reflux cooling, and the mass loss incurred compared with that using a completely closed method (where loss would be restricted to that due to oxidation reactions and release of carbon dioxide). The reflux and closed methods showed similar small mass losses, whereas those where reflux had not been used showed much higher losses, reflecting considerable evaporation of acid. Analysis of the digests for manganese by flame atomic absorption spectrometry showed no significant losses of analyte using either method.

Keywords: *Microwave digestion; sample preparation*

Microwave heating of various types of samples with concentrated acids under pressure is now a well established method for rapid, reproducible, and controllable digestion prior to elemental analysis, and has found many applications.[1,2] However, the closed digestion of samples of high organic content can lead to excessive build up of pressure (which may continue after the microwave power is switched off), leading to bursting of sealed vessels, or where pressure relief valves are present, venting of the hot vessels with potential loss of sample. This is a particular problem when digesting fairly large samples (1 g or above), which may be necessary to prevent errors owing to sample inhomogeneity.

To alleviate this problem, microwave pressure digestions of organic samples are often preceded by a pre-digestion step in unsealed vessels, either on a hot-plate[3–5] or, more quickly, by microwave heating.[6,7] This allows the escape of gaseous reaction products, notably carbon dioxide produced from the decomposition of easily oxidized 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 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 of 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 has been mentioned as a possibility,[8] it has 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,[9,10] or by the rapid pumping of chilled water through a small reflux condenser in the microwave cavity.[11] 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 the 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,[12] 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 the flammability, being essentially microwave transparent and having a useful liquid range ($-90\ °C$ to $+98\ °C$).

Experimental

Apparatus

Digestions were carried out using a Model MDS-81D microwave unit and 120 ml perfluoroalkoxy Teflon (Teflon PFA) vessels [CEM, Matthews, NC, USA, supplied by CEM (Microwave Technology), Buckingham, UK], with a maximum power output measured at 675 ± 15 W and pressure monitoring *via* an external transducer (Sensym SX150DN). The features and calibration of this equipment have been described previously.[13]

The coolant, heptane, was delivered to the microwave cavity from a pressurized 1 l shielded glass bottle with a three-valve cap (Omnifit, Cambridge, UK), cooled in a solid carbon dioxide (cardice)–methanol slurry, *via* $\frac{1}{8}$ in o.d. Teflon tubing which entered and left the microwave cavity through inlet/outlet ports at the rear of the unit. Temperature measurements of the heptane, before and after it flowed through the cavity, were made using a Technosyn temperature probe.

Analysis for manganese was accomplished using a Philips (Cambridge, UK) PU 9100 atomic absorption spectrometer, with operating parameters as given in Table 1.

Reagents

Digestions were carried out using Aristar grade concentrated nitric acid (BDH/Merck, Poole, Dorset, UK). Laboratory-

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grade heptane, dried over a molecular sieve, was used as coolant in the microwave cavity. Standards for flame atomic absorption spectrometry (FAAS) were prepared in 10% nitric acid by dilution of a standard solution containing 1000 mg l^{-1} of manganese (Pierce Inorganics, Rotterdam, The Netherlands). Water purified by a Liquipure Modulab system (Liquipure Europe, Bicester, UK) was used for all dilutions. The sample of sweet bay was provided by the Metallic Impurities in Organic Matter Sub-Committee of the Analytical Methods Committee of the Royal Society of Chemistry (Analytical Division).

Design of Reflux Apparatus

As an initial attempt, a 'cold finger' was designed and constructed from borosilicate glass (which is virtually transparent to microwaves), to fit onto a digestion vessel. This consisted of an open cup that sat on the rim of an uncapped vessel, leading into a finger-shaped tube protruding downwards into the vessel. The open top allowed the vessel to be filled with liquid nitrogen for cooling during a pre-digestion stage.

This simple glass finger was tested for its usefulness in preventing sample loss in an unsealed pre-digestion. Water was heated in the Teflon PFA vessel, with the finger, and the 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.

Owing to the highly flammable nature of heptane, the possibility of ignition from a spark from the electrics could not be excluded, so a completely enclosed system was constructed, with inlet and outlet ports for the coolant. 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. 1, which is used in conjunction with the cap from the vessel, making the vessel–condenser interface more stable. This design 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. However, as the condenser has to be fairly short to fit into the microwave cavity, it has the disadvantage of having 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.

After several design modifications, the assembly shown in Fig. 2 was constructed. This includes symmetrical inlet/outlet ports, and a broad rim to enable it to seat more firmly in the vessel. These features are necessary in order 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 the condensed vapours, as happened when cold heptane was pumped through the ungrooved finger while heating nitric acid in the vessel.

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 PFA jacket was constructed out of the screw-threaded section of an old digestion vessel and tied around the reservoir with poly-(tetrafluoroethylene) (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.

Delivery System for Heptane Coolant

The heptane was delivered from a 1 l bottle, cooled by immersion in a cardice–methanol slurry and pressurized using nitrogen to produce a flow rate of approximately 100 ml min^{-1}. It was necessary to use all-Teflon connections to the bottle cap, 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. The tubing carrying heptane out of the microwave unit was fed into a 1 l bottle through a vented stopper. The heptane was then returned to the pressure bottle and cooled for the next pre-digestion cycle.

Table 1 Operating parameters for the determination of Mn by FAAS

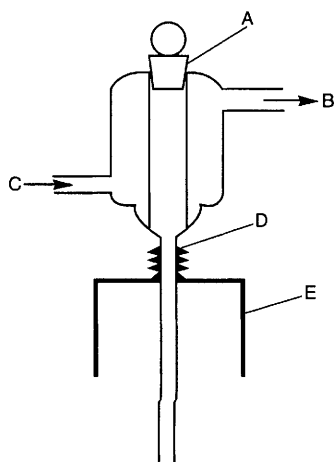|	
Flame	Air–acetylene
Lamp current	9 mA
Wavelength	279.5 nm
Slit-width	0.2 nm
Measurement mode	Integrated absorbance
Integration time	5 s

Fig. 1 Borosilicate glass reflux condenser. A, Glass stopper, loosely attached with PTFE tape (not shown); B, heptane out; C, heptane in; D, join sealed with PTFE tape; and E, vessel cap
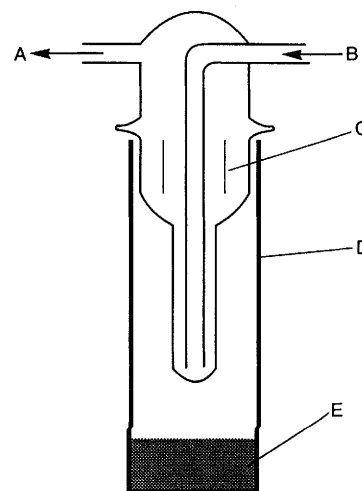

Fig. 2 Borosilicate glass cold finger assembly. A, Heptane out; B, heptane in; C, four equally spaced vertical grooves acting as vents; D, Teflon PFA vessel; and E, digestion mixture

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 programme. Suitable conditions for two vessels (one with the reflux condenser and one unsealed), each containing 10 ml of concentrated nitric acid, were found to be: coolant, 1 l of heptane, pre-cooled to about −50 °C in a cardice–methanol slurry; applied pressure, 83 kPa (12 lb in^{-2}); flow rate 100 ml min^{-1}; and microwave programme, 50% power for 7 min.

The efficiency of the reflux was tested by comparing the mass loss from 10 ml of acid in a vessel using the cold finger, to that from 10 ml of acid in an unsealed vessel (with the cap loosely fitted) heated simultaneously at 50% power for 7 min, measurements being made for six replicate runs.

Comparison of Reflux and Open Pre-digestion and Sealed Digestion of Sweet Bay

Using the 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 the 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. For comparison, the mass loss resulting from a completely sealed pressure digestion of a 1 g sample of sweet bay was measured, when the microwave power was adjusted to give a pressure of 550 kPa over three heating, cooling and venting stages.

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.

Analysis of Sweet Bay Digests

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 the 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 500–550 kPa for 15 min. Following pressure digestion, the digests were transferred into 25 ml calibrated flasks and made up to volume. The solutions were stored in 60 ml poly(propylene) screw-capped bottles prior to analysis.

Manganese levels were determined by FAAS. To check for matrix interferences, an aliquot of each digest was diluted by a factor of two and re-analysed; 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 2 Mass loss during microwave acid pre-digestion of sweet bay; comparison of reflux, unsealed and sealed vessel methods

	Mass loss/g				Equivalent volume of HNO$_3$/ml
Run	Reflux	Unsealed	Sealed	Difference/g	
A	2.3	10.5	—	8.2	5.8
B	2.2	11.8	—	9.6	6.8
C	—	—	2.8	—	—
Blank acid	0.2	1.0	—	0.8	0.5

Results and Discussion

Mass Loss Measurements

The six runs using acid blanks showed consistently low mass losses of 0–0.3 g from the reflux vessels, whilst losses from the unsealed vessels varied from 0.5 to 4.6 g (mean 2.1 g) for different vessels. This variation could be partially because 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 this 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 the normal boiling-point owing to differences in the condition of the internal vessel walls.[14] Fine scratches and abrasions on the walls of some vessels could 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. It was also found that the reflux vessel cooled much quicker than the open vessel, enabling easier handling.

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 2.

Comparison of mass loss from the reflux vessels with that from the sealed vessel strongly indicates that the mass 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.

For the unsealed (non-reflux) vessels, the evaporation losses from the acid blanks were much less than those from the sweet bay digestions, therefore more acid had to be added to the latter to replace these losses. Thus, for the unsealed pre-digestions, the level of impurities in the blank would not reflect that in the sample because of the differing volumes of acid used, and hence would be difficult to allow for in low-level analyses.

Coolant Temperature Measurements

The initial temperature of the heptane at the top of the pressure bottle lay between −25 and −40 °C. The maximum outlet temperature of the heptane coolant was measured at 12.8 °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 had ended.

Manganese Determinations

The results of analyses of the sweet bay samples by FAAS are summarized in Table 3. No significant differences were found between measured manganese concentrations following the various pre-digestion methods.

Alternative Reflux Condenser Design

The alternative design of the small reflux condenser, as shown in Fig. 1, was also tested for its efficiency as a pre-digestion

Table 3 Determination of manganese concentration in sweet bay powder, 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; and D, no pre-digestion (lengthy multi-stage pressure digestion). In all instances, 1 g samples were digested with 10 ml of concentrated HNO_3. Results are given as mean $\pm$ standard deviation

Digestion method	Mn concentration/ $\mu g\, g^{-1}$	Number of determinations
A	25.0 ± 0.6	8
B	24.9 ± 0.6	7
C	24.5	2
D	24.8	2

aid, and initial experiments were encouraging in terms of reduced evaporation losses of nitric acid, by comparison with heating digests in covered, but unsealed vessels (*i.e.*, with their caps loosely fitted). The outside of the condenser was insulated with polystyrene. However, because of the reduced surface area available 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 levels by FAAS in these digests showed no significant differences between the pre-digestion methods.

Conclusions

Reflux pre-digestion using a microwave-transparent coolant has 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 recovered quantitatively using the reflux method by comparison with an open-vessel pre-digestion method. Also, the lower volumes of acid required should lead to lower impurity levels and thus lower limits of detection.

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