

MICROCHEMICAL AND MOLECULAR DATING*

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ABSTRACT. The depth and reliability of archaeological and environmental information on ages, sources and pathways of carbon are being greatly enhanced through a new synergism between advances in “micro ^{14}C dating” and advances in micro-organic analytical chemistry and individual particle characterization. Recent activities at the National Institute of Standards and Technology (NIST, formerly NBS) involving this linkage include dating individual amino acids isolated from bone collagen and the apportionment or tracing of individual carbon compounds derived from anthropogenic sources. Important knowledge has been gained through “direct” (sequential) and “indirect” (parallel) links between microchemistry and ^{14}C measurement. The former is illustrated by ^{14}C measurements on specific amino acids and on the polycyclic aromatic hydrocarbon (PAH) class of compounds. Isolation of the respective molecular fractions from far greater quantities of extraneous carbon held the key to valid dating and source apportionment respectively. Parallel data on ^{14}C and molecular patterns promises new knowledge about the identity of sources of environmental carbon at the nanogram level through multivariate techniques such as principal component analysis and multiple linear regression. Examples are given for atmospheric particulate carbon, using PAH molecular patterns and laser microprobe mass spectral patterns.

INTRODUCTION

Progress in accelerator mass spectrometry (AMS) and correlative micro-analytical and target preparation techniques have enabled investigations of sources and pathways of environmental ^{14}C which were previously impracticable because of the large quantities of sample carbon required. The match between the new “microchemical ^{14}C ” needs and the advanced AMS capabilities is indicated in Table 1, drawn from studies in our laboratory (Currie, Klouda & Voorhees, 1984; Currie, Fletcher & Klouda, 1987; Klouda *et al.*, 1986; Sheffield, Currie & Klouda, 1988; Stafford *et al.*, 1989.). The link between isotopic composition (^{14}C concentration) and chemical composition falls into two categories: “serial” (direct), and “parallel” (multivariate). When sufficient carbon is available for direct measurement, *ie*, microgram to milligram amounts, we can assay ^{14}C directly in isolated chemical fractions. This is the case for our studies of atmospheric methane, the collagen and individual amino-acid fractions of ancient bones, atmospheric and exhaled (breath) benzene, and the class of polycyclic aromatic hydrocarbons (Tables 1, 2). Below the range of AMS (sub-microgram carbon), we must use parallel relationships where ^{14}C tracer information for age or source is conveyed indirectly through multivariable molecular (molecular, isomeric, structural) patterns. Such patterns may, in

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TABLE 1
Microchemical and molecular dating
(and environmental carbon source apportionment)

Needs: mg (CH ₄ , collagen, benzene) ng (benzo[ghi]perylene)	10 μg (NH ₂ CH ₂ COOH, ΣPAH) pg (individual particles: C-clusters)				
Capabilities (β decay counting; AMS atom counting):					
Conventional sample	Signal* limit (100c)	Background* (BEM)	Blank mass	Blank recovery	
Decay	5–10 mg	2.4mg	5mg	40 μg	95%
AMS	0.5–2mg	1.3 μg	0.2 μg	15 μg	30% (1984)
				1.4 μg	95% (1987)

*Mass equivalent limits arising from counting statistics (signal limit) are based on 100 (β particle or ¹⁴C atom) counts; the background limits are based on the background equivalent masses (BEM).

TABLE 2
Microchemical ¹⁴C dating, source resolution

Method	Chemical species	Medium
Serial [direct]	Amino acids	Bone collagen
	Benzene	Breath
	CO, CH ₄ , HCHO, VOC	Atmos gases
	C _e (graphitic), Σ (PAH)	Atmos particles
Approach		
Parallel [multivariable]	¹³ C × ¹⁴ C × Element	¹⁴ C = a variable
	(μg) K (wood), Pb (auto)	¹⁴ C & elemental patterns
	(ng) Polycyclic aromatic hydrocarbons (PAH)	¹⁴ C & molecular patterns
(pg)	Individual C-particles	¹⁴ C & structural patterns

some cases, be calibrated via source experiments (specimens of known age or origin) or theory, or by direct calibration via parallel measurements of ¹⁴C in microgram to milligram-size samples.

Table 1 indicates ¹⁴C measurement capabilities, using miniature gas proportional counters (decay counting) and AMS, respectively. Mini-gas-proportional counting attained its asymptotic capability (5–10mg natural ¹⁴C) several years ago; Table 1 shows it is limited “naturally” by counting statistics rather than by inefficiency, losses or the chemical blank (Oplet, Huxtable & Sanderson, 1986). AMS, however, is only now approaching this point, for the blank, especially that associated with complex microchemical isolation and target preparation, is still comparable to the machine

background and the natural counting statistics limit (Klouda *et al*, 1984; Verkouteren *et al*, 1987). Nevertheless, the current AMS capability of ca 10–200 μg modern carbon is significantly less than the typical accelerator sample size of ca 0.5–2 mg carbon. It should be noted that required Poisson counts, and thus, sample size, differ significantly for dating *vs* environmental applications. One percent imprecision (10^4 counts) is often required to provide adequate age discrimination, whereas 3–10% (1000–100 counts) is usually adequate for environmental source apportionment.

We describe here the decisive roles played by direct measurements of ^{14}C in microgram to milligram samples of isolated chemical fractions, and indirect ^{14}C measurements for the calibration of chemical patterns in picogram to nanogram amounts of individual submicrometer particles and individual molecular species. In all such cases of “microchemical dating,” isolation or assay of the individual fractions was mandatory for obtaining unique carbonaceous source identification, or reliable, uncontaminated ^{14}C dates.

MICROCHEMICAL MEASUREMENTS OF CARBON IN BONES, BREATH AND THE ATMOSPHERE

Table 2 shows the two primary classes of ^{14}C molecular measurements. Direct measurement is clearly the method of choice when sufficient carbon ($>10\mu\text{g}$) is present to isolate chemical fractions. This class is termed “serial” because of the two sequential stages: isolation of the relevant molecular fraction and ^{14}C measurement. The four application areas share a common dependence on quantitative chemical isolation for reliable inferences on sample age or origin. Also, the corresponding molecular concentrations in available samples commonly restrict one to sub-milligram quantities. The second, multivariable measurement class depends on parallel measurements of ^{14}C in “bulk” samples of the chemical (or particle) fractions for comparison with ancillary compositional or structural information often derived from sub-microgram samples. Through appropriate ^{14}C multivariate “calibration” experiments, *ie*, multiple linear regression, or multivariable molecular pattern recognition, it is possible to transfer knowledge derived from the ^{14}C measurements to future studies which must be performed from the picogram to nanogram samples, using the chemical patterns or signatures.

The four *serial* applications (Table 2) are important for: 1) reliable ^{14}C dating of ancient bones, in which the protein (collagen) may not be fully preserved; 2) assessment of the possible contributions of personal practices (diet, smoking) or metabolic processes to benzene sampled in breath, in contrast to exogenous contributions of this known carcinogen; 3) improved knowledge of the anthropogenic and natural sources of volatile and gaseous carbon compounds in the atmosphere; and 4) apportionment of sources of combustion carbon compounds in the atmosphere, such as graphitic carbon (“soot”) and the polycyclic aromatic hydrocarbons (PAH). Only the first of these applications will be discussed in detail here. The second, benzene in breath, relates to a cooperative project between NIST and the American Petroleum Institute, which is just beginning in our laboratory. Knowledge of the ^{14}C content of this benzene may be very important for controlling

sources of this toxic chemical (Krotoszynski, Gabriel & O'Neil, 1977). The last two application areas are central to the continuing quest to apportion the fossil and biospheric sources of the trace carbonaceous gases and particles, because of their profound effects on climate, health and atmospheric visibility (National Research Council, 1984). Finally, it should be noted that the chemical isolation techniques for these studies depend heavily on chromatography. For the larger molecular species, liquid chromatography (LC) and supercritical fluid chromatography (SCF), which is used in the benzene study, are the methods of choice. Gas chromatography may be used for molecular isolation, but more commonly, in our analyses of gases and volatiles, the requisite quantities of purified compounds are obtained via cryogenic, thermogravimetric and selective oxidation techniques.

For the four *parallel* applications (Table 2), ^{14}C as a variable has somewhat different functions. 1) The first ($^{13}\text{C} \times ^{14}\text{C} \times \text{Element}$) actually employs ^{14}C explicitly as one of the source classification variables. In cases where multi-elemental or -molecular data are available, "element" may be replaced by an elemental or molecular ratio or pattern. Besides its application to atmospheric particulate source apportionment (Currie, Klouda & Voorhees, 1984), this type of approach is widely used in isotopic organic biogeochemistry, eg, for investigations of petroleum maturation and contaminant sources (Deines, 1980), and ancient diets (Bumsted, 1985). 2) ^{14}C plays a rather special role in the second multivariable application, in that it can validate an alternative, multiple linear regression model for atmospheric particle origins (Stevens *et al*, 1989). The elemental regressors (K, Pb) are presumed to yield reliable apportionment of the total carbon into its fossil and biospheric sources, based on elemental tracers in combustion materials (wood, petrol); but complications can arise from a third local source (soil or mineral dust), long range transport, or from varying K and Pb concentrations in the respective fuels (Currie *et al*, 1986). In this application, ^{14}C is reliable and absolute; but K and Pb are attractive for large-scale pollution monitoring because they are convenient and inexpensive to measure using x-ray fluorescence spectrometry. Details of the validation procedure are beyond the scope of this article, but the basic strategy is to test model validity using ^{14}C as the dependent variable (Currie, Beebe & Klouda, 1988).

The final two parallel applications of ^{14}C will be discussed in more detail below, following the discussion of molecular dating of bone collagen. In both of these cases, ^{14}C functions as the independent variable used to calibrate a separately measured molecular or structural pattern in the samples of interest. Also, in both cases, the inherent measurement capabilities of the molecular and structural measurement techniques far exceed that of ^{14}C – AMS. Thus, following successful pattern calibration, we can use the organic patterns to identify sources contributing only nanograms of PAH, and structural patterns of carbonaceous particles may be applied at the picogram level.

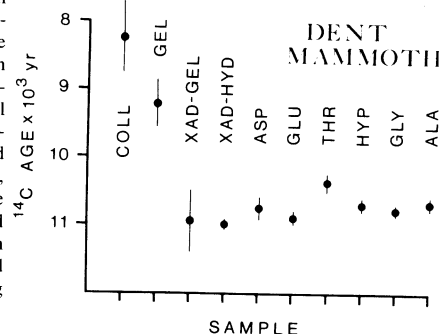
Molecular Dating

The impetus for ^{14}C dating of pure substances is quite simple. If we can identify chemical species uniquely associated with an artifact, especially if those species resist chemical decay or diagenesis, and if they can be chemically (or physically) isolated from foreign carbonaceous material, we are apt to get a reliable date. Standard pretreatment of samples for ^{14}C dating has long been used for this purpose, but isolation of individual source-derived molecular fractions is the more elegant solution, when practicable. A problem, which is diminishing with advances in AMS, has been collection of sufficient material for molecular dating. Dating of associated material, such as bits of charcoal, has not always provided reliable dates (Stafford & Tyson, 1989).

The thesis behind individual amino-acid dating of bone collagen is that the protein that survives diagenesis still contains carbon from the original living creature. Upon hydrolysis of the surviving collagenous protein, we should thus obtain contemporaneous amino acids. If foreign (environmental) organic matter is incorporated into the bone, the proteins and peptides will generally have different isotopic (age) and chemical composition. Thus, if significant original collagen remains, concordant ^{14}C dates between the collagen and the constituent amino acids should ensure reliability.

To test this hypothesis, we selected bones from reliably dated sites and applied sequential steps of chemical purification and molecular isolation. Figure 1 shows results for the Dent Mammoth, with a known age of 11,000 BP, based on its association with Clovis artifacts, ^{14}C dating of sediments and biostratigraphy (Haynes, 1984). These fossil bones represented an intermediate stage of preservation in that significant collagenous material was present, but important protein loss and diagenesis had occurred as reflected by the relatively low nitrogen content (0.8% in contrast to 4.5% for modern bone). The first two chemical fractions shown in Figure 1, weak acid insoluble collagen (COLL) and gelatin (GEL) represent the commonly dated organic fractions of bone. Their discordant ages, which are much too young for the Clovis culture, illustrate the effects of humate contamination which may occur with all but the best preserved fossil specimens. Removal of the non-contemporaneous humates can be accomplished by passing

Fig 1. ^{14}C ages for successively purified chemical fractions from Dent Mammoth bone extracts. The mammoth has a known age of 11,000 yr, based on association with the Clovis culture and biostratigraphy. Fractions shown are: weak acid insoluble collagen (COLL), and gelatin (GEL); XAD-2 resin purified gelatin (XAD-GEL) and collagen hydrolysates (XAD-HYD), the latter representing the total purified amino-acid fraction; and the chromatographically isolated amino acids: aspartic acid (ASP), glutamic acid (GLU), threonine (THR), hydroxyproline (HYP), glycine (GLY), and alanine (ALA). Age concordance among the individual amino acids and with the total amino-acid fraction (XAD-HYD) implies accuracy. Non-purified chemical fractions (COLL, GEL) are too young because of contamination with exogenous humates.



gelatin and collagen hydrolysates through XAD-2 resin with 6N HCl. These fractions (XAD-GEL and XAD-HYD in Fig 1) give the correct age for the mammoth, but for a fossil bone in a state of poor to moderate preservation, it is important to proceed to the molecular level. That is, to guard against exogenous amino acids, we should verify that the average age of the XAD-purified collagen fraction is consistent with that of the individual amino acids. ^{14}C dates for six amino acids, isolated from the purified collagen fraction of the Dent Mammoth by liquid chromatography, are generally self-consistent and concordant with the purified collagen age and the known age of this fossil. See Stafford *et al* (1989) for a discussion of experimental techniques, including methods for assuring adequate recovery and resolution, and minimal contamination.

An application to human fossil bones of unknown age in different states of preservation comes from ^{14}C dating of individual amino acids from skeletal remains from the Del Mar Early Man site in southern California (Stafford & Tyson, 1989). Table 3 indicates the states of preservation of two

TABLE 3
Percent nitrogen and amino acid composition of the Del Mar
sphenoid and tibia fossils

Sample	Del Mar sphenoid	Sign*	Del Mar tibia
Percent nitrogen [modern bone: 4.5%]	0.69	[-]	0.06
<i>Amino acid residues (%)</i>			
4-hydroxyproline	95	[-]	0
Aspartic acid	47	[+]	132
Threonine	17		32
Serine	29		50
Glutamic acid	78	[+]	111
Proline	121	[-]	74
Glycine	342	[-]	276
Alanine	118		126
Valine	31		37
Methionine	5		0
Isoleucine	10		37
Leucine	24		46
Tyrosine	0		0
Phenylalanine	7		26
Histidine	2		1
Hydroxylysine	13		5
Lysine	30		13
Arginine	30		32
Total	999		998

*Signs of the most important differences are given in brackets

samples from the San Diego Museum collection (SDM-16704). The sphenoid (cranial bone) is collagenous and moderately well preserved, but the tibia has lost most of its collagen as shown by the very low nitrogen content. Table 3 shows also the alteration of the characteristic pattern of amino acids in human bone collagen occurring during diagenesis. Most notable is the loss of hydroxyproline and the large relative increase in aspartic and glutamic acids. Alteration of the fundamental molecular pattern thus signals possible age heterogeneity among the residual amino acids, and thus, an "age" for the total amino-acid fraction which has little meaning. The molecular dating results bear this out. Figure 2 shows: 1) ^{14}C ages for the

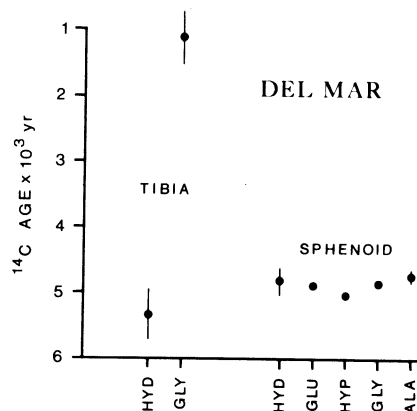


Fig 2. ^{14}C ages for the purified hydrolyzed amino acid fraction (HYD), and for individual amino acids from the tibia and sphenoid samples from the San Diego Museum collection (SD-16704), Del Mar Early Man site. The sphenoid age is reliable on the basis of amino-acid age concordance; the tibia is not. States of preservation for the sphenoid and tibia were moderate (0.69% N) and poor (0.06% N), respectively.

XAD-2 purified hydrolysates of the weak HCl insoluble fractions (HYD) from the Del Mar tibia and sphenoid, representing the total amino acids, and 2) dates for individual amino acids. The collagenous, amino-acid fraction from the sphenoid is chronologically homogenous, but that from the tibia is not. Similarity of the average "age" of the tibia amino-acid fraction to that of the sphenoid collagen is considered fortuitous; an independent dating method applied to the tibia, uranium series disequilibrium, yielded dates of ca 11,000 BP (Bischoff & Rosenbauer, 1981). It is worth noting that ability to test for age concordance at the molecular level required the most advanced capabilities in microchemical purification and accelerator target preparation, for dating fractions of individual amino acids were in some cases $< 80\mu\text{g}$.

Molecular Source Apportionment

Complementing the importance of ^{14}C microchemical measurements to the geological dating of individual organic fractions, is its value in discovering sources of classes of organic compounds in the environment. The polycyclic aromatic hydrocarbon (PAH) class is a case in point. These types of compounds are produced in certain geological processes, as well as all incomplete combustion processes; and their isomeric and homologous patterns are indicative of both the source material and the combustion condi-

tions (Blumer, 1976). Reliable links between fuel and molecular patterns, however, are lacking. For this reason, and because of the importance of this class of mutagenic compounds in atmospheric pollution, ^{14}C has been employed as a means for quantitative PAH source apportionment (Sheffield, 1988). An ideal opportunity for developing and testing this approach to organic molecular source apportionment arose with severe winter pollution episodes now common in several cities in Europe and America (Currie *et al.*, 1986). Samples of atmospheric particles were taken in December 1985 in Albuquerque, New Mexico – a city which has significant carbonaceous pollution from two sources, motor vehicle emissions and residential wood burning (Stevens *et al.*, 1989). The analytical scheme developed for this work is shown in Figure 3A. Structures of selected PAH are shown in Figure 3B. Isolation of the purified PAH fraction and the subsequent AMS target preparation required great care to ensure quantitative

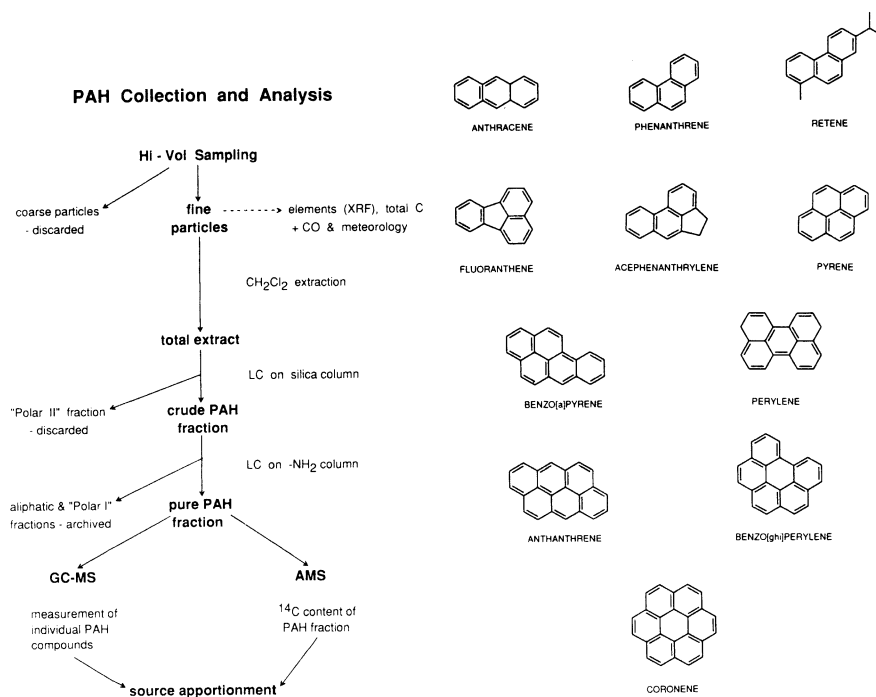


Fig 3A. Flow chart for the overall sampling and analysis process for PAH in fine (<2.5 μm) particles. (X-ray fluorescence analysis [XRF] of the samples is not yet complete.)

Fig 3B. Structures of selected PAH formed as combustion products. The principal molecular markers found for wood burning (soft woods) are abietic acid and its dehydrogenation products, culminating in retene (1-methyl, 7-isopropyl phenanthrene). This compound has the basic ring structure of phenanthrene, but it retains characteristic alkyl side chains. Benzo(ghi)perylene appears to be a useful discriminator for motor vehicle exhaust.

recovery and acceptable chemical blanks (Sheffield, 1988), for the atmospheric concentrations of the individual PAH's were typically only 4–10ng/m³. Resulting AMS samples for the class (Σ PAH) contained 37–470 μ g carbon (median, 130 μ g). Results of the ¹⁴C measurements confirmed that both motor vehicles and wood burning were major sources of the urban atmospheric PAH, during the day. At the residential sampling site, wood-burning contributed 63% of the PAH carbon; at the non-residential urban site, motor vehicle emissions contributed 53%. At night, wood burning PAH's predominated at both sites (79%).

The second phase of this research was to use ¹⁴C to discover and calibrate characteristic *molecular patterns* within the class of PAH molecules. We have special interest in this class of compounds, not only because of their serious health effects, but because they comprise unique tracers for carbonaceous combustion. Success in the pattern calibration effort would have great importance, because it would mean that sources of PAH's could be investigated on a global scale at concentrations far below those accessible to ¹⁴C measurement. Thus, the strategy was to calibrate PAH molecular patterns, for use at the nanogram level, by parallel measurements of ¹⁴C in the class Σ PAH at the microgram level. Our objective was to find individual PAH molecules or groups of molecules that could function as quantitative discriminators for this two-source environment. That was essentially achieved. Figures 3C, D show the results of two regressions, using normalized ¹⁴C data (percent of contemporary carbon in the PAH fraction) and normalized PAH concentrations for potential PAH tracers for motor vehicle PAH and wood burning PAH. Note that the abscissa f_c , which represents the percent of "living" carbon, is proportional to the percent of carbon derived from wood burning. Adjustments for firewood age and variations in atmospheric ¹⁴C indicate that wood burning carbon in this study would be equivalent to roughly 100–110% "contemporary" or 120–130% "modern." ("Modern" carbon is defined by reference to the international ¹⁴C dating standard [Kra, 1986]. See Glossary, below, for information on its relation to the derived quantities, contemporary carbon and wood burning carbon, which are important for atmospheric pollution studies.) Considering the chemistry of the formation processes (Blumer, 1976), these results are most reasonable. The wood burning PAH tracers, retene and its precursors, derive from thermally induced dehydrogenation reactions of the softwood constituent abietic acid (Ramdahl, 1983; Simoneit & Mazurek, 1982). In the Albuquerque air shed, in fact, softwood comprised some 86% of that used in fireplaces. The proposed motor vehicle PAH tracer benzo(ghi)perylene (Sawicki, 1962), is already suggestive of the graphite-like structure that we might expect from multiple acetylenic radical polymerization stages characteristic of high-temperature combustion (see Fig 3B for the structures of these molecules). Because of the complexity of the PAH production, transport and reaction processes, however, ¹⁴C measurements of the isolated Σ PAH class were essential to establish such relationships.

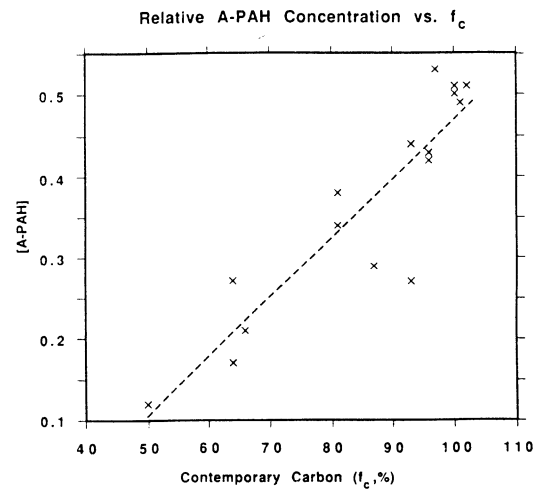


Fig 3C. Correlation observed between normalized abietane PAHs [A-PAH] and normalized ^{14}C [f_c]. Here, "A-PAH" includes retene and its principal precursors, the methyl esters of abietic acid and of dehydroabietic acid, normalized to the sum of all identified PAHs [ΣPAH] in each sample. The abscissa f_c indicates the percent of contemporary carbon in the ΣPAH fraction. f_c is proportional to the percent of wood-burning carbon; it would be zero for fossil carbon, 100% for "living" carbon (in isotopic equilibrium with atmospheric CO_2), and ca 100–110% for wood-burning carbon in this study. (See Glossary.)

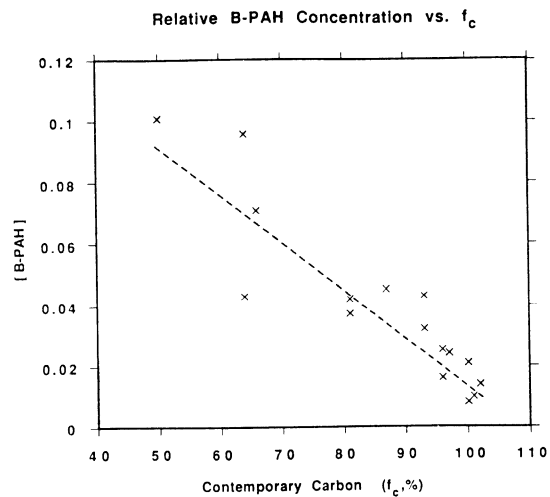


Fig 3D. Correlation observed between normalized benzo(ghi)perylene [B-PAH] and normalized ^{14}C . Normalization and symbols follow Fig 3C.

¹⁴C Calibration of Individual Particle Structures

The origins of individual carbonaceous particles in the atmosphere are of considerable interest, on the local scale, for identifying pollution sources that threaten health and visibility, and on the global scale, because of potential effects on climate (Rosen, Hansen & Novakov, 1982). Despite the remarkable advances in AMS which make possible microgram carbonaceous source apportionment, there is no chance of reaching the picogram levels needed for classifying or apportioning small groups of particles. In a second investigation of ¹⁴C – microchemical pattern calibration, analogous to the PAH study described above, we sought to link individual particle molecular-structural patterns to ¹⁴C data on “bulk,” microgram-level samples of the same particles. The means of making this comparison is laser microprobe mass spectrometry (LAMMS), where we can generate complete positive and negative ion mass spectra from individual laser “shots” or irradiances of isolated particles. Figure 4 depicts the laser microprobe ion formation process. Our initial work with this technique used soot particles collected from moderate scale, laboratory test burns by the Center for Fire Research at NIST on pure combustion source materials (Currie, Fletcher & Klouda, 1987; Fletcher & Currie, 1988). More recently, we studied mass spectral patterns from atmospheric particles collected in Albuquerque, where, as described above, ¹⁴C measurements on actual ambient samples were made to determine the wood burning – motor vehicle source contributions (Currie, Fletcher & Klouda, 1989).

Some brief insight into the results of the ¹⁴C – LAMMS individual particle pattern recognition/calibration is given in Figures 5 and 6. Figure 5 is based on particles from the test burns mentioned above, for “pure” sources including wood, heptane and polyurethane. The figure shows negative-ion cluster patterns produced from laser irradiation of individual soot particles from the respective laboratory combustion experiments. Although, in all cases, the pyrosynthetic graphitization process occurs in the flame, there are distinct differences in the resultant LAMMS patterns. The formation of cluster ions extending at least to C₁₀ is observed in all spectra, and a common, characteristic even/odd predominance is also seen. It is interesting that an analogous odd/even preference occurs in homologous series of aliphatic hydrocarbons (n-alkanes) found in atmospheric particles arising from natural biospheric emissions. Apportionment of those emissions, in comparison to fossil hydrocarbons, has been approached via a Carbon Preference Index (CPI) (Simoneit & Mazurek, 1982). As a first-order attempt to test LAMMS pattern recognition capabilities for the apportionment of actual atmospheric soot particles, we applied an analogous pattern index (Cluster Ratio Index, CRI) to the Albuquerque field data.

Figure 6 shows the results of the ¹⁴C – individual particle pattern calibration experiment for the Albuquerque samples. Using the CRI in its most elementary form (C₄ / C₂), we find an apparently linear relationship between the CRI of individual groups of particles, representing sub-nanogram amounts of carbon, and the fraction of contemporary carbon derived from wood burning, based on ¹⁴C measurements of the bulk particle samples. These results should be viewed as exploratory and suggestive; con-

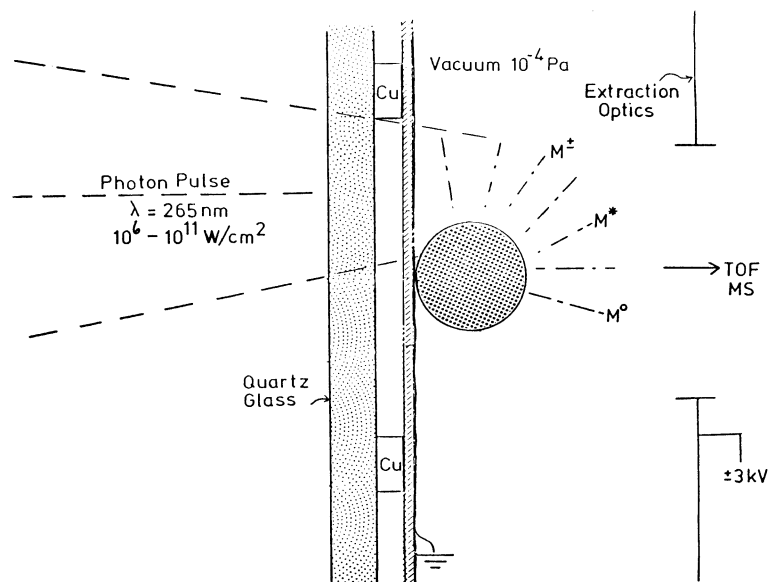


Fig 4. Laser microprobe mass spectrometry (LAMMS), single-particle ion-formation process. The sketch indicates qualitatively the laser irradiance of a single particle of micrometer size, resulting in neutral mass fragments (M^0), excited neutrals (M^*) and positive and negative ions (M^\pm).

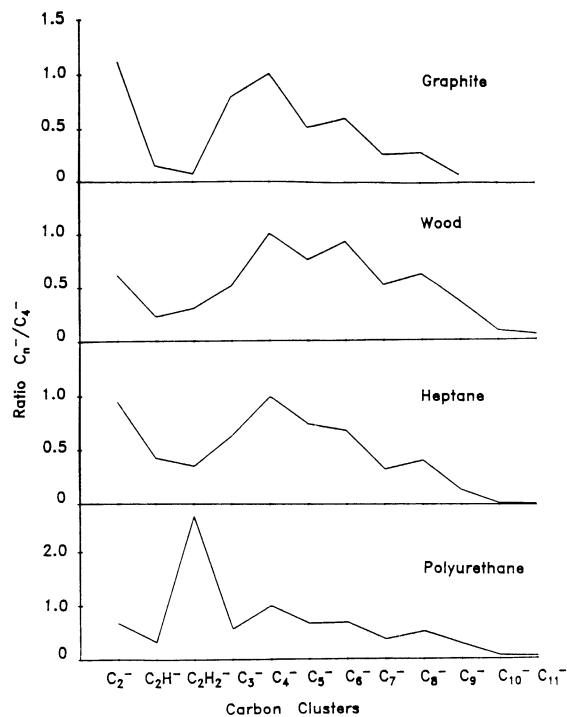


Fig 5. Negative ion LAMMS carbon cluster spectra characteristic of combustion particles ("soot") from the burning of wood, heptane and polyurethane. A negative ion cluster spectrum of pure graphite is given for comparison.

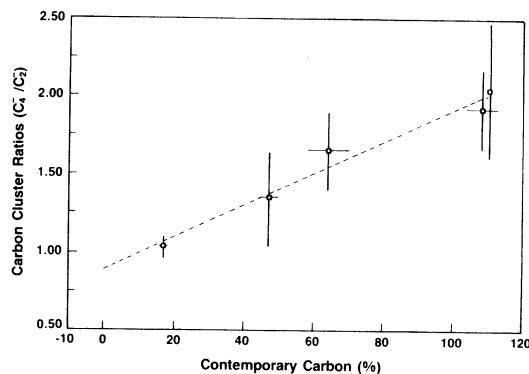


Fig 6. ^{14}C – LAMMS calibration curve for ambient combustion particles. The plot shows the cluster ratio index, C_4/C_2 (CRI), vs percent of contemporary carbon (f_c). CRI uncertainties correspond to the replication standard errors for groups of 9–12 particles. f_c uncertainties represent the Poisson standard errors for ^{14}C measurements made on the bulk samples of fine particles. The sample at the extreme right (largest f_c) comes from the laboratory test burn of wood; the other four come from fine-particle filter samples collected from the Albuquerque atmosphere.

firmatory experiments will involve additional variables (masses), larger numbers of particles, and sensitivity analysis of instrumental parameters. As with the ^{14}C calibration of individual PAH molecular patterns, this research linking ^{14}C and LAMMS structural patterns offers the potential of extending micro- ^{14}C AMS “dating” to far smaller quantities of atmospheric carbonaceous particles, with the possibility of better understanding the particulate carbon cycle in the global atmosphere.

CONCLUSION

The measurement of ^{14}C in 10–100 μg quantities of pure molecular fractions of archaeological or environmental samples ensures new levels of reliability for inferred ages or origins for rare or sparse carbonaceous material. Important manifestations of this new link between microchemistry and ^{14}C AMS are found in the direct dating of individual amino acids from collagenous bone, and in the direct apportionment of the PAH class of combustion products in an urban atmosphere. A major future development in this union of techniques will come with advances in the direct injection of CO_2 into the tandem accelerator ion source (Bronk & Hedges, 1987). As seen in Figure 3A, the pure chemical fraction isolation proceeds by a continuous flow, chromatographic process, so oxidation and injection of the resultant CO_2 into an AMS ion source would be an ideal second step. Such combinations of chromatography and mass spectrometry have become major, established methods in analytical chemistry, eg, gas chromatography/mass spectrometry (GC/MS) (Sievers *et al*, 1980). Among the most

important *chemical* advances in ^{14}C “dating” will be the development of GC/AMS, LC/AMS, SCF/AMS, and even GC/MS/AMS.

^{14}C calibration of molecular and structural patterns for very low concentration classes of organic compounds and individual carbonaceous particles will become extremely important as we attempt to trace natural and anthropogenic sources of these species in the present and past environment. Using only a small fraction of the information contained in such patterns, we have shown the feasibility of discriminating between sources of atmospheric PAH at the sub-microgram level, and sources of atmospheric carbonaceous particles at the sub-nanogram level.

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GLOSSARY

By international convention, measured activities (and isotope ratios) of natural ^{14}C are normalized to 0.95 times the ^{14}C specific activity of the original NBS Oxalic Acid Standard Reference Material (SRM 4990B), adjusted to $\delta^{13}\text{C} = -19\text{‰}$. This is defined as “modern carbon.” (See Kra, 1986, and references therein.) Further definitions for derived quantities, such as ^{14}C age, are important for specific areas of application. Those employed in this article follow.

modern carbon – 0.95 times ^{14}C specific activity of SRM 4990B, normalized to $\delta^{13}\text{C} = -19\text{‰}$ (PDB). Sample carbon is normalized to $\delta^{13}\text{C} = -25\text{‰}$ (PDB); the per mil difference of the normalized sample / modern-carbon ratio from unity is denoted $\Delta^{14}\text{C}$.

age – for applications to archaeology, geophysics, etc, the (conventional) “ ^{14}C age” is defined using a half-life of 5568 years (Libby half-life), units of years BP (before present), and a reference date of AD 1950 (0 BP). This is the basis for the “ages” reported in Figures 1 and 2. For discussion of dendrochronologically “calibrated ages” (cal AD/BC/BP), and historical ages (AD/BC), see Mook (1986).

environmental levels – as with dating applications, environmental ^{14}C measurements are first normalized to the international standard for modern carbon, and we employ the symbol “ f_M ” to denote the ^{14}C activity relative to this standard. That is, if the activity exceeds that of modern carbon by 10%, $f_M = 1.10$ (or 110% on a percentage basis.) This is consistent with the statement in Kra (1986) that “results which reflect the post AD 1955 rise in atmospheric ^{14}C are reported as ratios of the modern standard value.”

contemporary carbon – a direct indication of the relative contributions of fossil carbon and “living” biospheric carbon can be expressed as the fraction (or percentage) of contemporary carbon, symbol f_C . This is derived from f_M through the use of the observed input function for atmospheric ^{14}C over recent decades, representing the combined effects of fossil dilution of atmospheric ^{14}C (minor) and nuclear testing enhancement (major). The relation between f_C and f_M is necessarily a function of time. By 1985, when the particulate sampling discussed in this article took place, the ratio had decreased to ca 1.20 (Levin *et al*, 1985), *ie*, in 1985 a newly born biospheric source would have $f_C = 1.00$ (by definition) and $f_M = 1.20$.

wood burning carbon – because of the considerable environmental concern with carbonaceous particle pollution resulting from wood burning, a number of atmospheric scientists are in need of a measure for the fraction of carbon from wood burning in the common two-source (wood fuel – fossil fuel) environment. Unfortunately, this measure is more uncertain than f_C , because it depends not only upon the time of sampling, but it requires assumptions for the average wood age and the tree-ring model (equal ring width, equal ring area, ...) employed for integrating over the atmospheric ^{14}C input function (Currie, Klouda & Gerlach, 1981). For the experiments discussed here (Figs 3C, D and 6), conversion to the fraction of residential wood combustion carbon (f_{RWC}) entails an added uncertainty of ca $\pm 4\%$.

Comment: results expressed in terms of f_C or f_{RWC} are very useful for assessing the significance of local environmental studies. Studies carried out at different times may thus be compared directly for environmental impact without possible confusion from the changing level of ^{14}C in biospheric carbon. These measures are not appropriate, however, for global studies (in time or space), because rather more complicated modeling is necessary to take into account such factors as ^{14}C variations over the scale of the study, transport pathways and reservoir time constants. In all cases, we recommend that formal records (Reports of Analysis) contain results expressed in terms of f_M , fraction of modern carbon.

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