

Appendix 2

DTMS and DTMS/MS Study of Solid Organic Residues

Modified after:

T.F.M. Oudemans, G.B. Eijkel, & J.J. Boon 2005, 'DTMS and DTMS/MS study of solid organic residues preserved on ancient vessels', in: *Proceedings of the 33rd International Symposium on Archaeometry, 22-26 April 2002*, H. Kars & E. Burke (eds.), Vrije Universiteit, Amsterdam, 501-505.

1. Introduction

1.1. Organic residue analysis in ceramic studies

The chemical characterisation of solid organic residues found in association with ancient pottery can give direct information about the original prehistoric vessel use. Chemical analysis of solid surface residues and absorbed organic residues alike, has taken place as early as the 1920s (Rottländer & Schlichtherle 1980), and has greatly expanded in the last two decennia (Evershed *et al.* 1992; Heron & Evershed 1993; Evershed *et al.* 1999) due to improvements in analytical instrumentation and an increasing interest in the functional aspects of pottery in ceramic studies. Many analytical techniques have been applied to the analysis of specific classes of compounds such as solvent extractable lipids (Evershed *et al.* 1999), waxes (Heron *et al.* 1994; Evershed *et al.* 1997; Regert *et al.* 2001), terpenoids (Charters *et al.* 1993a; McGovern *et al.* 1996; Dudd & Evershed 1998) and amino acids (Evershed & Tuross 1996) and, more recently, to immunologically detectable proteins (Craig & Collins 2000; Craig *et al.* 2000). Although these studies have facilitated the identification of a whole range of compounds in archaeological residues, and have provided plausible identifications for particular groups of residues, they are limited to a specific group of compounds. For the study of the non-soluble solid macromolecular fraction that forms the matrix of solid organic residues, a non-selective analytical technique is required to characterise the overall chemical composition of residues and to identify the cross-linked components otherwise ignored.

1.2. PyMS studies of solid organic residues

Analytical pyrolysis mass spectrometry (PyMS) has been applied to the study of solid biomaterials from very different origins and has proven to be a fast fingerprinting method particularly suited for the recognition of different classes of compounds in complex materials (Boon 1992).

In earlier studies of residues preserved on ancient vessels, indicative markers for a broad range of bioorganic moieties were detected using Curie point PyMS mass spectra and subsequently identified using Curie point PyGC/MS (Oudemans & Boon 1991). The Curie point PyMS spectra are obtained through very rapid heating of the sample to a set temperature of 610 °C, creating a chemical fingerprint indicative of the overall chemical composition of the residues. A comparative statistical study of these Curie point PyMS fingerprints (up to mass weight m/z 220) showed the chemical composition of the residues to be correlated to the form and size of the vessel. Since no indications could be found for severe post-depositional degradation or contamination of the samples, this correlation reflects a difference in original use between vessels of different forms and sizes (Oudemans & Boon 1996).

1.3. DTMS and DTMS/MS

Developments in instrumentation have opened up new possibilities for the study of solid biomaterials. Direct temperature-resolved mass spectrometry (DTMS) achieves a physical separation between low molecular weight compounds by evaporation and the cross-linked fraction of a sample by pyrolysis, by gradually raising the temperature of the sample on the probe.

DTMS not only makes it possible to chemically categorise small amounts of organic residues on pots, but can also give an insight into the physical conditions that have allowed compounds to survive long-term burial in archaeological context. The temperature at which compounds are freed from the solid, is indicative of the preservation processes - encapsulation in an inert material, adsorption within the pores of a matrix, and cross-linking or condensation - that may play a role in the survival of foodstuffs in ancient vessels. Tentative identifications of the chemical composition of residues can be made by comparison with modern (fresh or experimentally charred) reference materials.

Subsequent tandem mass spectrometry makes it possible to identify individual characteristic ions in the DTMS spectra of residues. This Direct Temperature-resolved Mass Spectrometry/Mass Spectrometry (DTMS/MS) option can thus identify ions and confirm earlier tentative identifications based on comparison with standards.

In this study, a short account is given of the DTMS and DTMS/MS characterisation and comparison of 34 charred and non-charred solid organic residues preserved on exterior and interior of vessels recovered from indigenous settlements from the Roman period (0 - 300 AD) at Uitgeest-Groot Dorregeest (Abbink 1999). A more extended article on this topic is appearing soon (Oudemans *et al.* in press-b).

2. Experimental

2.1. Sample treatment

Samples (5 - 10 μg) for DTMS and DTMS/MS were ground up in 15 - 25 μl of ultra pure water. An aliquot of 1 - 2 μl of this suspension was used per analysis.

2.2. Instrumental

DTMS experiments were carried out on a JEOL DX - 303 double focussing (E/B) mass spectrometer equipped with a JEOL DA - 5000 data system. The sample suspension was applied to the filament (Pt/Rh 9:1, 100 μm) of a direct insertion probe, which is inductively heated at

1A/min to a maximum temperature of 800 °C. Ions were generated by low voltage (16 eV) EI conditions in an ionisation chamber kept at 180 °C and accelerated by 3 kV before being measured over a range of m/z 20 – 1000 at 1 s full range cycle time. Quadruple measurements were done for each sample.

DTMS/MS experiments were carried out on a JEOL JMS - SX/SX 102A tandem mass spectrometer (B/E/B/E) with a JEOL MS - MP 9020D data system. The filament (Pt/Rh 9:1, 100 μ m) was heated at a rate of 0.5 A/min to an end temperature of about 800 °C. Ions were generated by EI (16 eV) in an ionisation chamber kept at 190 °C and accelerated to 8 kV. Measurements were performed over a mass range appropriate for the ion under scrutiny at a full range cycle time of 3 s and post-accelerated to 10 kV. Collision induced dissociation (CID) was performed in the third field free region using helium as a collision gas (0.5 - 1.0 10^{-3} Pa). The resolution used was 1000.

2.3. Multivariate Analysis

Discriminant analysis was performed using a modified version of the package ARTHUR (infometrix, Seattle) that calculates discriminant functions (DF), linear recombinations of highly correlated masses, that express main similarities and dissimilarities between groups of mass spectra. A hierarchical Q-mode Complete Link Cluster Analysis (CLCA) was applied to the discriminant scores of the samples weighted according to the relative variance they explain.

3. Results and Discussion

3.1. Chemotyping

Multivariate analysis of the DTMS spectra of residues (chars, red-brown residues, cream coloured residues and black residues on exterior vessel walls) shows the occurrence of several 'chemotypes': i.e. groups of residues with similar chemical composition (Fig. 1), which are in general agreement with earlier CuPyMS classifications based on more limited mass range measurements (Oudemans & Boon 1996). The clustering in Figure 1 is based on the discriminating chemical features (masses of characteristic fragments) from the DTMS spectra. In the DTMS spectra of a typical charred residue from Chemotype A₁, sample 7-7 (Fig 2), a distinction between desorption and pyrolysis is clearly visible. The volatile lower molecular weight part of the residues (Fig. 2, spectrum A) includes lower temperature desorption products such as fatty acids (m/z 129, 256 from C16 FA, 284 from C18 FA), sterols (m/z 368 from cholestadiene) and acylglycerols (m/z 523, 550, 551, 577, 579), while the compounds formed at higher temperatures (spectrum B) are derived from native proteins (m/z 108, 117, 131) and

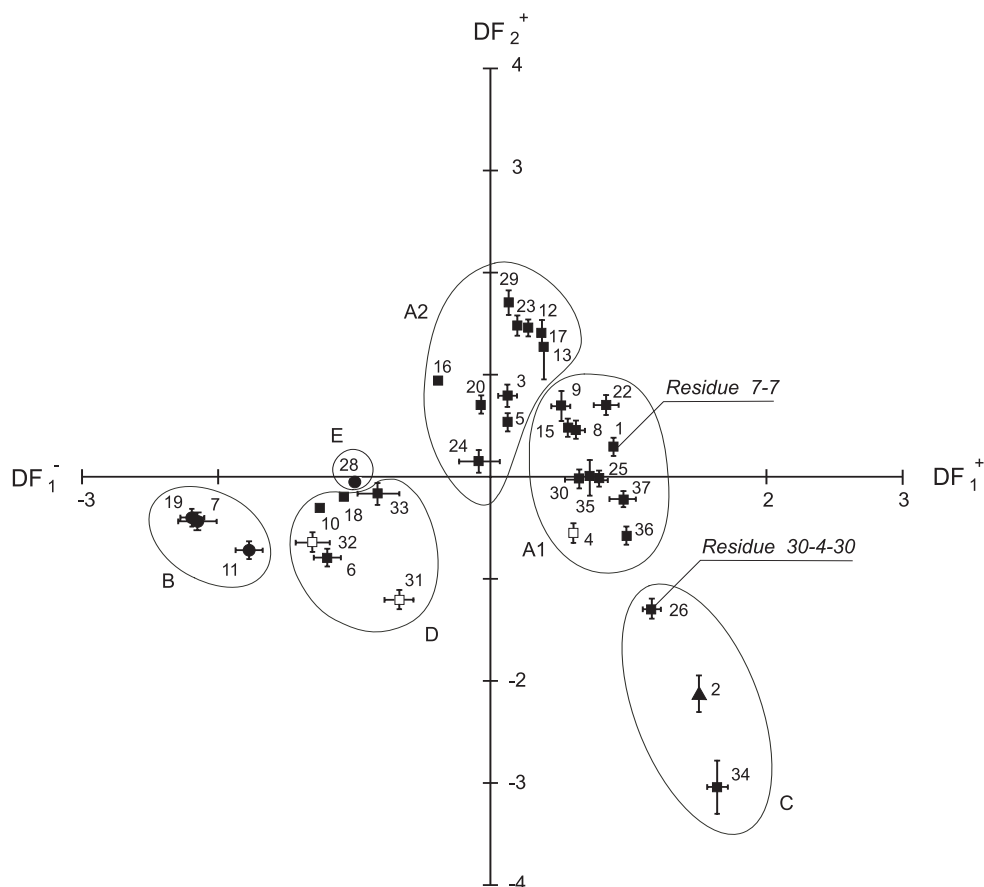


Figure 1: Chemotyping Residues based on MS spectra.

Discriminant analysis and cluster analysis were used to classify residues according to discriminating chemical features (m/z values of characteristic fragments) in the DTMS data. Each cluster represents a particular chemotype. Discriminating features in Chemotype: A: lipids, polysaccharide fragments; B: aliphatic and aromatic hydrocarbons; C: proteins and protein fragments; D: aliphatic and aromatic hydrocarbons; E: contamination. Residue types: Chars on vessel interior (■); black residues on vessel exterior (●); red-brown residues (▲); and cream coloured residues (□).

polysaccharides (m/z 95, 96, 109, 110, 126) and more condensed cross-linked materials evolved during partial charring (unresolved envelope of mass peaks from m/z 100 – 500).

Charring of polysaccharides and proteins leads to a newly formed thermally stable cross-linked network (Boon *et al.* 1994) that can be addressed at higher temperatures with DTMS. The DTMS data show that the char is a solid matrix of cross-linked organic compounds in which lipids are incorporated. Lipids may be incorporated in the charred matrix through absorption into pores in the matrix or by encapsulation within the matrix. Their liberation at evaporation temperatures does indicate they are not covalently bound to the matrix.

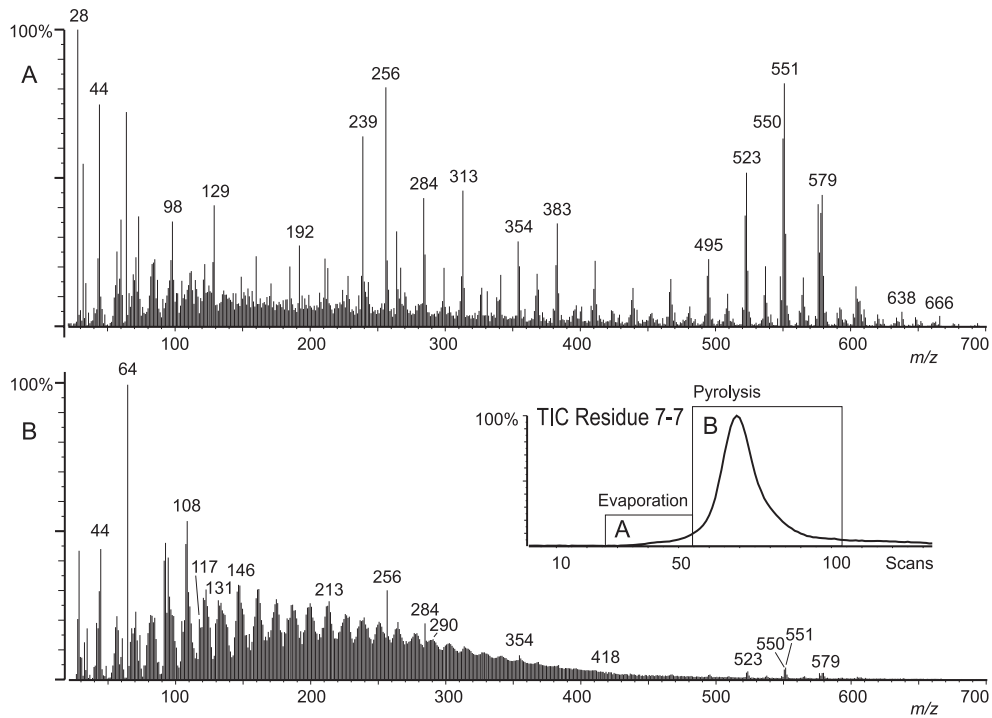


Figure 2: DTMS results for residue 7-7.

Total Ion Current (TIC) and Mass Spectra for A: Evaporation, containing fatty acids and acylglycerol fragments; and B: Pyrolysis, containing markers for cross-linked components created from proteins and polysaccharides during the original food preparation.

Residues situated on the exterior surfaces of vessels Chemotype B, show a different mass spectrum typically containing series of aromatic compounds in the higher temperature region. These aromatic compounds are indicative of wood smoke and soot.

Residues from Chemotype C, predominantly show compounds derived from native proteins (m/z 108, 117, 131) and the condensed cross-linked materials evolved during partial charring (m/z 144, 145, 146, 159, 160, 161, 173, 174, 175, etc). A typical sample from this chemotype, residue 34-0-30, is a charred residue preserved on the interior of a vessel. The DTMS spectrum of the high temperature pyrolysis fraction (Fig. 3) shows such a pattern in detail. The liberation temperature of these compounds indicates that the fragments indicative of proteins such as indole (m/z 117) and methyl indole (m/z 131) are incorporated in a network of cross-linked compounds and are chemically bound to the matrix.

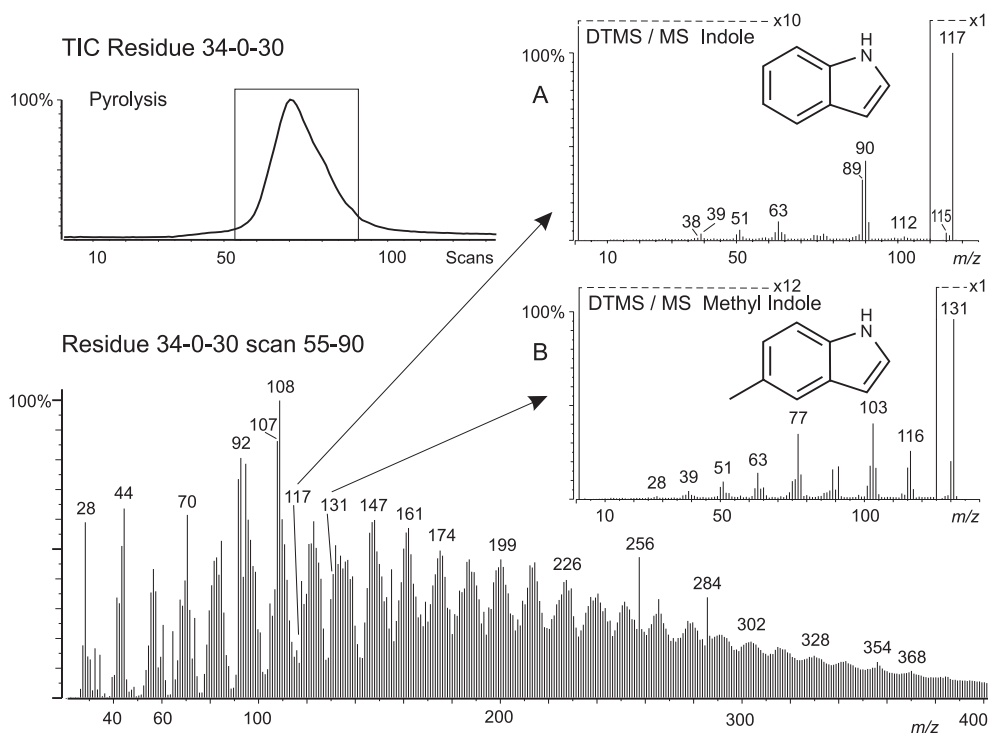


Figure 3: DTMS spectrum of the pyrolysis fraction of residue 34-0-30.

DTMS/MS identifications for two DTMS/MS spectra for the ion peaks of A: indole (m/z 117) and B: methyl-indole (m/z 131).

3.2. Identification

Although the spectra of samples were compared to the spectra of fresh biological materials such as Amylose and Bovine Albumin and their experimentally charred counterparts (Oudemans *et al.* in press-a), DTMS/MS provided the tool for more solid identification of compounds. A typical Chemotype C sample, 34-0-30 (Fig. 3) illustrates the identifying power of DTMS/MS. During the DTMS/MS experiment, a preselected ion was isolated and introduced into a collision chamber to collide with helium. The fragments were detected in the second sector MS to give a mass spectrum of the fragmented ion. Figure 3 shows two MS/MS spectra for indole (m/z 117) and methyl-indole (m/z 131), two fragments originating from the tryptophane (Tsuge & Matsubara 1985) in the native proteins.

4. Conclusions

DTMS is providing a rapid analytical technique to obtain information about a broad range of chemical compounds in solid organic samples. Compounds as varied as lipids, proteins, polysaccharides, polynuclear hydrocarbons, and fragments of complex cross-linked compounds were detected in microgram amounts of archaeological samples.

In addition, DTMS gives information about the chemical composition of the sample and renders new understanding of the kind of chemical complexes under study. Solid organic residues are now proven to consist of a matrix of cross-linked compounds (probably consisting of a combination of partially heated proteins and polysaccharides) in which lipids are incorporated either through adsorption or through encapsulation within the matrix, but without being chemically bound to this matrix.

The addition of DTMS/MS greatly increases the identifying potential of DTMS techniques and gives a more definite character to the tentative identifications obtained through comparison with standards and known (fresh or experimentally charred) biomaterials.