

An innovative molybdenum column liner for oxygen and hydrogen stable isotope analysis by pyrolysis

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The most widely used method for pyrolysing samples for hydrogen or oxygen isotopic analysis involves heating them to greater than 1300°C in a helium stream passed through a glassy carbon tube in an alumina casing. There are a number of difficulties with this. Glassy carbon tubes are expensive and interaction between the carbon tube and the outer casing produces unwanted carbon monoxide by reduction of the alumina at high temperatures. The latter effect is overwhelming if temperatures of 1400°C or greater are used for pyrolysis. We experimented with lining alumina casings with pure molybdenum sheet. It is relatively cheap, conforms well to the interior of the reactor tube (to avoid carrier and sample bypassing of the carbon pack), resists high temperatures and neither oxidises excessively nor absorbs the gases. The main disadvantages are that silver sample cups must be used and that the molybdenum degrades over time by formation of the carbide. We can maintain sharp peaks, high precision and good accuracy over more than 700 solid samples for both hydrogen and oxygen. The reactors last longer for water injections. The molybdenum in the columns does not contribute greatly to memory effects. The precision of analysis is dependent on other factors as well as the pyrolysis column, but for oxygen we typically achieve approximately <0.2‰ (sucrose), <0.25‰ (water) and <0.25‰ (leaf), sometimes using only a linear correction of drift, after dividing the run into 1 to 3 segments. Copyright © 2008 John Wiley & Sons, Ltd.

The use of carbon to reduce organic samples to CO and H₂, etc., at high temperatures is well known in stable isotope analysis. A good summary is found in Saurer and Siegwolf.¹ Further discussions of the chemistry and methodology of pyrolysis for oxygen isotope analysis can be found in the literature.^{2–4} Several workers have suggested improvements to give better precision and accuracy.^{5,6} The main problem areas affecting precision are low/variable yield, incomplete conversion of the sample into carbon monoxide and/or hydrogen, memory effects and size dependency. In addition some or all materials (especially mineral salts) are best analysed at temperatures higher than 1400°C⁴ but, at these temperatures, although the equilibrium CO:CO₂ is very favourable,⁷ background levels of CO in the reactor are unacceptable. Background CO is derived from a reaction between the alumina outer casing of the furnace tubes (which has a significant vapour pressure at these temperatures) and the glassy carbon liner (Refs 8 and 9 and our own analyses) by the reaction $\text{Al}_2\text{O}_3 + 3\text{C} \rightarrow 2\text{Al} + 3\text{CO}$. Typically after the reaction of a number of samples the glassy carbon tube shows extensive surface erosion with deposits of metallic aluminium and aluminium carbide. In addition it is often very difficult to prevent bypassing of the packed part of the

pyrolysis column by carrier gas, resulting in peak tailing and improper flushing of the sample gas. Various re-routings of the carrier gas have been suggested.^{1,6} We have also experimented with double-walled reactors, where helium flows between the inner and outer tubes, greatly reducing atmospheric diffusion into the reactor. The improvement in the results was not significant.

We have conducted a research programme to work around the problems noted above. Initially we concentrated on making a better seal between the glassy carbon tube and the reactor wall to reduce bypassing but the experiments produced unreliable results, and high background concentrations of CO were still present at elevated temperatures even when the glassy carbon tube and alumina casing were separated by several millimetres. This resulted in variation in the apparent composition of samples, proportional to their size ('non-linearity') and excessive scale compression, when the range of values from the analyses is less than the true range of values. This effect is typically linked to non-linearity and results from the addition of a contaminant of constant composition, such as from a leak. Past experience with nickel liners suggested that the most effective material would be an affordable metal that did not decompose or form stable oxides at high temperatures. Metal sheet can be made to give a good seal with the reactor walls, takes up little room in the tube (so that blockage by sample debris and melted cups is minimised) and provides an oxidation-neutral barrier

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between glassy carbon chips within the reactor (as a carbon source) and the oxidised aluminium (alumina) outer casing.

Platinum group metals appear attractive in this context but are far too expensive and may not have high enough melting temperatures. Slivers of 0.05 mm rhenium foil placed in the furnace appeared to be extremely resistant but platinum at 1400°C (melting point 1772°C¹⁰) failed within one day. Rhenium might be cost-effective for water-injection furnaces where contamination is minimal and they might have a very long life but our budget did not extend to testing this. Unfortunately, experiments with nickel (used for pyrolysis at lower temperatures²) showed that it decomposed rapidly – the decomposition apparently being hastened by forming a eutectic with tin sample cups. We also tested Inconel 625 but, although this material and nickel resist oxidation, they melt at close to 1400°C and failed rapidly causing a high background and peak-tailing. We tried tantalum foil in a similar manner but it removed all sample oxygen by oxidation in addition to absorbing nearly all the hydrogen, causing severe tailing. The best results were obtained with molybdenum sheet and we have refined our method using this. Molybdenum appears to resist oxidation under the conditions of pyrolysis furnaces and has a high melting point – 2610°C¹⁰ – but fails, typically after several weeks, primarily by carbide formation.

After discussion with us in 2005, Sercon Ltd., Crewe, UK, made available liners based on our design. These are shorter than ours and designed to fit a high-temperature elemental analyser.

EXPERIMENTAL

Sample preparation system

Two mass spectrometers were used for testing in continuous-flow mode: an Isoprime (Micromass, Middlewich, UK) for hydrogen and an Isochrom (Fisons, Middlewich, UK) for oxygen. These are connected to high-temperature pyrolysis systems. Solid samples are folded into silver cups (tin is unsuitable for the molybdenum liner, see section 'Pyrolysis columns' below) and dropped into the pyrolysis column using a pneumatic AS200 autosampler (Carlo Erba, Milan, Italy). Water samples were treated the same way except that 0.75 µL of water was placed carefully into silver cups using a micro-pipette and the cups were then crimped manually, using a modified crimper supplied by Tyler Coplen (USGS, Reston, VA, USA). Cups were not flushed with argon as tests showed no improvement in precision and a statistically insignificant offset, while we had concerns that the argon flow might cause unpredictable evaporation. We typically analyse 147 samples (three carousels) in a run. The AS200 is mounted on top of a high-temperature furnace (B. & L. Tetlow Pty Ltd., Blackburn, Victoria, Australia). The furnace consists of an insulated 250 mm high cavity with three pairs of silicon carbide helical elements running horizontally across it, one above the other. Each pair is regulated using individual thermocouples and current-ramping controllers. We have kept the furnace at about 1400°C for >15000 h without element failure when this paper was written, lowering the temperature to 200°C only when we need to service the two reaction columns which pass vertically

through the pairs of elements. The alumina furnace tubes are 700 mm long and 18 mm o.d. (Ceramic Oxide Fabricators, Eaglehawk, Victoria, Australia). These tubes are 250 mm longer than the standard elemental analyser tubes but with the same o.d.: the extra length results from the perceived requirement for a larger 'hot-spot' and the extra thickness of insulation on the high-temperature furnaces. The broad hot-spot (>200 mm) has a relatively flat temperature profile across it (Fig. 1). Note that it is not necessary to have the longer furnace tubes to use the molybdenum liners – our alumina casings are 700 mm long because that is the size required to fit our furnaces. The column pack (Fig. 2) will be described in detail below but in essence the hot part of the alumina column is lined with molybdenum sheet and packed with glassy carbon chips (Sigradur G; Hochtemperatur-Werkstoffe, Thierhaupten, Germany). For analysing water, an additional carbon source is placed on top of the chips (see discussion below). The high-purity helium (99.999%; BOC Gases, Sydney, Australia) carrier flows down through the furnace at a rate of 120 mL min⁻¹, regulated by a mass-flow controller, and flows to the mass spectrometer through a 1.6 mm o.d. stainless steel tube.

The sample gases, mixed with the carrier, pass through a scrubber of AlphaSolve (sodium hydroxide on silicate carrier; Alpha Resources Inc., Stevensville, MI, USA) and magnesium perchlorate (Alpha Resources Inc.) to remove carbon dioxide, water, and other acid gases. The carrier and scrubbed sample gas then pass through a gas chromatograph (model GC-8A; Shimadzu Corporation, Kyoto, Japan) incorporating a thermal conductivity detector (TCD) and

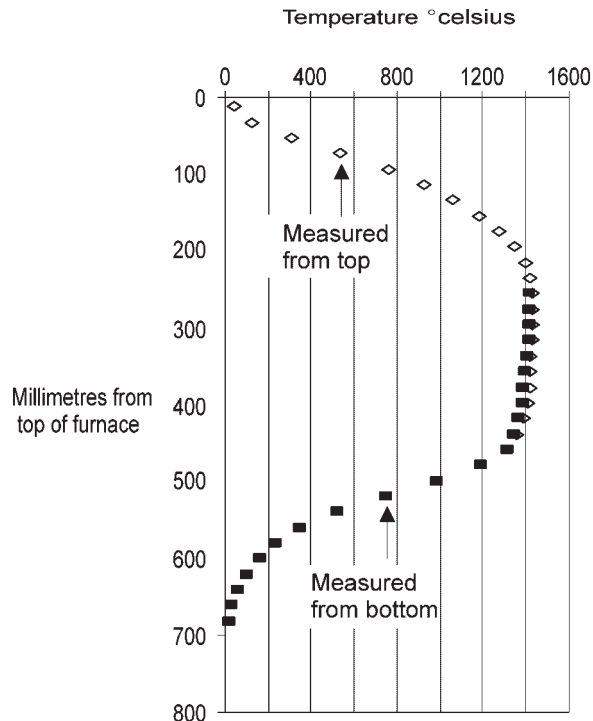


Figure 1. Temperature profile across the high-temperature furnace. Temperatures were measured down from the top and up from the bottom using a type S thermocouple probe. The temperature is essentially constant across the 250 mm open cavity. Note that temperature shown in the hotspot is slightly in excess of 1400°C as the thermocouple was uncalibrated.

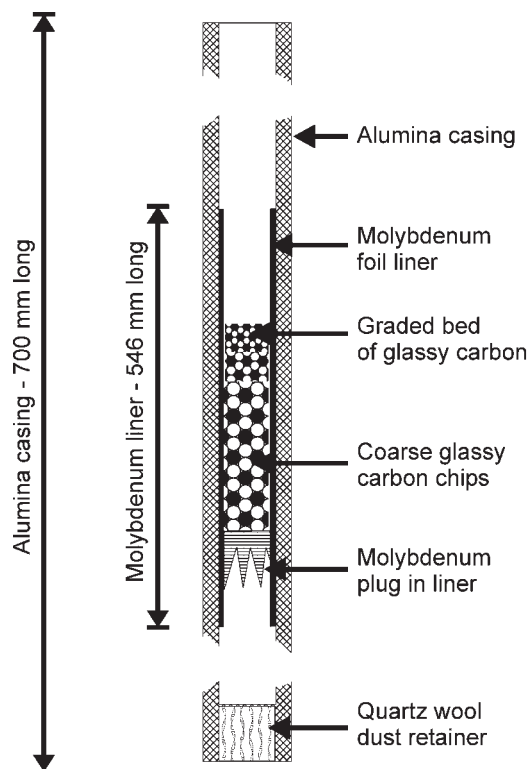


Figure 2. Construction of the reactor tube. The molybdenum plug is pushed well up into the molybdenum liner so that the carbon chips cannot come in contact with the alumina casing and react.

packed GC column. The column is 1.5 m of 4 mm i.d. (0.25" o.d.) stainless steel tube packed with 5 Å molecular sieve of 40/60 grade/mesh (Alltech Associates Inc., Deerfield, IL, USA). The temperature of the GC column is set at 60°C for solid materials and 80°C for water because there is very little nitrogen to be separated from CO for the water injections and a higher GC temperature gives a shorter sample analysis time. During the analysis of water the gas flows from the gas

chromatograph to an open split where the sample enters the mass spectrometer through an approximately 1.5 m long 100 µm i.d. fused-silica capillary (SGE International Pty Ltd., Rignwood, Victoria, Australia). For solids, the gases leave the gas chromatograph through a modified VG diluter (VG, Middlewich, UK). The diluter switches between exhaust from the gas chromatograph and pure helium, so that gases from the furnace pass through the capillary to the mass spectrometer only during the period that the sample gas peak is emerging. A typical oxygen isotopic analysis of a sunflower leaf sample on the Isochrom is shown in Fig. 3. The thermal conductivity detector (TCD) spectrum shows undesirable gases being directed away from the mass spectrometer during the first 190 s, during which time the machine receives only clean carrier gas and analyses the reference pulse. From 190 to 400 s the gas leaving the gas chromatograph is directed to the mass spectrometer and the sample CO pulse is analysed. Following that the mass spectrometer is again switched to pure helium. The sample gas pulse has returned to background levels before being switched away from the mass spectrometer. This redirection of furnace effluent gas minimises the amount of contaminant, including nitrogen, entering the mass spectrometer. Exclusion of nitrogen is highly beneficial as N₂ and its oxides (formed by combination with oxygen sources, such as water vapour) are isobaric with CO but have very different isotopic ratios: e.g. ¹⁴N¹⁶O has the same mass as ¹²C¹⁸O. Gases for the analyses (He carrier, CO and H₂ reference gases) are stored two storeys down outside the building and are supplied through 3.2 mm o.d. copper tube.

The columns are not ashed or opened during their lives as they do not function properly after exposure to the atmosphere at high temperatures. Plugging with sample debris is seldom a problem as the reactor occupies almost the entire i.d. of the alumina casing. We regard a column as being used up when the background of mass 28 increases significantly, the peaks begin to tail badly, memory or drift become problematic or the precision is worse than 0.35‰ on

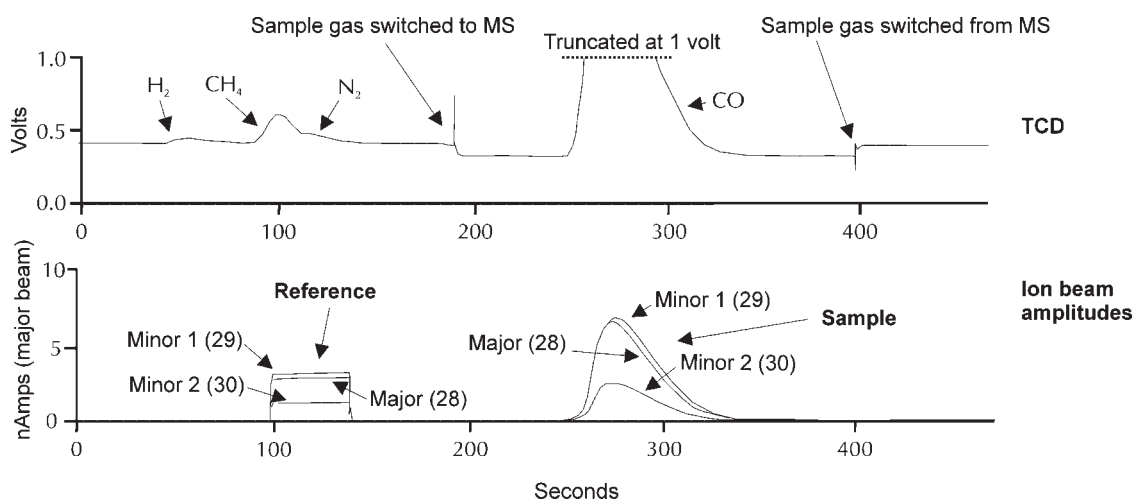


Figure 3. (a) A spectrum showing analysis of a leaf sample on the Isochrom using a modified diluter. The mass spectrometer receives only the carbon monoxide pulse, while the other gases are directed away. The gases not analysed include hydrogen (under-represented by the thermal conductivity detector, TCD), methane and nitrogen. The switching points show on the TCD curve as a result of variations in the reference helium flow in the TCD. The amplitudes of minor 1 and minor 2 are scaled to fit on the graph.

standards. Failure is usually abrupt rather than progressive. We have not made extensive tests as to whether column life can be increased by reducing the furnace temperature between runs, but this does not seem to be the case. On several occasions rapid failure after temperature change suggests that the different thermal expansion rates of molybdenum and alumina ceramic may cause cracking of the molybdenum due to the adhesion of solidified silver.

Pyrolysis columns

The ceramic columns (Fig. 2) are lined over the area of the hotspot with 0.1 mm (0.004") thick high-purity molybdenum supplied in 305 mm wide by 546 mm (12" × 21.5") long sheets (ESPI Metals, Ashland, OR, USA). The large rectangles are cut into five strips of 61 mm width which when rolled into the column seem to provide adequate (about 10 mm) overlap, giving an effective gas seal between the inside and outside of the liner. We have not tested whether a strip twice as wide, giving a double jacket, might extend column life but it is unlikely as once the inner liner cracked, gas would be trapped in the unflushed region between the sheets. We use the full length of 546 mm for water reactors and half the length for solids. Lining the column to the top slightly reduces memory effects for water when injected through a septum (at the top of the tube) rather than dropped as sealed capsules. This may be because the molybdenum conducts the heat further up the column, water adsorbs to molybdenum less than to the alumina or because the vapour cannot bypass the pack by getting over the top of the molybdenum tube. The molybdenum needs to be rolled carefully to keep the overlapping edges perfectly smooth (preventing leakage) and to conform to the alumina casing to prevent by-passing of the packed column by the carrier gas. The molybdenum remains elastic even at high temperatures and will spring out to make a good fit in columns of slightly different sizes if the curvature is of essentially the correct radius. We cannot use badly bent alumina casings or ones with very rough surfaces. Bending of the molybdenum before insertion into the casing takes place in two stages. During the first stage the edge of the sheet is pressed into a former so that 7 mm along the side conforms to a 15 mm radius of curvature. This is done because the roller tends to leave the last few millimetres unrolled. Our roller comprises a stainless steel tube with an i.d. of 15 mm and a slot cut along one side. Inside the tube is a steel rod with a 5 mm deep, 0.7 mm wide cut along its length from the end. The tube is held rigidly to a bench with two clamps and the unbent edge of the sheet is inserted through the slot and into the cut in the rod. When the rod is rotated with a wrench, the sheet is drawn in and wound around it, the pre-curved portion entering last. Once the sheet is rolled into the tube, the end of the alumina column is lined up on it and the rod is rotated while it is pushed into the ceramic column, with the sheet wound around the rod like a watch-spring. When the coil is precisely located the rod is drawn straight out, leaving the molybdenum coil inside the alumina column. As the molybdenum is very tough, attempts to insert it by hand, once it is expanded, are invariably unsuccessful. We place a plug in the bottom of the molybdenum-lined section so that the glassy carbon chips which will fill it are in contact only with the metal and not

with the ceramic or silica glass. The plug is made using a star of molybdenum with the arms folded back and pushed up inside the molybdenum tube. The column is then filled with glassy carbon chips up to the centre of the hot spot. For water analyses this carbon pack is constructed as a graded bed, fining upwards. The 3 to 4 mm chips from the supplier are crushed and sieved into grades of approximately <0.5 mm, 0.5 to 0.8, 0.8 to 1.3 and 1.3 to 2.0 mm. Uncrushed chips are placed at the bottom of the pack on the molybdenum plug. Then the top 20 to 30 mm are constructed from layers of increasingly small chips. Above this is approximately 2 g of reactive carbon (see below). For analysis of carbon-containing solids, the column is only packed with ungraded, coarse glassy-carbon chips. The purpose of the graded pack in our water-analysis columns is two-fold: it stops the fine reactive carbon from falling through the packing of coarse chips and it gives the glassy carbon a much higher surface area and smaller passages to ensure complete equilibration of the reactive sample gases by minimising the distance gases must diffuse to a reactive surface, although there will be a corresponding increase in flow velocity through the interstices. This is in contrast with Gehre *et al.*⁹ who use only chips >3 mm to preserve gas flow and reduce differential pressure across the column pack. We have not specifically examined how much gas bypasses the reactor, between the molybdenum and the alumina casing. As vapour bypassing the column would not encounter reactive carbon we would expect it to emerge from the base of the column in its original state (water, not CO and H₂) but there is no excessive consumption of the magnesium perchlorate or AlphaSolve in the moisture and CO₂ scrubber: we typically replace 3 mL of each material every 294 samples (2 × 3-carousel runs). The column is left empty below the molybdenum plug except for a wad of silica wool (IVA Analysentechnik E.K., Meerbusch, Germany) in the bottom end to prevent debris from entering the 1.6 mm o.d. tube through which the gases exit. The peaks that emerge from the column are very sharp (contracted in time) and do not appear to be smeared by diffusion in the large open cross-section – indeed one of the main functions of the GC column in the water-analysis setup is to broaden the peaks slightly to give better integration. Carbon monoxide passing through the gas chromatograph suffers some isotopic separation: the heavier isotopologues emerge after the light ones. To get precise analyses it is important that the isotope distribution in the peak is uniform from sample to sample or the varying composition of the gases lost in the excluded part of the peak below the baseline or in the small tail will alter the composition of the part of the peak that is integrated.

One of the major difficulties that we have encountered is the provision of available carbon for reduction of the sample. Initially we used glassy carbon as the carbon source but found that yields were variable and low, indicating incomplete conversion of the sample gas. This was not entirely unexpected as glassy carbon is known not to be an adequate carbon source at 1400°C.³ However, several improved methods^{3,9} use only 10 to 20 mg or less of nickelised/reactive carbon in the construction of the column and if any significant number of samples is run, the conversion must be deriving the vast majority of the carbon

from the glassy carbon chips and tube. Nickelised carbon did not function correctly in our columns: the efficiency of the carbon seemed to be reduced rapidly (with correspondingly decreasing gas yields) and the nickel reacted with the molybdenum liner. This led to pinholes which caused severe peak-tailing and poor precision (Fig. 4). Tin causes the same problem but to a greater extent – whereas we have always used tin cups in our nickel and carbon columns we are forced to use silver cups with the molybdenum or the precision declines rapidly. Experimentation with other forms of carbon such as platinised carbon and various charcoals showed low yields and unacceptable memory between samples, as well as poor chromatography. Our preferred carbon in the reactors is the same carbon substrate that is used to produce nickelised carbon but without the nickel treatment. This carbon was kindly supplied to us for testing by IVA Analysentechnik E.K.

Testing

Testing of the columns focused on verifying the accuracy and precision of analysis of the product gases. One of the key parameters is that a peak of a predictable shape and amplitude, with negligible tailing, must emerge from the pyrolysis reaction. As noted above, the distribution of carbon monoxide isotopologues is not uniform through a peak as isotopically light gases emerge before heavier variants. This means that the tail of gas that may be lost is not isotopically identical to the head: thus head to tail proportions and tailing must be uniform through a run. Furthermore, if sample gas is trapped within the column and emerges slowly, it will be mixed into the peak in such a way as to produce an isotopic bias and poor precision.

As noted above, it is essential to separate nitrogen from carbon monoxide before the mass spectrometer. Gas chromatographic issues are not relevant to this paper but even the best gas chromatograph cannot separate N_2 and CO if the peaks do not emerge from the pyrolysis reactor cleanly,

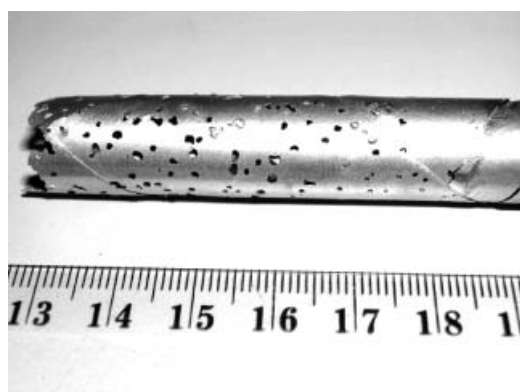


Figure 4. Problems with tin pellets and nickelised carbon – a molybdenum tube showing perforations. The holes correspond with contact points of the glassy carbon filling but probably result from the formation of a low-temperature eutectic with the tin meniscus, rather than reaction of the molybdenum with the carbon. The shattered top (left) results from contact with nickelised carbon. Note that this tube was inserted into the casing incorrectly so that the seam is helical, not straight.

with little tailing. Achieving effective baseline separation of the N_2 and CO requires rapid and complete decomposition of the sample as well as the exclusion of residual gas from the column.

Memory is related to tailing in some circumstances: if gas is trapped within the column and released slowly it will mix with subsequent samples. A more common form of isotopic memory is found where either the sample is adsorbed onto the walls or pack of the pyrolysis column, or it forms compounds stable enough to be able to exchange with subsequent pulses of gas. Memory is therefore a key parameter to be tested, especially when its presence makes it necessary to run otherwise unnecessary duplicates or triplicates of samples.

All measurements are made relative to pyrolysed standard materials. We do not attempt to correct results to a non-pyrolysed standard, such as a reference gas, for a number of reasons, including that the apparent composition of a gas is commonly dependent on GC separation.

Testing: oxygen

Initial testing for this paper has been concentrated on oxygen, rather than hydrogen, isotopes. Most analyses were performed on the Isochrom (which is not equipped for hydrogen isotopic analysis) with the remainder and the hydrogen samples being processed by the Isoprime. Two classes of material are considered: plant solids where carbon is generally present in the sample itself and water, where carbon must be supplied to the reaction. Plant material formed the main focus of the tests as our research group concentrates on that area. All tests were conducted at 1400°C with samples dropped in silver cups. The molybdenum liner could withstand much higher temperatures but the life of the furnace and heating elements would be considerably reduced.

Solids

Groups (typically 5–6 samples) of ANU (Cane) sucrose and beet sucrose of known $\delta^{18}\text{O}$ were measured, interspersed with sets of methionine samples (Fig. 5). While sucrose is hygroscopic, affecting its $\delta^{18}\text{O}$, we have found that by keeping it dry it can be used satisfactorily as a laboratory standard. Methionine has a high N content and therefore tests the ability of the system to deal with overlap between N_2 and CO. The range of $\delta^{18}\text{O}$ provided by our sugars (30.81 to 36.40‰ with respect to Vienna Standard Mean Ocean Water (VSMOW) (as for all isotopic values in this paper)) is rather narrow so this was extended by the addition of Ag_3PO_4 of known composition: standards Na-1 and NH4-2, shown by fluorination and bromination¹¹ to be 7.81 and 20.97 ‰ VSMOW, respectively. The sample mass for organic solids is typically 1.5 mg. The mass spectrometer is set up for this relatively large amount of gas to reduce variability due to sample heterogeneity, which is particularly noticeable when analysing oxygen isotopes in plant material. Samples are weighed to a specific weight ± 0.1 mg to reduce size-dependency but as they are not precisely weighed, some size-related effects are present. Note also that when dealing with plant material, an identical weight does not mean an identical amount of CO due to variability in the proportion of

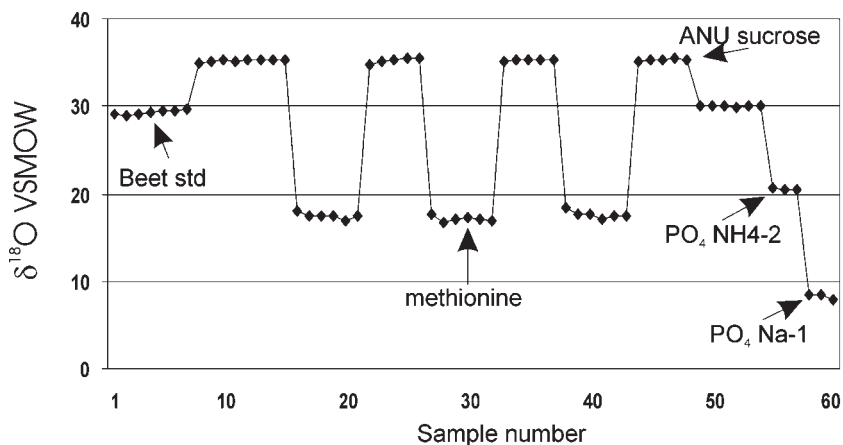


Figure 5. The $\delta^{18}\text{O}$ of standards (sucrose and phosphates) and methionine samples. The relationship between tested and accepted values for sugars and phosphates is shown in Fig. 9. The data are uncorrected and unedited so as to show the small inaccuracies in the first samples in the run, the minor memory effect, the drift visible in the beet sugar along the run and the less good precision of the methionine analyses.

oxygen between components such as carbohydrates and lignin.

A more typical production run with plant material interspersed with 83 beet sugar and some sunflower leaf standards is shown in Fig. 6. This longer run tests the ability of the column to withstand the development of low-turnover memory (drift) during a run.

Liquids

It is possible to put solids and liquids in the same run (Fig. 7). A run is examined where the first 20 samples are cane (36.40‰) and beet sugar (30.81‰), the remaining 34 are water standards – Standard Light Antarctic Precipitation (SLAP) and VSMOW (–55.5 and 0‰) and LEAF, a laboratory water standard with an approximate value of 31.7‰ tested by equilibration with CO_2 .

Testing: hydrogen

All hydrogen analyses were performed on the Isoprime. The majority of testing was aimed at verifying that the molybdenum column was suitable for oxygen isotopic analysis so only a small number of oils were analysed for $\delta^2\text{H}$. Analysis of the $\delta^{18}\text{O}$ of water worked well with this column, but the increased amount of reactive carbon required resulted in tailing of the hydrogen peak. For this reason we have continued to analyse hydrogen isotopes in water by reduction on chromium. The hydrogen peak shapes and precision from carbon-containing solids and liquids have been good in our tests (Fig. 8). In the two runs examined here the most representative data are from analysis of a polyethylene tubing used as a laboratory standard. We also tested oil from *Melaleuca* sp., IAEA-C3 cellulose and dried sunflower leaf. The polyethylene tubing was the most

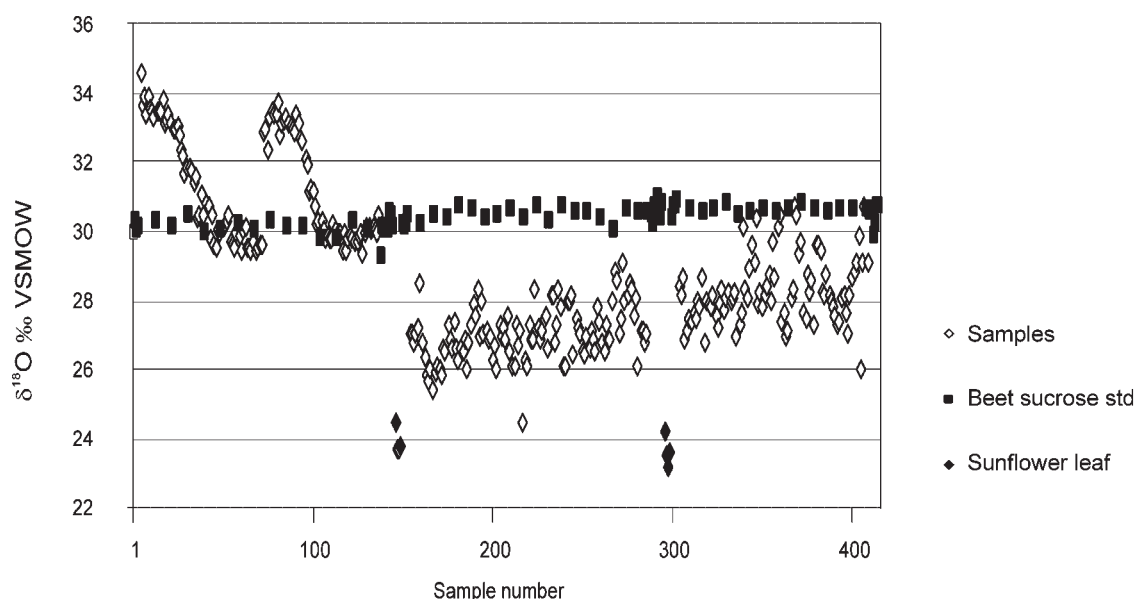


Figure 6. The $\delta^{18}\text{O}$ of plant solids analysed by pyrolysis. The graph shows results for samples (phloem sugar), beet sugar standards and sunflower leaf samples.

suitable for these tests as it does not contain exchangeable hydrogen, unlike the other materials which were not equilibrated with vapour or derivatised.

RESULTS

The precisions presented here are those of the entire analysis: they include errors from many other causes than those resulting directly from the column structure. Examples of other sources of error are leaks, non-linear response to varying sample size, temperature variation in the laboratory and sample evaporation/desiccation. Oxygen isotopic assessment of the reference gas composition typically has a precision of about 0.1‰ or better within a run.

$\delta^{18}\text{O}$ of solids

Figure 5 shows a run of sugars, phosphates and methionine. No corrections have been performed. From visual inspection of the changes from batches of ANU Cane sucrose samples to methionine batches and back, the memory effects are small, with only some of the first samples after a large change showing any appreciable influence from the previous sample. Precision (one standard deviation) on all ANU Sucrose is 0.16‰, with 0.07, 0.12, 0.06 and 0.08‰ for the groups. Some of the error may result from heterogeneity within the materials (including some residual water after drying) or the influence of the high nitrogen content in the methionine, although the 5 Å molecular sieve in the gas chromatograph was held at 45°C and the separation of the N_2 and CO peaks was excellent – about 220 s between N_2 and CO peak centres. The uncorrected precision on the beet sugars is 0.46‰ for all the samples, including the first samples in a run – we normally exclude the first three samples of a run – when this is done the precision for this run is 0.31‰; the precision of the two groups separately is 0.1 and 0.09‰.

The run shown in Fig. 5, with four different standards – ANU cane, beet and two silver phosphates (the methionine was unknown) – is useful for examining the accuracy of the analyses as well as the precision. After correction for drift, tested values (derived by our pyrolysis) with the knowns (derived by fluorination, bromination and pyrolysis) give a slope to the relationship of 1.0035 and a R^2 of 0.998 (Fig. 9). This demonstrates an almost complete absence of scale stretch and a very low contribution of background gas which would cause the slope to be less than 1. The different materials have pyrolysed completely, with efficient conversion of the oxygen they contained into CO .

Figure 6 shows the small amount of drift that normally occurs with a typical run of solid samples.

$\delta^{18}\text{O}$ of liquids

Figure 7 shows a range of waters and sugars with a $\delta^{18}\text{O}$ range of over 90‰. The results as displayed have been corrected for about 5% scale stretch – an effect usually caused by the addition of a proportion of material of a constant composition (such as a leak) which results in analytical results having a smaller range of values than the true range of the materials. The precision in this run (Fig. 7) is generally good (with no corrections performed) except for two particular 'legs' in the run – leg 1 (ANU Cane) and leg 5

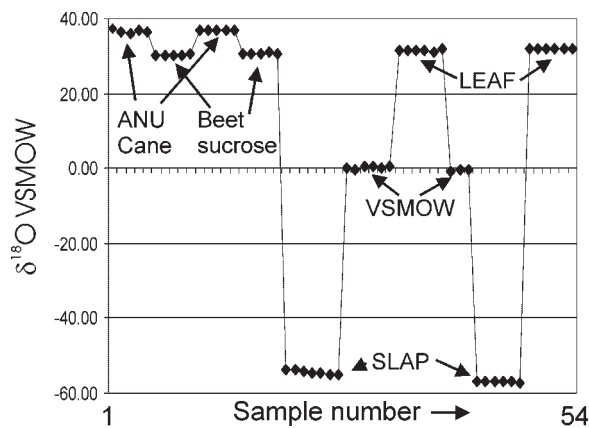


Figure 7. Analysis of sugar and water samples. The run starts with alternating groups of cane and beet sugar, followed by SLAP, VSMOW and laboratory standard LEAF waters. Note the lower precision at the start of the run and at the transition to carbon-consuming water analyses.

(SLAP). Memory effects are also negligible. The poor legs are shown here as they demonstrate characteristic problems of oxygen isotope analysis. The first five samples of ANU Cane have a precision of 0.44‰, resulting from the initial instability of pyrolysis, while the precision of the second leg of ANU Cane is 0.09‰. The first leg of SLAP has a precision of 0.32‰ (and an offset) compared with 0.2‰ for the next leg. The poor initial precision for the water results from the transition from pyrolysing sugars, which provide their own carbon, to water, which strips carbon from the column, perhaps starting with the very fine carbon left behind by the sugar pyrolysis. This results in pyrolysis conditions for the water which initially change very rapidly. Once the more reactive carbon has been stripped, conditions stabilise and the precisions of the subsequent water analyses are better: 0.23, 0.34, 0.19, 0.20 and 0.09‰. The series of LEAF with a 0.34‰ precision improves to 0.26‰ if the last, rather small and probably leaky, pellet is removed. We typically run hundreds of samples through a column and this stability improves through the early part of the column life; these tests were performed when the column was first started.

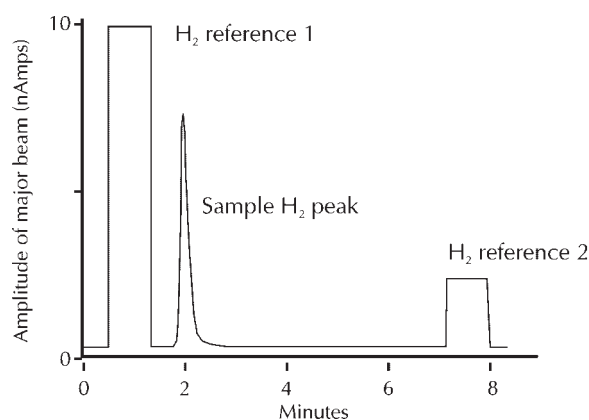


Figure 8. Hydrogen isotope analysis of oil standard NBS 22. Note the sharp hydrogen peak from the sample. There are two pulses of hydrogen reference gas to enable calculation of the H^{3+} correction.

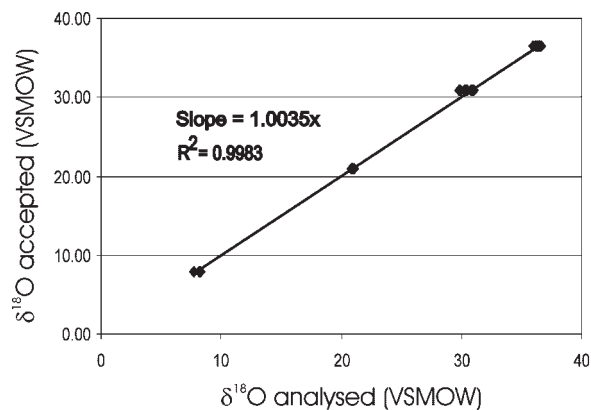


Figure 9. The tested and recognised $\delta^{18}\text{O}$ of the sucrose and phosphate from Fig. 6. ANU Sucrose is identical with IAEA-CH-6. RSBS Beet has been repeatedly analysed with numerous other standards. The two phosphate standards are Ag_3PO_4 prepared by HSW in 1994, analysed by fluorination and bromination and published in Stuart-Williams and Schwarcz.¹¹

Hydrogen

The precisions of the analyses of the polyethylene tubing (without exchangeable hydrogen) from several runs were: 0.67‰, $n = 9$; 0.17‰, $n = 5$; 0.2‰, $n = 3$; and 0.35‰, $n = 3$. The oil from *Melaleuca* sp. showed a precision of 0.53‰ ($n = 4$), while IAEA cellulose C3 had a precision of 1.1‰ ($n = 8$). Dried sunflower leaf gave a precision of 1.1‰ ($n = 9$). The worse precision for C3 and sunflower leaf may be partly due to the hygroscopic nature of cellulose and other carbohydrates – we were not able to ensure that the samples did not absorb moisture while waiting on the machine to be analysed. While such limited tests are not conclusive, the excellent shape of the hydrogen peaks and the precisions obtained suggest that the molybdenum column is suitable for hydrogen isotope analysis of solids (Fig. 8). Approximately 1% to 2% of memory was observed in the column during hydrogen isotope analysis of these carbon-containing materials.

DISCUSSION

The primary concerns in the selection of a pyrolysis column are probably precision, accuracy and cost per sample. The pack and lining of a water column using our design costs about US\$62 and a column for pyrolysis of solids about US\$31. As we typically get at least 500 samples through a column, the sample cost is just a few US cents. We have shown above that the precision of repeated analyses of the same material on a column is good for the $\delta^{18}\text{O}$ of solids and liquids and for the $\delta^2\text{H}$ of carbon-containing compounds, but it is also apparent that memory, drift and scale compression may be issues. Before continuing it must again be noted that the results given here are tests of the whole system, not just tests of the molybdenum column alone. This becomes very important when examining memory, drift and scale compression as these are not entirely dependent on the molybdenum column build. For example, $\delta^{18}\text{O}$ memory seldom exceeds 2% for solids and is fairly easily corrected but it can be substantially greater for water injections, especially in an aging column. One of the key differences is that water reaction requires the presence of

reactive carbon whereas the column for typical plant solids contains only molybdenum and glassy carbon. Experiments with varying the amount of glassy carbon or the length of the molybdenum liner have not been found to alter substantially the amount of memory, whereas altering the type or amount of carbon has a large effect. Experiments with syringe washing and evacuation between samples did little to help. A large part of the memory was therefore in the injection port and septum. We have not experimented with electropolishing in the injection port or similar⁹ and there is undoubtedly much room for improved design in this area. To overcome the problem of memory in water analyses as much as possible, we frequently inject in triplicate or quadruplicate and use the average of the last two values, which is acceptable given the low unit cost, although it makes the analyses more time-consuming. It is probable that memory to some extent relates to surface area and the extra length of our columns may be a hindrance in this respect.

It is difficult to separate the effects of drift and memory: sample-to-sample (short-term) memory is easily detectable but in our experience there are usually at least two sources of memory in a typical run, contributing different amounts of mixing over different time frames. Any memory correction must assess three variables: the size of the pool exchanging, its isotopic composition and the proportion that exchanges. The longest-term of these memories is drift, unless it results from changes in the mass spectrometer response, but most of the change in mass spectrometer response should be compensated for by the use of a reference gas. While it is easy to write an algorithm to self-optimize a short-term memory effect and give the best precision the problem becomes much more difficult when trying to solve for more than one memory proportion and period. Memory over a different period also demonstrates a different phase relationship. The data presented here are uncorrected (except where specified) to emphasise the characteristics of the reaction column. This is also typical of most of our production runs on plant material, except that multiple standards are analysed and a slope and offset are calculated from known delta values. Sample analyses are then corrected with this slope and offset which are typically constant through a whole run. Drift corrections are seldom required for the $\delta^{18}\text{O}$ of solids or water, or the $\delta^2\text{H}$ of solids.

Scale compression/stretch (where the apparent difference in isotopic composition between two materials is less than its true value) is typically a result of adulterating the sample gas with an extraneous material of relatively constant composition. Usually this is due to isotopic exchange with the preparation system or to leaks, and can be treated as a memory effect resulting from exchange with an extremely large pool. As most high-temperature furnace materials are oxides, it is difficult to avoid some exchange of oxygen with them. Glassy carbon liners separate the sample gas from the high-temperature alumina casing but in a conventional system the steady flow of CO with a constant composition from the reaction between the carbon tube and the alumina liner itself frequently creates substantial difficulties. Atmospheric leaks are also a major problem as N_2 has the same mass as CO and cannot be separated from CO by the gas chromatograph if it arrives in a constant stream. Carbon

monoxide based systems are particularly sensitive to leaks as the ratio of $^{15}\text{N}^{15}\text{N}$ to $^{14}\text{N}^{14}\text{N}$ (mass 30:mass 28) is very different from the ratio of $^{12}\text{C}^{18}\text{O}$ to $^{12}\text{C}^{16}\text{O}$ – approximately 0.00133%:0.204%.

Our Isochrom combined with the prep system tends to suffer from nonlinearity/size dependency at the moment. This is often linked to scale contraction as leakage (which tends to be a relatively constant amount of gas with a relatively constant value) will also cause the apparent isotopic value of the sample to differ systematically related to its size. In our machine the size dependency is usually related to source pressure effects as background gas levels are very low and the size dependency that we experience is very sensitive to tuning. Unfortunately, although the effect is minimised by tuning and we run calibrated corrections, size dependency does tend to decrease the precision of the analyses. This may be because the isotopic ratio is determined from the integrated area of the peaks. A peak with a given amplitude comprises the sum of a large number of values between close to zero (the baseline) and the given maximum value, each of which was measured at a different source pressure and needs its own correction. If the peak shape changes then the contributions and required corrections for each point defining the peak also change. It is not only the maximum value that requires correction.

One other issue should be noted here with regard to the effects of chemistry and stoichiometry. As noted above these tests were all run at 1400°C. At this temperature an absence of methane was reported.⁹ This was true in our tests for pure carbohydrates such as sucrose and cellulose, but did not apply to some other materials such as dried leaf, which may produce very substantial amounts of methane (Fig. 3). We postulate that the production of methane to some extent depends on the carbon:oxygen stoichiometry as well as the molecular architecture. In carbohydrates the ratio for C:O is approximately 1:1 but leaves contain a substantial amount of lignin which is composed of a variety of alcohols where C:O = 1:~0.8. Under these circumstances it would appear that some carbon satisfies valency requirements with H instead of O and methane is generated. To the extent that H reacts to form methane, measurement of the isotopic composition of H in a sample by analysis of the H_2 peak is compromised.

We also tested whether molybdenum might be unsuitable for oxygen isotopic analyses due to forming compounds with oxygen having long or short lives – long-lived oxides causing drift by slow exchange or decay and short-lived oxides causing sample to sample memory. Silver-wrapped solid samples do not have high sample-to-sample memory, indicating that CO/molybdenum interaction is negligible. In addition the rapid return to baseline after pyrolysis of solid or liquid samples indicates that short-lived oxides are not formed. The presence of longer-term oxides might be the cause of drift but interactions with other parts of the system are equally probable and the drift is never large enough to suggest substantial oxide formation. Liquid and solid columns both produce good results almost from the first sample dropped and there is no initial increase in the amount of sample gas produced for a given mass of sample, which might indicate initial loss of oxygen as oxides. Column aging is associated with brittleness of the molybdenum liner and



Figure 10. Molybdenum tube after 8 weeks and 700 samples. The top of the tube is to the left, with the top of the hotspot at the right. The alumina tube was shattered with a hammer and fragments of alumina are cemented to the molybdenum with silver. Larger drops of silver at the left may be associated with pin-holes, the smaller droplets at the right show surface diffusion from right to left, to the cooler end of the tube.

the formation of small holes and cracks. As memory, tailing and drift increase with column aging it is more probable that they result from interactions in the space between the molybdenum liner and the alumina column after the gases pass through the pinholes and microscopic cracks. Silver collects between the alumina column and the molybdenum liner (Fig. 10), accumulating where the column temperature is close to the melting point of silver – about 962°C¹¹ – by random-walk effects. This occurs even when the molybdenum liner is long enough that the top and bottom are cooler than 962°C but it is not clear whether the silver passes through the join of the rolled molybdenum sheet tube or through pinholes and cracks at a late stage in the life of the column. The small amount of memory noted in the analysis of the $\delta^2\text{H}$ of solids is probably associated with adsorption of hydrogen onto carbon in the column. Note that as embrittlement of the column is a product of age as well as use, it is desirable to run as many samples as possible through the column as soon as it is commissioned. For this reason we prefer to load a new column only when we have enough samples stockpiled to use it up.

CONCLUSIONS

While much work remains to be done to refine the use of molybdenum as a liner of pyrolysis columns, results so far have been excellent. Molybdenum has consistently outperformed carbon as a liner on our systems and is now used for all our pyrolysis work. Advantages are high precision analyses, low cost, ease of construction and high sample count between column changes. The potential exists to work at higher temperatures as molybdenum melts at >2600°C: we are currently operating 1200°C below that, at 1400°C. Disadvantages are that silver (instead of tin) cups must be used and that columns age over several weeks at high temperatures even if unused. The columns can only be used

once – it is not recommended to vacuum out the liner and, once cooled, the molybdenum liner is firmly welded to the alumina casing by the melted silver cups.

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REFERENCES

1. Saurer M, Siegwolf R. In *Handbook of Stable Isotope Analytical Techniques*, vol. 1, de Groot PA (ed). Elsevier: Amsterdam, 2004; 497–506.
2. Farquhar GD, Henry BK, Styles JM. *Rapid Commun. Mass Spectrom.* 1997; **11**: 1554.
3. Kornexl BE, Gehre M, Höfling R, Werner R. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1685.
4. Gehre M, Strauch G. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1497. DOI: 10.1002/rcm.1076.
5. Brooks PD, Dawson TE. *Presentation and Abstract at Joint European Stable Isotope Users Group Meeting*, Vienna, 2004.
6. Gehre M. *Presentation and Abstract at Joint European Stable Isotope Users Group Meeting*, Vienna, 2004.
7. Santrock J, Hayes JM. *Anal. Chem.* 1987; **59**: 119. DOI: 10.1021/ac00128a025.
8. Available <http://www.uvm.edu/geology/geowww/isogeochem.html>.
9. Gehre M, Geilmann H, Richter J, Werner RA, Brand WA. *Rapid Commun. Mass Spectrom.* 2004; **18**: 2650. DOI: 10.1002/rcm.1672.
10. *Handbook of Chemistry and Physics*, Weast R (ed). CRC Press: Boca Raton, 1985.
11. Stuart-Williams HLeQ, Schwarcz HP. *Geochim. Cosmochim. Acta* 1995; **59**: 3837. DOI: 10.1016/0016-7037(95)00304-1.