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Use of a 700 MHz NMR microcryoprobe for the identification and quantification of exogenous carbon in compounds purified by preparative capillary gas chromatography for radiocarbon determinations

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ABSTRACT

Preparative capillary gas chromatography (PCGC) is the central technique used for the purification of volatile or semi-volatile organic compounds for radiocarbon analysis using accelerator mass spectrometry (AMS). While thicker film columns offer efficient separations, column bleed of cyclic poly(dimethyl siloxane) (PDMS) stationary phase has been highlighted as a potential source of contaminant carbon in 'trapped' compounds. The dimethylpolysiloxane

CH_3 groups are of 'infinite' radiocarbon age due to the fossil carbon origin of the feedstock used in production. Hence, column bleed, if present at sufficiently high concentrations, would shift the radiocarbon ages of trapped compounds to older ages. Quantification of the column bleed in trapped samples, however, is extremely challenging and up to now has only been achieved through indirect ^{14}C determinations of chromatographic blanks, which are used for post ^{14}C determination 'corrections'. As part of wider investigations aimed at better understanding the chemical nature of contamination in compound-specific ^{14}C-determinations, herein, we report a rigorous approach to column bleed identification and quantification. Using reference fatty acid methyl esters (FAMEs) ^{1}H nuclear magnetic resonance spectroscopy (NMR), employing a 700 MHz instrument equipped with a 1.7 mm microcryoprobe optimised for ^{1}H observation, was able to detect low sub-microgram amounts of low molecular weight compounds (<500 Da). Direct quantification of PCGC 'trapped' FAMEs was achieved based on the recorded ^{1}H NMR spectra. Gravimetrically prepared calibration mixtures of cyclic PMDSs and FAMEs, showed column bleed abundance to be below 0.03% *w/w* of the 'trapped' FAMEs, which would lead to a maximum shift in radiocarbon age of <3 years toward older values. We therefore conclude that column bleed contamination has a negligible effect on the ^{14}C determination of FAMEs prepared using the chromatographic method described. The ^{1}H NMR analysis also revealed the absence of other protonated carbon-containing components that would affect radiocarbon determinations at the precisions achievable by AMS.

INTRODUCTION

A critical concern when preparing samples for radiocarbon dating is contamination of the sample through the introduction of exogenous carbon. Such contamination from sample treatment can lead to significant offsets (older or younger) of the actual sample age leading to

erroneous dates[1,2,3]. Contamination becomes particularly problematic with the small sample sizes, i.e. less than 1 mg of C, that are increasingly commonly analysed due to advances in sample preparation methods and AMS technologies.

Identification and quantification of exogenous carbon in samples for radiocarbon analysis has been attempted using various approaches, e.g. FTIR has been used to identify contamination in bone collagen[4] and Raman to determine soil carbon contamination of charcoal[5,6]. However, these techniques are ineffective in the case of low-level contamination of small samples, due to a fundamental lack of sensitivity, precluding quantification of contamination at the part per thousand level, which would affect radiocarbon determinations.

The question of exogenous contamination is especially critical in compound-specific ^{14}C determinations in which preparative capillary gas chromatography (PCGC) is used to isolate compounds from extracts of various environmental matrices[7,8,9,10], and archaeological pottery vessels[11,12,13]. The compound-specific approach routinely involves trapping sub-milligram amounts of analyte for ^{14}C determinations, hence, the use of PCGC requires assessment of all potential sources of exogenous carbon likely to arise during the sample pre-treatment. As the analytes are purified exogenous carbon could potentially be introduced either from the PCGC used for compound purification, the handling of compounds between isolation, oxidation and graphitisation. A recognised source of exogenous carbon to compounds isolated by PCGC is column "bleed"[14,15,16] (figure 1), derived from thermal degradation of the commonly used PDMS stationary phase coating the column through heating in the GC oven[15,16]. The cyclic degradation products of the polymer released typically are $n = 3$ and $n = 4$ cyclic oligomers of the monomer unit ($-[Si(CH_3)_2-O]_n-$), with the possibility of higher homologues up to an $n = 7$[17,18].

Several approaches have been considered to identify, limit, and correct for the effects of 'column bleed' from the GC column. Eglinton and co-workers[7], who reported the first use of PCGC to isolate compounds for radiocarbon dating, suggested using columns coated with a thin film of ultra-low bleed stationary phase (≤0.5 μm). Stott *et al.*[12] attempted to determine the column bleed concentration by the preparation of chromatographic blanks when isolating $C_{16:0}$ and $C_{18:0}$ FAMEs from archaeological pottery. This was achieved by trapping the column eluent for almost an entire run after injection of solvent only (~40 times longer than typical trapping windows). The blanks contained insufficient carbon for radiocarbon analysis (~0.9 μg), thus, the column bleed was assumed to exert an insignificant effect on compound-specific ^{14}C age determinations. In a later study Ziolkowski *et al.*[19] used a column coated with a thicker stationary phase film (1.5 μm) and generated blanks based on 400 dry injections and trapping 7 min retention time 'windows' then normalised the amount of exogenous carbon to 50 injections. This approach assumes that the column releases its stationary phase at constant rate regardless of time or temperature, thus the amount of column bleed associated with a trapped compound is proportional the length of the trapping window. It is however known that that the release of PDMS from GC columns increases with temperature. Another approach adopted by Ziolkowski *et al.*[19], and probably the most effective of the approaches used up to now[20,21] to assess the effect of column bleed, involved the isolating of reference compounds of known modern age then investigating shifts in ^{14}C content by radiocarbon analysis. The influence of column bleed was estimated from deviations in the ^{14}C content of reference materials compared to those of dry injections, i.e. blanks. The summary is that all the methods used to date to assess the potential effects of column bleed on ^{14}C determinations are indirect and unable to identify and quantify the specific chemical contaminants of the PCGC trapped analytes. The specific analytical hurdle thwarting direct characterisation and quantification until now is the small quantity of compound isolated by PCGC, typically sub-milligram, combined with the low

concentration of contaminant, i.e. ppt, that could potentially effect ^{14}C determinations. The latter highlights the need for a new approach for assessing analyte purity for compound-specific ^{14}C determinations.

Nuclear magnetic resonance (NMR) offers the possibility of detecting and quantifying the presence of contaminant species but has not typically been employed due to perceived problems with sensitivity. The latest generation of high field NMR spectrometers equipped with microcryoprobes, however, extends the lower limits of detection of protonated species to the picomol scale[22,23]. The extreme sensitivity makes these probes ideal tools to study mass limited samples such as isolated low abundance proteins, peptides and small molecules and difficult to express proteins. For example, this technology has been used to determine the structures of molecules that can only be isolated in minute amounts (micrograms or less) from natural sources such as deep sea sponges[22,23], red algae[24] and plants[25]. It is also used by structural genomic consortia for high throughput microscale screening of protein targets[26,27]. In addition to analysing the structure and purity of molecules, NMR can also be used to determine the concentration of one more chemical species in solution with a high level of precision in a non-destructive manner[23,28]. qNMR has proven to be a reliable, specific and linear over a wide concentration range with limits of accuracy and precision in the order of 0.5-1%[29,30,31]. The method is particularly suited for the simultaneous determination of the active constituents and impurities in samples from the food, pharmaceutical and chemical industries[30,32,33,34]. Furthermore a number of studies have used qNMR to specifically identify and quantify impurities in agrochemicals[35], pharmaceuticals[36] and amino acids and peptides[37], however this is the first time to our knowledge that NMR has been used in the field of radiocarbon analysis.

The advent of this analytical technology opens the way for a new approach to the qualitative and quantitative assessment of contamination in analytes for radiocarbon analysis.

The sensitivity and dynamic range of high field NMR instruments, combined with their capability for compound identification and quantification, offers hitherto unattainable potential for use in assessing contamination at the ‰ level in sub-milligram amounts of purified compounds, trapped in ca. 1 min 'windows', instead of extended sequences. This allows for the first time, the assessment of exogenous carbon in trapped analytes, specifically the definitive assessment of the degree to which column bleed and other potential sources of carbon could affect radiocarbon date determinations Herein, we demonstrate: (i) the use of microcryoprobe-equipped 700 MHz ^{1}H NMR to determine the purity of compounds isolated with PCGC, (ii) identification of exogenous carbon in the trapped analytes by comparisons with authentic standards, (iii) quantification of the contamination present using calibration mixtures, and (iv) the impact of such contamination on high precision ^{14}C dates using AMS and conclude whether corrections are required.

EXPERIMENTAL

Reference materials and sample preparation

All reference materials were purchased from Sigma Aldrich (Poole, UK). HPLC grade solvents were purchased from Rathburn Chemical Ltd (Walkerburn, UK) and deuterated chloroform ("100 %", 99.96 atom % D) from Sigma Aldrich (Poole, UK).

Organic residues from the pottery vessel were extracted using the method described by Correa-Ascensio and Evershed[38].

Stock solutions containing known concentrations of $C_{18:0}$ FAME and hexamethylcyclotrisiloxane were prepared to produce a NMR calibration curve for its quantification by NMR. These solutions contained the FAME at 1 $mg.mL^{-1}$ and siloxane in varying concentrations, from 1 $mg.mL^{-1}$ to 1.10^{-6} $mg.mL^{-1}$ (see table 1), diluted in chloroform-d solvent.

PCGC analysis

The $C_{16:0}$ and $C_{18:0}$ FAMEs from a standard solution (5 $\mu g.\mu l^{-1}$) were isolated by PCGC using a Hewlett Packard 5890 series II gas chromatograph coupled to a Gerstel preparative fraction collector. Aliquots (1 µL) of the standard FAME solution were injected repeatedly using an autosampler (40 runs in a continuous sequence) into a non-polar fused silica capillary column (Rxi-1ms, 30 m x 0.53 mm i.d., DB1 stationary phase, 1.5 µm film thickness, Restek (High Wycombe, UK)). The thickness of the column stationary phases is optimal for the injection of large samples (5 µg per injection), combined with low bleed properties. The GC temperature program for the standard solution started with an isothermal hold at 50 ºC for 2 min, followed by an increase in oven temperature at 10 ºC min^{-1} up to 300 ºC, followed by a 3 min hold. For the archaeological pottery extract the temperature programme was modified slightly due to the presence of compounds with higher boiling points; the temperature programme started with an isothermal period at 50 ºC for 2 min followed by programming a temperature ramp at 40 ºC/min to 200 ºC, then 10 ºC/min to 270 ºC, and finally 30 ºC/min to 300 ºC, followed by a hold for 9 min. Helium was used as carrier gas at constant pressure (7 psi for the standard solutions and 10 psi for the archaeological sample). The column terminated at a zero dead volume effluent splitter to divert 1 % of the eluent to the flame ionisation detector

(FID) to monitor column effluent and 99 % through a deactivated silica transfer line to silica traps. The FAMEs were isolated in one minute windows based on their respective retention times. The $C_{16:0}$ and $C_{18:0}$ FAMEs were isolated into individual traps, denoted $TC_{16:0}$and $TC_{18:0}$, respectively.

The isolated compounds were recovered from the traps with 1 mL of deuterated chloroform. The solutions were blown down to dryness under a gentle stream of N_2, re-dissolved in 200 µL of deuterated chloroform, of which a 50 µL aliquot was transferred to a 1.7 mm NMR tube for analysis (see below). Further 50 µL aliquots of each trapped analyte solution were combined, separately, with 50 µg of an internal standard (*n*-tetratriacontane), then blown down and dissolved in 500 µL of hexane for quantification by GC-FID (see below). The same protocol was applied to an archaeological pottery vessel containing an absorbed residue comprising high concentrations of $C_{16:0}$ and $C_{18:0}$ fatty acids (FAs).

GC, GC/MS and GC-Q-TOF analysis

Aliquots of the standard trapped FAME solutions, containing 50 µg of internal standard (IS), were submitted to GC analysis to determine trapping efficiencies and quantification of the amount of FAMEs trapped. The GC analyses were performed using an Agilent technologies 7890A GC fitted with an FID to monitor the effluent. Helium was used as carrier gas at a flow rate of 2 $mL.min^{-1}$. The samples were injected into a non-polar fused silica capillary column (50 m x 0.32 mm i.d., DB1, 0.17 µm film thickness, Agilent technologies (Cheadle, UK)). The GC oven temperature programme started with a 50 ºC hold for 2 min, followed by a ramp at 13 ºC min^{-1} to 350 ºC, then an isothermal hold for 2 min. The amount of trapped FAME was calculated using equation 1:

$$m_{FAME} = A_{FAME} * (m_{IS}/A_{IS}) \quad (1)$$

Where A_x and m_x are, respectively, the peak area and the mass of the FAME and IS.

GC/MS analyses used a Trace 1300 coupled to a Thermo Scientific ISQ LT single quadrupole MS. The GC column was the same as used in the GC/FID instrument and the temperature program the same as used for PCGC analysis. The MS was operated in electron ionisation mode at 70 eV. Samples were acquired over the range of *m/z* 50-650 Da. Data were processed by XCalibur software and the NIST (National Institute of Standards and Technology) mass spectral library was used to identify eluting compounds.

GC-Q-TOF analyses were performed on an Agilent technologies 7890B GC instrument coupled to an Agilent technologies 7200 Accurate Mass Q/TOF GC/MS device. The GC column was the same as the one used above in the GC/FID analysis. The temperature program started at 50ºC and increased to 300 ºC at a rate of 30º min^{-1}. Data were acquired over the range of *m/z* 50-1200 Da, from 5-16 min, to capture the elution window of the cylic poly(dimethyl siloxane) oligomers and avoid saturation of the instrument with FAMEs and IS. Data were processed using the NIST database

NMR analysis

The analyses were performed on a Brucker Avance III HD 700 MHz NMR instrument equipped with a 1.7 mm TCI microcryoprobe. The samples dissolved in $CDCl_3$ (99.96%D) were transferred to 1.7 mm NMR tubes using a SGE gas tight syringe. Standard 1D ^1H spectra (zg30) were acquired at 25 ºC using a spectral width of 15 ppm and 65536 complex points to

give an acquisition time of 3.12 sec per scans. 5120 scans were acquired per experiment with an interscan delay of 1 sec. Spectra were referenced to residual solvent signals (^{1}H, δ 7.26 ppm) and the total measurement time per experiment was 5 hrs 55 mins. The spectra were processed and analysed in MestreNova v 9. A multiple point background correction was performed by subtraction of a cubic spline function, on a sample-by-sample basis adjusted by adding manual points to achieve a flattened area adjacent to the peaks used for quantification. Phase correction was performed manually using the peak at 1.28 ppm as a pivot position. The spectra were normalised to the peak at 1.28 ppm. Integration was performed using a peak-by-peak calculation method, with the cyclic poly(dimethyl siloxane) peaks requiring manual integration.

Special care was taken to select deuterated solvent without added TMS (tetramethylsilane) standard as this would have interfered with the resonances of siloxanes likely to arise from GC column bleed. NMR analysis of the solvent was performed to confirm its purity and eliminate TMS as a possible source of interference.

RESULTS AND DISCUSSION

The investigation proceeded in three parts: (i) determination of NMR resonances for cyclic poly(dimethyl siloxanes) appropriate for quantifying column bleed against a background of FAME signals at three to four orders of magnitude higher abundance; (ii) establishing a calibration graph for mixtures of FAMEs and cyclic poly(dimethyl siloxane) in varying proportions, and (iii) determination of the concentrations of cyclic poly(dimethyl siloxane) in

modern reference FAMEs and those of archaeological origin in order to assess the potential impact of column bleed on ^{14}C determinations by AMS.

Reference spectra and calibration

Each of the reference compounds was analysed by NMR to establish their chemical shifts (see figure 2 for spectra). The reference degradation products of stationary phase exhibit peaks at 0.20, 0.12, and 0.10 ppm, respectively, for the $n = 3$, $n = 4$, and $n = 5$ membered ring cyclic poly(dimethyl siloxanes). The FAMEs, CH_3(A)-COO-CH_2(B)-CH_2 (C)-$(CH_2)_{12\ (or\ 14)}$ (D)-CH_3(E), display a singlet at 3.69 ppm (A), a triplet centred on 2.32 ppm (B), a singlet at 1.64 ppm (C), a range of peaks at 1.35 - 1.20 ppm (D), and a triplet centred on 0.9 ppm (E). For the reference FAMEs used for preparing the standard solutions, the baseline was rigorously inspected to confirm that no impurities were present in the region where the resonances of protons from column bleed cyclic poly(dimethyl siloxanes) occur, i.e. 0 to 0.3 ppm.

In order to quantify the amount of column bleed collected in the traps together with the FAMEs during PCGC, a calibration curve was generated covering the concentration range 1 $mg.mL^{-1}$ to 1.10^{-6} $mg.mL^{-1}$ based on the peak areas of the methyl group protons (singlet at 3.69 ppm) of the $C_{18:0}$ FAME and that of the cyclic poly(dimethyl siloxane) methyl group protons (singlet at 0.20 ppm) for the hexamethylcyclotrisiloxane (see table 1). The lowest concentration, i.e. [siloxane]/[FAME] = [siloxane]/[FAME] = 1/100,000 produced hexamethylcyclotrisiloxane signals too weak for integration and was therefore not included in the calibration.

The concentration of the standard solutions in $mg.mL^{-1}$ were converted into the mass of carbon of the compounds using equation 2:

$$mC_x = [x] * V_x * M_C * nbr_C / M_x \quad (2)$$

Where x is either the $C_{18:0}$ FAME or the hexamethylcyclotrisiloxane, C is carbon atom, mC_x the mass of carbon in the compound in mg, [x] the concentration in $mg.ml^{-1}$, V_x is the volume in mL, nbr_C the number of carbon atoms in the molecule, and M_x is the molar mass in $mg.mol^{-1}$.

Plotting {peak area siloxane / peak area of FAME} against the ratio {mass of C siloxane/mass of C FAME} (table 1), showed the curve to be described by a linear regression with a slope of 25.843 ± 0.612 and correlation coefficient R^2 = 0.999. The correlation coefficient confirms a linear calibration curve has been obtained, which can be used for the required quantitative analyses.

Quantification of exogenous carbon

The $C_{16:0}$ and $C_{18:0}$ FAMEs mixtures analysed by 700 MHz microcryoprobe NMR were formulated to reproduce the trapping of real archaeological samples, i.e. PCGC of FAMEs containing 200 μg of carbon from 40 injections of a solution containing $C_{16:0}$ and $C_{18:0}$ FAMEs at concentrations of 5 $\mu g.\mu L^{-1}$. The amount of exogenous carbon is defined as the percentage of exogenous carbon contaminating the compound isolated by PCGC. GC/FID analysis of the trap contents demonstrated that the FAMEs isolated by PCGC contained only one FAME, i.e. no $C_{18:0}$ was detected in the trap intended to trap $C_{16:0}$ and *vice versa*. The carbon content of the isolated FAMEs varied from 112 μg to 264 μg of C (table 2). The variation in sample

recovery from the traps could have number of causes; most likely being the increasing concentration of the standard solution in the autosampler vial due to sample evaporation over a sequence, or the loss of sample during solvent evaporation under a stream of N_2. Overall the average of sample recovery was of 202 μg of C which was very close to the targeted trapping amount.

The NMR spectrum shown in figure 3a contained resonances at chemical shifts corresponding to the isolated $C_{18:0}$ FAME (3.69 ppm, 2.32 ppm, 1.64 ppm, 1.35 - 1.20 ppm, and 0.9 ppm). In spectrum displayed at full scale, i.e. normalised to the FAME 1.31 ppm peak only resonances of the FAME are evident. However, unlike the references and blanks, close inspection of the baseline revealed, in all of the FAMEs isolated by PCGC, peaks were clearly visible at 0.09 and 0.11 ppm at a relative intensities lower than 0.06%; these are exogenous carbon derived from the PCGC instrument. Due to their very low intensity, 5120 scans (corresponding to just under 6 h of analysis) were required to obtain acceptable signal-to-noise for meaningful comparison between samples. The data collection requires therefore a long time-slot with the instrument, it is however necessary as more typical NMR spectra (resulting from 1024 scans recorded over 1 hr 11 mins) were too noisy to perform rigorous integration of the low intensity contaminant peaks. Knowing that the stationary phase of the column fitted into the PCGC was a poly(dimethyl siloxane) polymer, and based on known ^{1}H chemical shifts, the observed resonances can be confidently assigned to cyclic poly(dimethyl siloxane) products resulting from the thermal degradation of the stationary phase. The chemical shift values of 0.09 and 0.11 ppm indicate that the column derived compounds trapped are likely a mixture of octamethylcyclotetrasiloxane and decamethycyclopentasiloxane derived from column bleed. The ^{1}H NMR spectra of some of the trapped compounds also contained a weaker peak at δ 0.07 ppm likely to correspond to cyclic compounds with $n > 6$ membered ring. This identification

was confirmed by GC-Q-TOF analysis, that showed the presence of the *n* = 4, 5, 6, and 7 membered ring cyclic poly(dimethyl siloxane) degradation products in different proportions in the isolated FAMEs; the *n* = 3 homologue was undetectable in all the trapped FAMEs. It should be noted that while other very minor peaks of similar intensity to the cyclic poly(dimethyl siloxane) are present in the NMR spectra, they also occur in the reference compounds and/or blank spectra. Therefore these components can be eliminated as deriving from the PCGC, confirming that column bleed is the only source of exogenous carbon detectable from the isolation process.

Based on the mass of carbon trapped, the NMR peak areas and the calibration curve slope, the amount of carbon from the column bleed has been determined using equation 3:

$$mC_{cb} = (A_{cb}/A_{FAME}) * (mC_{FAME}/S) \quad (3)$$

Where mC_{cb} is the mass of carbon of the column bleed, mC_{FAME} the mass of carbon of the FAME, A_{cb} the peak area of column bleed, A_{FAME} the peak area of methyl group from the FAME and S the slope of the calibration curve.

The carbon contributing from column bleed in the PCGC trapped FAMEs ranged from 5 ng (sample 2 - $TC_{16:0}$) to 69 ng (sample 3 – $TC_{16:0}$; table 2), with a mean of 28 ng. It is noteworthy that no significant difference was observed in the amount of column bleed detected in the traps $TC_{16:0}$ and $TC_{18:0}$. This confirms that for these trapping windows, under these conditions, the temperature of the GC oven had no appreciable effect on the amount of column bleed eluting from the GC column.

The coating of the column was composed of 100% poly (dimethyl siloxane). The main monomers used for polymerisation are of synthetic origin[39], and the industrial precursors are commonly petroleum derived (i.e. radiocarbon dead) which means that the impact of column bleed on the isolated FAME, i.e. shift in years to older age, can be calculated based on equation 4[3]:

$$\Delta Age = -8033*\ln(1-f_c) \quad (4)$$

Where ΔAge is the shift in years on the real age of the sample and f_c is the fraction of contamination from the column bleed (i.e. mC_{cb}/mC_{FAME}).

The amount of carbon introduced into the trapped FAMEs from column bleed ranges from 0.03 ‰ to 0.32 ‰, with a mean of 0.14 ‰; this is well below the critical theoretical limit of 1 ‰ radiocarbon dead contamination that would significantly affect high precision radiocarbon dates (see figure 4). The level of contamination observed is equivalent to a shift of 0 to 3 years towards older dates (table 2), which is well within the maximum precision achievable by AMS. The significant conclusion is, therefore, that column bleed will not have a significant effect on ^{14}C dates of compounds isolated by PCGC and, hence, its effect can be neglected with regards to corrections of dates.

Application to lipids preserved in archaeological pottery vessels

The NMR method was then applied to a solvent extract of an archaeological pottery vessel, i.e. where invisible food residues are preserved by absorption into the ceramic fabric. The lipid extract of the pottery vessel was first analysed using GC and GC-MS to identify and quantify the compounds present in the extract. The lipid concentration of the pottery vessel was 4.6 $mg.g^{-1}$ of sherd fabric, with $C_{16:0}$ and $C_{18:0}$ FAs dominating the extract (see figure 5). The GC temperature program was modified slightly due to presence of other compounds in the extract eluting at higher temperature. The trapping windows were shortened (~30 sec) due to the presence of compounds eluting close to the target FAMEs, especially the presence of a $C_{18:1}$ FAME eluting immediately before the $C_{18:0}$ FAME. The results obtained with NMR analysis of the archaeological FAMEs are entirely analogous to those obtained from the standard FAMEs (figure 4.b). The cyclic poly(dimethyl siloxane) bleed content was determined to be 16 ng and 15 ng of C for the $C_{16:0}$ ($TC_{16:0}$) and the $C_{18:0}$ ($TC_{18:0}$), respectively. This would theoretically offset the dates of the FAMEs by only 1 year towards older values (see table 2 and figure 4).

Low intensity peaks were present in the NMR spectrum of $C_{18:0}$ isolated from Pot-$TC_{18:0}$, of similar intensity, but different shift to the column bleed, at: 5.37 ppm, 4.13 ppm, and 1.98 ppm. These appear to correspond to very low concentrations of isomeric monounsaturated FAMEs resulting from the tail of the components eluting just before the $C_{18:0}$[40] (confirmed by a GC-MS analysis based on manual interpretation and comparison to the NIST database). Hence, this is archaeological and contemporaneous with the trapped $C_{18:0}$ FAME, i.e. it is *not* exogenous carbon introduced through the isolation protocol. This latter finding further emphasises the usefulness of NMR in assessing peak purity prior to AMS analysis.

CONCLUSIONS

The results presented herein represent the first direct identification and quantification of nanogram quantities of exogenous carbon in samples for radiocarbon dating by AMS. This novel NMR-based approach overcomes the shortcomings of all pre-existing techniques for monitoring the presence of exogenous carbon, and other spectroscopic techniques, i.e. FTIR, which lack the required sensitivity. While other approaches have attempted to indirectly account for column bleed by monitoring the effect of column bleed on standards with known radiocarbon content and radiocarbon dead samples, these approaches generally require excessive/unrealistic trapping windows to provide sufficient column bleed carbon for making corrections. Microcryoprobe NMR at high field (≥ 700 MHz) is uniquely suited to this application since it possesses the sensitivity and dynamic range perfectly matched to assessing the purity of organic compounds for radiocarbon analysis. Our results confirm that exogenous carbon is readily detectable by 700 MHz NMR at the ‰ concentrations that would affect high precision radiocarbon determinations by AMS.

Furthermore, our method is preferable to using corrections based on the age shift of standards isolated via PCGC, as the 700 MHz NMR spectrum provides a comprehensive overview of all the protonated chemical species present and, thus, is able to molecularly-identify other sources of extraneous carbon in trapped compounds, in addition to co-eluting column bleed. The archaeological example given above, which revealed ‰ concentrations of unsaturated FAMEs, in the $C_{18:0}$ FAME, resulting from the tailing of the earlier eluting GC peak, is a case in point. Our NMR results also demonstrate that changing the temperature programme had no significant effect on column bleed.

Finally, while the results presented here demonstrate that the column bleed from the DB-1 column coated with a 1.5 μm thickness film is negligible, it is important to be aware that other phases, phase thickness or even deteriorating cross-linked poly(dimethyl siloxane) coated columns, or temperature programmes using higher temperatures, may produce sufficient bleed to have a significant effects on radiocarbon determinations. If the nature of a stationary phase is not different than 100%-dimethylpolysiloxane then the presence of other 'bleed' products should be considered when applying this method. Thus, to guarantee the reliability of radiocarbon dates of compounds isolated by PCGC, determinations, such as those described here should be performed: (i) every time a new column is installed into a PCGC instrument, or (ii) when a compound with a different boiling point is isolated using adjusted temperature parameters in the PCGC program.

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TABLES AND FIGURES

Table 1: Concentration, mass of carbon in the FAMEs and siloxanes and peak areas of the standards in the solutions prepared for the NMR calibration curve. The NMR peak area of the FAME corresponds to the CH_3 group at 3.6 ppm. The siloxane was hexamethylcyclotrisiloxane (shift at 0.20 ppm).

[FAME] ($mg.mL^{-1}$)	[Siloxane] ($mg.mL^{-1}$)	Area FAME	Area siloxane	A Siloxane / A FAME	mass C FAME (mg)	mass C siloxane (mg)	mass C siloxane / mass C FAME
1	1.00E+00	1.12	12.23	1.09E+01	3.82E-02	1.62E-02	4.24E-01
1	1.00E-01	0.15	0.21	1.40E+00	3.82E-02	1.62E-03	4.24E-02
1	1.00E-02	0.15	0.02	1.33E-01	3.82E-02	1.62E-04	4.24E-03
1	1.00E-03	4.01	0.05	1.25E-02	3.82E-02	1.62E-05	4.24E-04
1	1.00E-04	3.97	0.01	2.52E-03	3.82E-02	1.62E-06	4.24E-05
1	1.00E-05	1.06	0	0	3.82E-02	1.62E-07	4.24E-06

Table 2: Details of FAMEs trapped by PCGC, associated column bleed in trapped compounds and calculated shifts towards older values in years. The trap $TC_{16:0}$and $TC_{18:0}$ contained the $C_{16:0}$ and $C_{18:0}$ FAMEs, respectively.

Sample[§]	Trap	A Si /A FAME	Mass of trapped FAME (µg)	Mass of C in FAME (µg)	Mass of C in siloxane (ng)	Mass C Si/ Mass C FAME	ΔAge (y)
1	$TC_{16:0}$	3.02E-03	286	205	24	1.17E-04	1
	$TC_{18:0}$	1.41E-03	232	177	10	5.46E-05	1
2	$TC_{16:0}$	7.09E-04	242	173	5	2.74E-05	0
	$TC_{18:0}$	5.01E-03	276	211	41	1.94E-04	2
3	$TC_{16:0}$	7.73E-03	322	231	69	2.99E-04	2
	$TC_{18:0}$	8.29E-03	184	141	45	3.21E-04	3
4	$TC_{16:0}$	8.32E-04	378	271	9	3.22E-05	0
	$TC_{18:0}$	5.09E-03	345	264	52	1.97E-04	2
5	$TC_{16:0}$	3.40E-03	157	112	15	1.32E-04	1
	$TC_{18:0}$	1.39E-03	307	234	13	5.37E-05	0
Pot	$TC_{16:0}$	2.91E-03	197	141	16	1.13E-04	1
	$TC_{18:0}$	1.68E-03	293	224	15	6.49E-05	1

[§]1 to 5 correspond to the prepared $C_{16:0}$ and $C_{18:0}$ FAME mixtures; Pot is an extract of an archaeological cooking vessel shown by GC and GC/MS to contain high abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids (analysed as FAMEs).

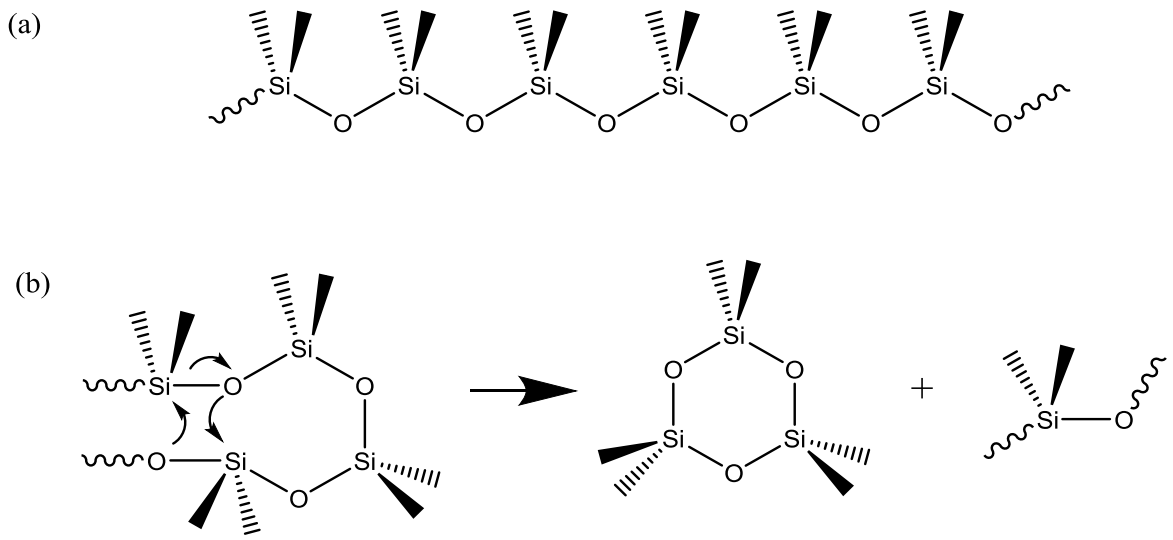


Figure 1. (a) Structure of the poly(dimethyl siloxane) GC stationary phase. (b) Proposed mechanism for thermal degradation of poly(dimethyl siloxane) by mid-chain back-biting elimination leading the formation of hexamethylcyclotrisoloxane as an example of cyclic poly(dimethyl siloxane) product from column bleed (adapted from Grassie and Macfarlane[14]).

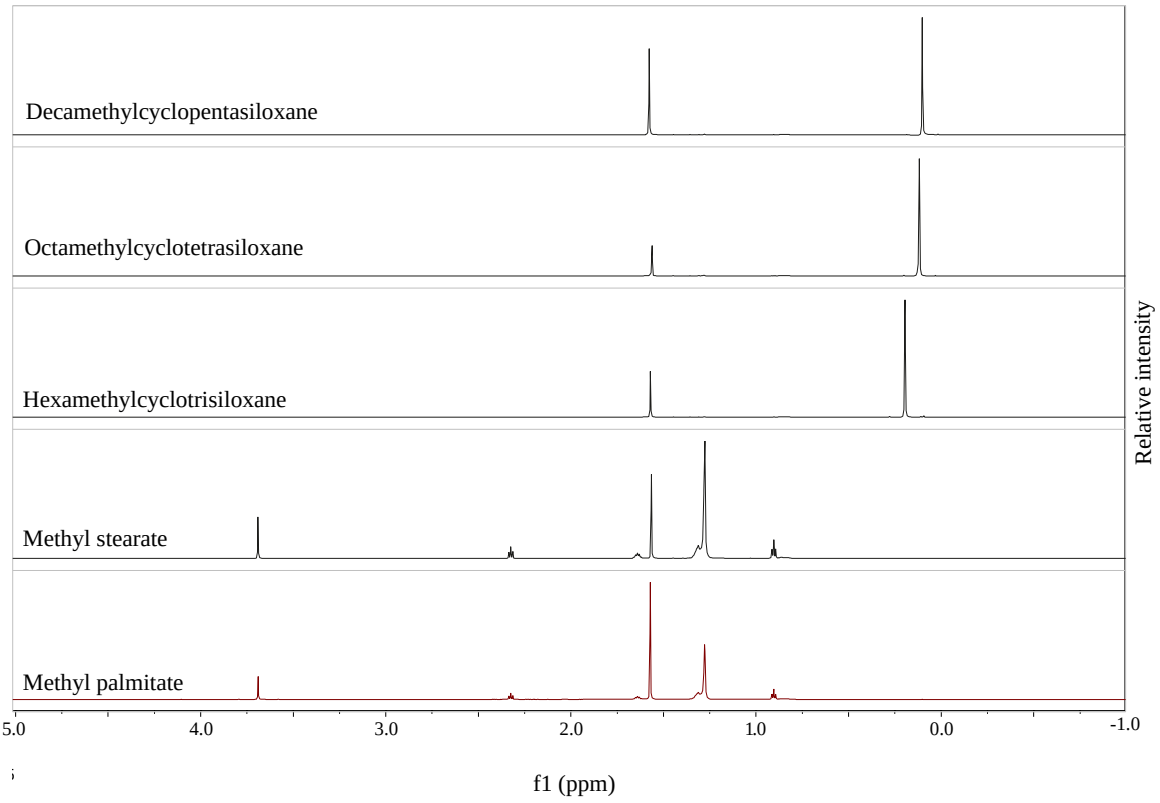
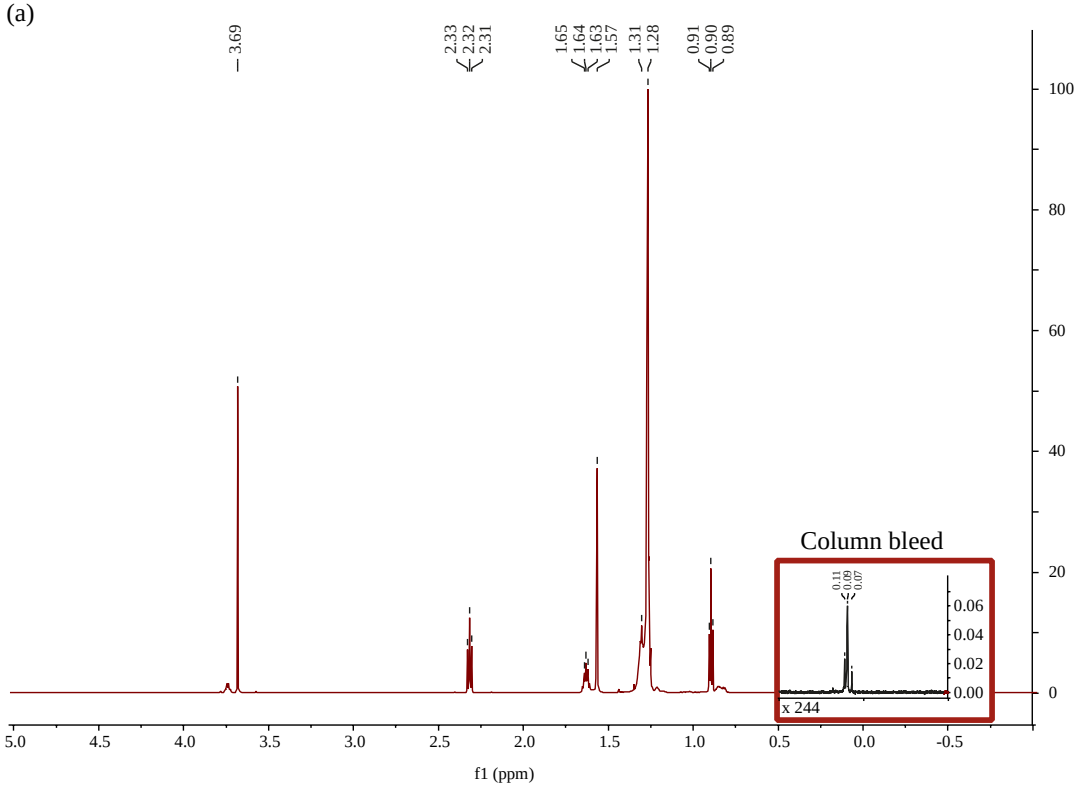


Figure 2. ^{1}H NMR spectra of reference compounds: hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane have, respectively, shifts at 0.20 ppm, 0.12 ppm and 0.10 ppm. The FAMEs display a singlet at 3.69 ppm, a triplet centred on 2.32 ppm, a singlet at 1.64 ppm, a range of peaks at 1.35 - 1.20 ppm and a triplet centred on 0.9 ppm. The resonance present in all spectra at 1.57 ppm corresponds to water.

(a)


3.69
2.33
2.32
2.31
1.65
1.64
1.63
1.57
1.31
1.28
0.91
0.90
0.89
Column bleed
0.11
0.09
0.07
x 244
f1 (ppm)

(b)

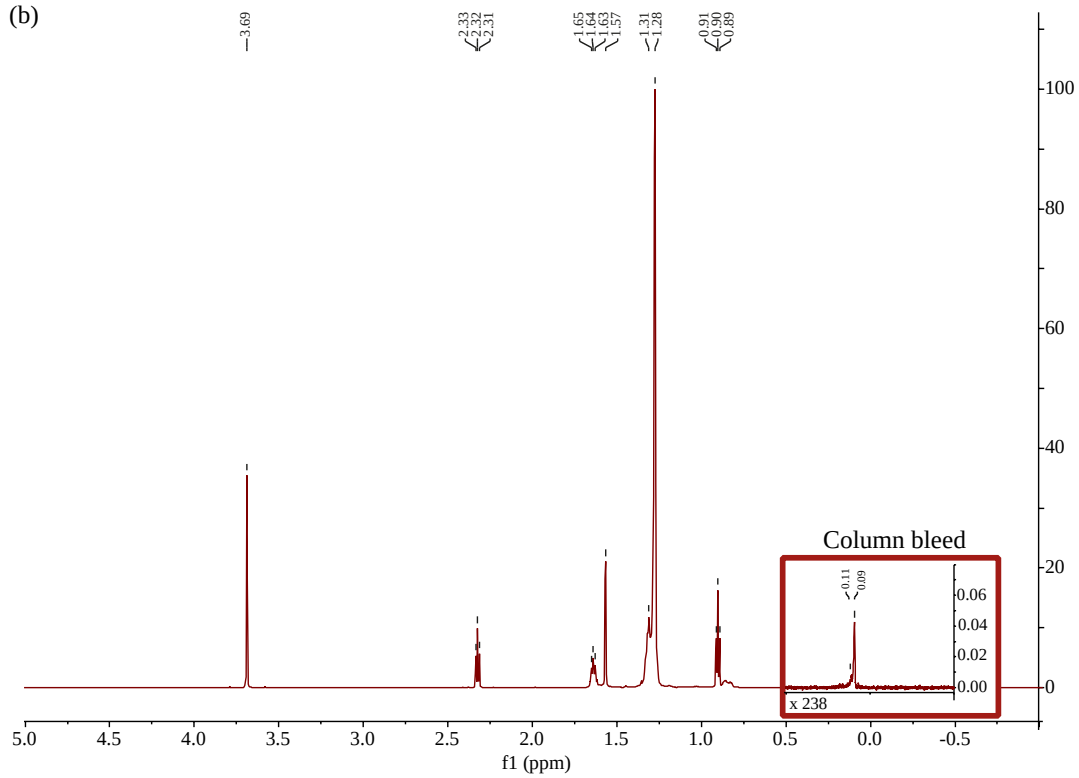

3.69
2.33
2.32
2.31
1.65
1.64
1.63
1.57
1.31
1.28
0.91
0.90
0.89
Column bleed
0.11
0.09
x 238
f1 (ppm)

Figure 3: Normalised NMR spectra of (a) sample 2-$TC_{18:0}$ with magnification of the column bleed resonances at 0.07, 0.09 and 0.11 ppm, and (b) sample Pot-$TC_{18:0}$ with magnification of the column bleed resonances at 0.09 and 0.11 ppm.

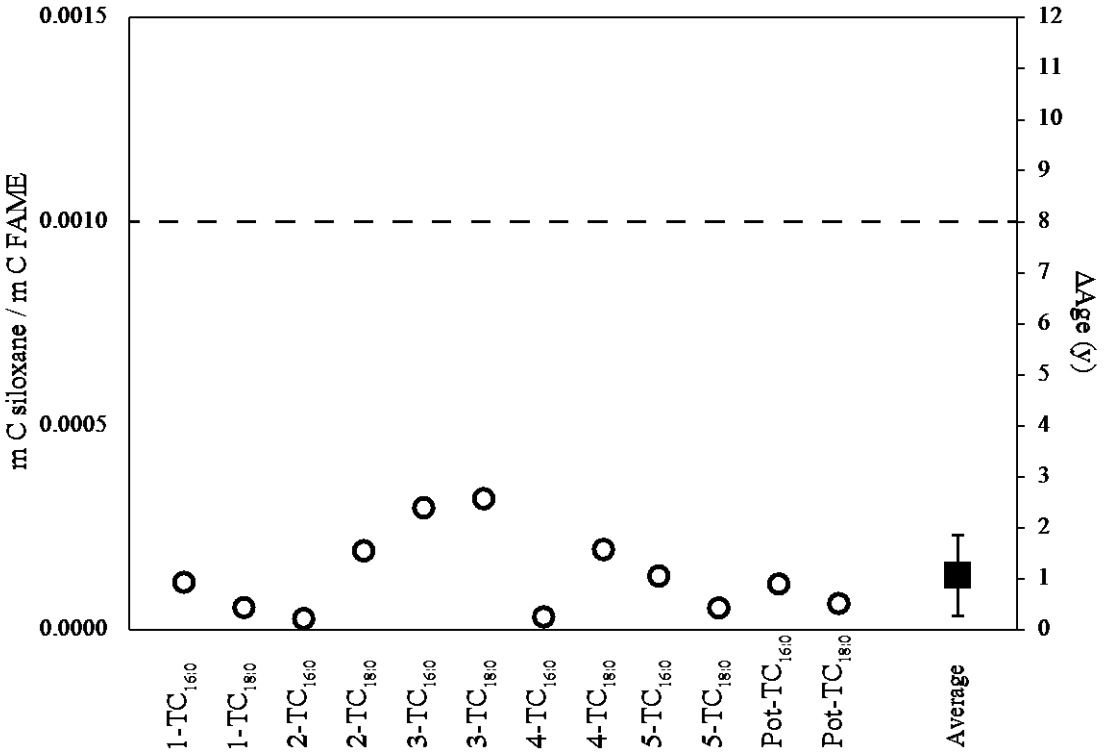


Figure 4: Ratio of the mass of carbon from poly(dimethyl cyclosiloxane) to the mass of carbon from the trapped FAME, for all the samples investigated (dot). The square corresponds to the average and error bar shows the standard deviation. The dashed line corresponds to the threshold of column bleed contamination of 1 ‰ that would affect ^{14}C determinations by AMS.

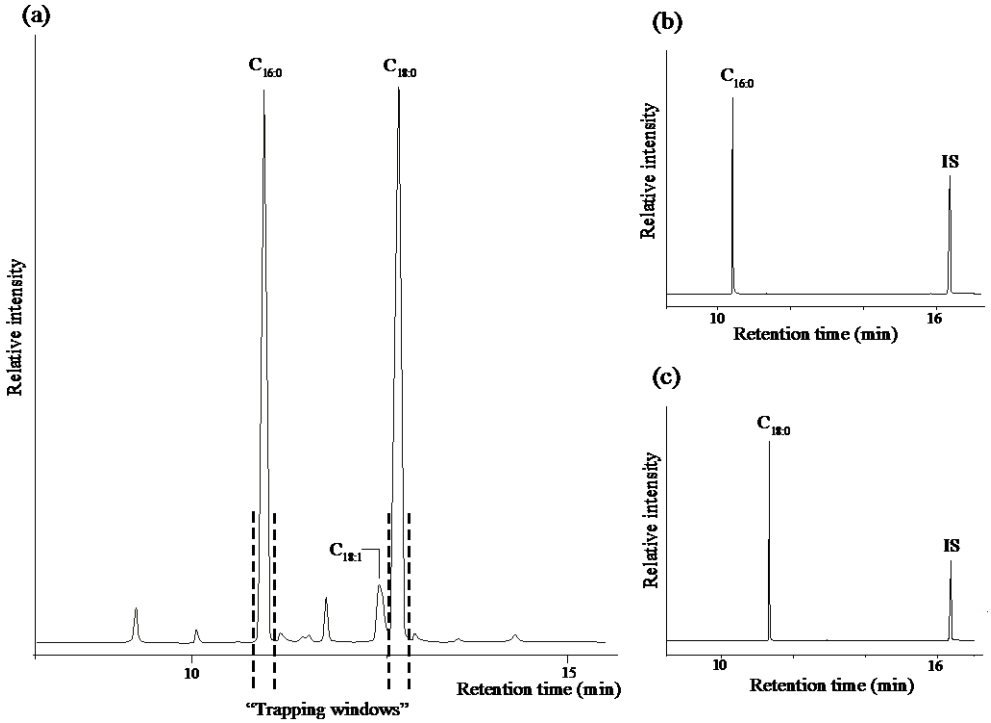


Figure 5: (a) Partial gas chromatogram of the invisible residues from the pottery vessel ROSC4695 with the dashed line correspond to the trapping windows chosen for isolation. (b) and (c) partial gas chromatograms of the contents of the traps, respectively, Pot-$TC_{16:0}$ and Pot-$TC_{18:0}$ corresponding to the $C_{16:0}$ and $C_{18:0}$ trapping windows indicated and (a). IS is the internal standard added for quantification.

For TOC only