Chemical Pretreatment Methods

DISCLAIMER: The pretreatments described below are not universally applied in all radiocarbon laboratories, there may be slight variations in some of the procedures between different laboratories and radiocarbon specialists. For detailed information regarding the pretreatment procedures employed by a specific laboratory it is necessary to contact the laboratory in question. Data given below is merely intended as a guide only.

Pretreatment of organic matter: Charcoal, peat, lake muds.

Many samples from terrestrial environments, such as wood, charcoal and peat, will often contain small amounts of absorbed carbonates from percolating groundwater. This material is non-contemporaneous and must be removed. Usually, dilute HCl (10% conc.) is used in this treatment. It is added to the sample in a beaker which is placed on a hot plate and heated until slowly boiling. After approximately one hour it is removed and placed into a buchner funnel. The buchner apparatus uses the pressure of flowing water to create a vacuum. By increasing the rate of flow, one can increase the effect of the vacuum. A glass filter is placed at the bottom of the funnel and dampened with distilled water (dist. H_2O). Afterwards, the sample is placed on top of the filter paper and a number of litres of distilled water poured in and drawn through the sample by the vacuum effect. The aim is to reduce the pH levels of the sample to a neutral level by continual rinsing. During this treatment, regular litmus readings are taken to determine the extent of the acidity remaining. Once the pH level is reduced, two sample fractions are left; an acid insoluble fraction and an acid soluble fraction. The soluble fraction should contain the carbonate contaminants and as such is seldom used for dating purposes, except when there is a need to know the age and nature of the contamination. The acid insoluble fraction should contain the original, pristine sample, minus the carbonate contaminants, if the acid wash has had its desired effect. It is placed in a petrie dish or beaker and dried in an oven prior to combustion or further pretreatment.

HCl acid washing is usually applied to samples destined for combustion. Because HCl reacts with carbonate to produce CO_2, its use in pretreatment work is restricted to non-carbonate samples.

Sodium hydroxide (NaOH) treatment is usually associated with the removal of humic acid contamination from soils, wood, charcoal and peat samples. Humic acids are the mobile decay products of biological materials deposited in the vicinity of the sample matrix. They are easily incorporated by sample materials, affecting the ages of each. There are two major decay contaminants; humic acids and fulvic acids. The humic fraction is acid insoluble and is removed using a base extraction method. The fulvic fraction however, is soluble in acid and may be removed using an HCl wash.

The most common method of treating samples thought to be contaminated with these substances is the acid-base-acid method (ABA), sometimes called the acid-alkali-acid (AAA) method. After being physically pretreated and reduced in size, the sample is washed in hot diluted (10%) HCl in a beaker for approximately one hour, or until the reaction appears to have ceased. It is then rinsed in a buchner funnel with distilled water to reduce the pH levels towards neutral. Following this, the sample is immersed in a 5% diluted, boiling NaOH solution for approximately one hour, after which it is rinsed or centrifuged again. The NaOH treatment produces two fractions, base soluble and insoluble. The former may be kept for dating purposes by being acidified, rinsed and dried in an oven. The latter too, must be acidified because the NaOH pretreatment sometimes involves an exchange between the NaOH and atmospheric CO_2. The NaOH absorbs CO_2 from the surrounding air. The final acid wash ensures that any such contamination is removed. The insoluble fraction usually contains the sample minus the contaminant and is the dateable component.

Sometimes, samples undergo a solvent extraction prior to ABA pretreatment to remove contaminants such as resins and waxes. A soxhlet extractor is the most common apparatus used in the extraction method. This apparatus continually recycles the solvents being used so that they do not have to be replaced. Solvents are heated in a round-bottomed flask and evaporate up through a siphon into a condenser. Upon condensing they drip down through the pyrex wool covering the sample and then through the sample itself, leaching the contaminants through the profile and dissolving them. As the pressure increases in the sample tube, the solvents are gradually deposited back into the original flask, whereupon the process is repeated. The contaminants are collected in the flask and either dated, stored for analysis, or discarded. The samples most often given solvent pretreatments such as this are wood, soil organics, peat and charcoal, from specific environments.

A variety of solvents may be used in the extraction process, depending on the type of material and the contaminants present.

The most common involves using three solvents, beginning with a chloroform/ethanol mixture ($CHCl_3$ and ETOH) at a ratio of 2:1. This is run through the sample until the solvents appear muddy and dirty. The solvent soluble materials are removed and dried in an oven. A second extraction is carried out using alcohol (C_2H_5OH) which continues until the siphon is clear. The procedure is repeated with water and the sample is removed and dried in an oven before further pretreatment or combustion. Other solvent extractions using ethyl acetate, acetone and benzene/ethanol will be dicussed later.

Bone

CONTRIBUTED BY: FIONA PETCHEY

(For more detail see Taylor 1982, 1992; Stafford et al 1987:25; 1988:2257; Brown et al. 1988:171; Gurfinkel 1987:46; van Klinken and Mook 1990:155).

The first radiocarbon measurements on bone were on naturally burned bone (Arnold and Libby 1951; De Vries and Barendesen 1954). Soon after Libby (1952:44) stressed concern over the low organic carbon content, porous structure and possible effects of putrefaction and chemical alteration on the bone. Only two samples of whole bone had been measured at this time, and both gave young dates. More recently the context of one of the samples (C-558 initially thought to be from a Folsom level at Lubbock Lake, Texas) has been placed in doubt (Taylor 1992:376). Consequently, while there had been little work in this area , bone did not appear in Libby's 1952 listing of suitable sample materials, though burned bone was ranked alongside charcoal at the top.

However, the obvious importance of bone to the chronology of many sites saw a continued interest in bone as a dating medium. The major problem was traced to the use of whole bone to generate CO_2 for 14C measurements, whereby contamination from both carbonates and organics could enter the date. Initial efforts to remove the indigenous organics from the bone included techniques such as the artificial pyrolysis of bone by May (1955) whose process was designed to minimise loss of residual organics, acid digestion and dialysis (Munnich 1957), and the gelatinization of "collagen" (Sinex and Faris 1959). Despite these attempts problematic dates still persisted. In a review of the literature up to 1960, Olson (1963:61-65, in Taylor 1992:377) noted that bone dates were most often rejected. Proof that humates were the predominant contaminant in decalcified bone was finally given by De Vries (in Vogel and Waterbolk 1963). A variety of techniques were developed to remove this matter: Initially the pretreatment procedure used on charcoal was adopted whereby decalcified bone is extracted with 0.1 to 0.5 M NaOH (Berger and Libby 1966; Haynes 1967); conversion of the sample to gelatin by Longin (1971); and later Protsch (1975) combined the HCl, NaOH and gelatinization steps. This is the general "collagen" extraction procedure used today in carbon dating and dietary analysis (i.e. DeNiro and Epstein 1981).

By the mid-70's a number of reviews and evaluations of bone dating were being undertaken (e.g. Olsson et al. 1974; El-Daoushy et al. 1978). One group at the Uppsala laboratory proposed the use of different fractions (acid soluble and acid insoluble) for the majority of bones on the basis that it would be improbable for contaminants to cause the same error in different fractions. Unfortunately the yields from the different fractions were often insufficient for conventional dating techniques (Taylor 1992:381-3).

Initial descriptions of experiments demonstrating the feasibility of accelerator or cyclotron-radiocarbon-based isotopic measurements appeared in 1977 (Muller 1977). The advent of AMS enabled dating of small amounts of material, of material with very low organic carbon content, and multiple 14C determinations of different organic fractions (Taylor 1982:46, 1992:37; Gillespie et al. 1984:165). While this was a clear advantage in the dating of bone, the use of smaller samples required a clearer separation of the organic and inorganic portions. Therefore, more emphasis had to be placed on the purity of the sample (Protsch 1991:284).

Cation exchange chromatography had initially been introduced for the dating of problem samples (Ho *et al.* 1969). The large sample sizes and excellent preservation of collagen in the bones at the La Brea tar pits, California, made these C14 measurements possible, but proved to be too expensive and impractical for the large samples required with conventional dating methods. The advent of AMS changed this. Initial chromatographic techniques involved the hydrolysis of the extracted "collagen", but the incomplete removal of humic acids by gelatinization, alkali and acid treatments often resulted in cross linkages with residual impurities when hydrolysed. Attempts to remove humates prior to hydrolysis using XAD resins (Stafford et al. 1988; Law and Hedges 1989; Law et al. 1991) and decolourising charcoal (Gillespie et al. 1984) still failed to remove exogenous amino acids associated with soil contaminants (Hassan and Hare 1978). More recently to aid in the understanding of the series of reactions that can take place during diagenesis and pretreatment, van Klinken (1994) has used sample yields during enzymatic cleavage to screen the degree of cross linking.

To counteract possible contamination products, techniques based on the molecular weight and size of the collagen molecule have been used. Brown, Nelson, Vogel and Southon (1988) modified the Longin method of "collagen" extraction by adding an ultrafiltration step (gel electrophoresis) designed to exclude low molecular weight species (see also Gillespie 1989). Another approach developed to purify collagen for stable isotope analysis involves the use of collagenase, which preferentially isolates tripeptides of known length from the surviving collagen fragments (DeNiro and Weiner 1988a, b; van Klinken and Hedges 1992; van Klinken 1994).

Other attempts have concentrated on identifying relatively uncontaminated parts of bone. The isolation of "aggregates" which

were identified as having potentially a better protected environment for collagen survival was undertaken by DeNiro and Weiner (1988b), but do not give reliable results from bone with a low collagen content (Hedges and van Klinken 1992:285-6). Recently several researchers have noted the use of non-collagenous components for dating seriously degraded bone. Long, Wilson, Ernst, Gore and Hare (1989:238) have suggested that phosphoproteins may be protected from degradation as they bond to the apatite structure. Gillespie (1989:240) noted the existence of osteocalcin, osteonectin and other phosphoproteins, proteoglycans, and glycoproteins as well as blood proteins, which may display differential survival characteristics to collagen.

The first suite of 14C measurements of a non-collagenous protein were undertaken on osteocalcin by Ajie, Kaplan, Slota and Taylor (1990). Osteocalcin makes up 1% of total bone protein and appears to bind tightly to hydroxyapatite, suggesting a good possibility of being protected from contamination. Further, it has not been detected in many species of bacteria, plants or invertebrates (Hauschka 1980 in Taylor 1992:389). However, osteocalcin values on two skeletons from the Haverty site (Los Angeles) gave disproportionably old values, which if correct would signify the oldest human remains in the western hemisphere (Taylor 1992:396). It may be that for osteocalcin to be a suitable medium, isolation of essential amino acids may need to be performed (Sobel and Berger 1994).

Apatite Fraction.

Early C14 studies using the inorganic or carbonate fraction of bone were in most cases clearly false, usually too young (Berger et al. 1964; Tamers and Pearson 1965). Haynes investigated the reliability of using the bone apatite fraction and concluded that erroneous apatite dates can result from carbon exchange in the apatite structure during recrystallization, and/or surface exchange reactions (Haynes 1968:688). Studies into separation of the in situ primary apatite fraction from diagenetic carbonates were initiated in the 1960's and 1970's (e.g. Haynes 1968; Hassan 1976; Hassan et al. 1977). Hass and Banewics (1980) reported more encouraging results, and the demonstration that careful etching with acetic acid can enable the residual carbonate to maintain a biogenetic d13C signal (Lee-Thorp et al. 1989) suggests possibilities. But no-one has so far demonstrated that the indigenous carbonate can be extracted reliably and separated from diagenetic carbonate (see Stafford et al. 1991; Hedges and van Klinken 1992:285; Taylor 1982:458; Gillespie et al. 1984:165).

Teeth

Good results have generally been obtained from teeth (e.g. van Klinken and Mook 1990:158), though CO2 exchange with the atmosphere may be more efficient in teeth than initially thought (John Head pers. comms. 27/6/95). Recent studies on CO2 from teeth do, however, indicate that secondary carbonates may be identified from stable isotope values, suggesting that reliable 14C determinations may possibly be obtained on tooth enamel (Hedges et al. 1994).

Analytical chemistry of Bone

As it became obvious that the state of preservation of collagen is vital for 14C accuracy, researchers began to examine biochemical indices that might be useful in characterising collagen (Taylor 1992:380). Those "finger-prints" which have been adopted to assess the degradation of bones include measurement of the nitrogen content of bone, stable carbon and nitrogen isotopes and the nitrogen/carbon ratio, a collagen like amino acid pattern, the presence and relative concentration of hydroxyproline, infra red spectra and tests for metal ions derived from humic contaminants.

Total collagen content

"Collagen" can be estimated by percentage nitrogen in the whole sample, or by measuring the nitrogen content in the decalcified extract. Fresh, dry, defatted, compact bone from large mammals contains on average between 4 and 5% organic nitrogen by weight, though variations do occur depending on maturity and size of mammal (Garlick 1969:503, 509). However, such measurements do not indicate if the nitrogen is wholly present as collagen, nor the extent of non-nitrogenous organic material (Hedges and van Klinken 1992:282).

Stable C and N isotopes

A basic assumption in the stable or radiometric isotope analysis of bone is that collagen is thought to retain 13C/12C and 15N/14N values postmortem even though collagen is known to degrade with time after death. However, as each amino acid has a unique isotopic value, diagenesis of collagen will theoretically alter the isotope value of the resulting organic fraction (Hare and Estep 1983; Tuross et al. 1988), while humates also have an effect on the isotopic composition of bone depending upon their concentration, 13C , 14C and 15N compositions (Stafford et al. 1988:2257). In some cases, the traditional pretreatments (i.e. HCl, EDTA, NaOH and gelatinization) may further change the observed isotopic values (Tuross et al. 1988:929, 934), though ion exchange chromatography does not seem to cause any major variations (Stafford et al. 1988).

C/N ratio

Carbon/nitrogen values can be taken either on the whole bone or extract of. Carbon/nitrogen values of 2.9-3.6 from gelatinous extracts of bone are though to be indicative of collagen with diagenetically unaltered carbon and nitrogen values, while high values (ie >>4) indicate extensive diagenesis, or a high proportion of exogenous carbon possibly from sample preparation, non-collagenous proteins or contaminants (Stafford et al. 1988:2266; Tuross et al. 1988:931; Hedges and van Klinken 1992:282-3).

Amino acids

Several studies have investigated the possibility of using amino acid composition and/or racemisation values as a means of characterising indigenous organics in bone samples (Hassan and Hare 1978:115-116). Some workers suggest that the absence of the collagen amino acid signature indicates the presence of contamination (Wyckoff 1972). Others (e.g. Hare 1980) have suggested that in some cases where the organic content is extremely low (below 0.4 to 0.1% N), the amino acid pattern may reflect the indigenous non-collagenous protein residue rather than contamination. A number of factors may also alter the collagenous amino acid "finger-print": The different pretreatments effect the total amino-acid composition of the bone, while differential loss of amino acids and peptides may occur during diagenesis due to differences in solubility, effect of temperature and susceptibility to oxidation or deamination, to name a few (Hedges and van Klinken 1992:283, 285). Attempts to identify a non-collagenous composition has seen the use of the Gly/Asp ratio (DeNiro and Weiner 1988a; Long et al. 1989; Law and Hedges 1989; Weiner and Bar-Yosef 1990; Hedges and van Klinken 1992:282-3). Glycine is abundant in collagen, whereas aspartate is abundant both in bone non-collagenous proteins and in most (including bacterial) protein, and therefore discrepancies in the relative amounts of each are a sensitive test for contaminants.

Infra red spectroscopy

Qualitative IR spectroscopy has been used to estimate the purity of the protein under analysis (DeNiro and Weiner 1988a, b; Law et al. 1991), as well as to assess the degree of recrystallization of hydroxyapatite (Weiner and Bar-Yosef 1990). However, with archaeological materials complex spectra may be obtained due to diagenesis and contamination (Law et al. 1991:308, 311), so at present this technique cannot identify impurities less than the >5-10% level (Hedges and van Klinken 1992:283).

Ion beam analysis

Analysis of light elements (F, N, P and Na) and trace metals using X-ray specta has been done by Redvers-Newton and Coote (1994) in order to identify the presence of metal complexes which form in the presence of humic materials. Again the complex spectra may be obtained and due to diagenesis and exogenous organic matter.

All these analytical techniques for collagen assessment have met with only limited success, depending on the preservation state of the bone itself. In an attempt to achieve a better chemical characterisation of the fraction selected for dating Stafford, Brendel and Duhamel (1988) used a number of these criteria to classify bone preservation (see Table 1). Unfortunately, there is currently no consensus as to biogeochemical methods which can be routinely used in bones exhibiting very low or trace amounts of collagen (i.e. lost >95% of their protein)(Taylor 1992:386-7). As a consequence Hedges and van Klinken (1992:282) suggest an age limit of 18ka as older dates are more sensitive to modern contamination.

	Class 1: [Modern]	Class II: [very well to well preserved]	Class III: [Moderately well preserved]	Class IV: [Poorly preserved]	Class V: [Extremely poorly preserved]
Whole bone % Nitrogen	4.5-3.5	3.5-0.6	0.9-0.4	0.5-0.1	0.1-<0.01
Characteristic amino acids: Residues per 1000 nominal values	Hydroxyproline Aspartic acid Glutamic acid Proline Glycine Alanine Arginine	90 50 70 120 330 105-110 55	90 50 70 120 330 120 45	90 50 70 120 300-330 120 45	30-80 50-100 70-130 100-180 260-300 100-120 40-45
Physical characteristics of whole bone	High compressive and tensile strength; spiral and conchoidal fracturing; dense mineral matrix.	Bone becomes white and chalky w. loss of concoidal fracturing: exteria hard and waxy. Less N than fracturing becomes uneven, perpendicular to the bone axis. >80wt% of protein.	Interior and exteria chalky, surface hardness decreases and porosity increases with decreasing %N. Uneven hackly fractures.	Continued decrease in hardness and increase in porosity.	Soft. Hard if inorganic replacement has occurred.
Gelatin	>or=90wt% of collagen	>80wt%of protein	>50wt% protein	2-50wt% protein	<10-20% of protein

(After Stafford et al. 1988:2258).

Wood

In instances where there is contamination by secondary carbonate deposition or humic acids the pretreatments described above for charcoal are applied as equally to wood. Water is used to collect the remains of starch from wood and bone samples. Starch consists of two fractions, μ-amylose and amylopectin, the former is soluble in water, the latter not. When aqueous, the μ-amylose forms a precipitate and changes into the insoluble form which sits in a layer above the water solution where it is collected for dating. The process usually begins with the ground or milled sample being placed in a beaker of distilled water and

either left standing for 3-5 days or slowly boiled for several hours and left overnight. The sample and water are then filtered into a buchner flask and the process repeated; fresh water is added and the solution heated and left to stand. A test for starch presence is conducted using iodine and 10% potassium iodine solution. The solution will turn blue if starch is present.

Holocellulose

The holocellulose fraction is targetted for dating when the laboratory requires the autochthonous sample carbon or in cases where the wood for dating is old or severely contaminated. It is the carbohydrate fraction, the structural element of the wood and is considered most reliable for dating. The use of cellulose is widely used in dendrochronological studies (Taylor, 1988:47). Pearson (1983), for example, used a holocellulose pretreatment in his important work on the Irish bog oak chronology where it was crucial that all contaminants were removed and only pristine sample dated (Pearson, 1983:21). There is a variety of different methods for cellulose extraction beginning with the removal of resins and waxes. The key apparatus in these techniques is again the soxhlet extractor.

Wood sample is usually chopped and milled prior to pretreatment. Organic solvents are used initially to remove resins and waxes from the wood. As mentioned above, ethanol, ethyl acetate, benzene and acetone may be used in the solvent extractions. The samples should also be thoroughly washed in water to remove any carbon absorbed from the solvents used.

After the waxes and resins have been removed by solvent extraction, the milled wood is placed into a buchner flask containing 800 mls of distilled water and 3 mls of concentrated HCl (or 30 mls 10% conc.). Into this is added an amount of $NaClO_2$ (sodium chlorite). The actual amount varies with the size and density of the sample in question. About 7.5 grams of sodium chlorite is used for every 25 grams of sample but this varies between labs. The sample, distilled water, HCl and $NaClO_2$ are placed on an 70-80° C heat source (warming flask), with a cover glass for approximately four hours. The sample is then rinsed in a buchner flask using distilled water. White cellulose should remain. This is placed in a beaker to which is added 5%w/v concentrated NaOH. This is heated. The base treatment will remove any further contaminants from the wood, but also absorbs atmospheric carbon, therefore an acid wash using 10% HCl is always implemented afterwards. Often, the laboratory will wash in the base in a Nitrogen (N_2) environment thus minimising atmospheric exchange. Finally, the sample is rinsed with distilled water until the pH level is neutral (pH=7). The sample cellulose is then removed, placed in a petrie dish and stored in an oven to dry before combustion and dating.

Lignin is a wood substance that makes up 25-35% of softwood species and 17-25% of hardwood species. According to Head (1982:221), lignin is made up of polymer chains formed into a 3-dimensional network. It is resistant to certain of the chemical reactions causing degradation in wood samples in the natural environment. It is not hydrolysed by acids, for example, whereas cellulose is. Many lignin substances are soluble in alkalis, however, they absorb metal ions in solution and may be vulnerable to degredation from bacterial action. Head (1982) has shown that the extent and characteristics of degradation can be analysed using x-ray diffraction, to examine the pattern and structure of wood, although these techniques require some refinement before they become generally used. These analyses have shown that it is possible to reconstruct the post-depositional environment of certain wood fractions and be able to recommend applicable pretreatments, once that information is known.

Lignin can be extracted from wood cellulose using a strong acid such as sulphuric acid.

Shell

The most common fraction of shellfish remains which have been dated is the inorganic calcium carbonate. Certain samples may show evidence of isotopic exchange, or recrystallisation. Recrystallisation involves the dissolution of primary aragonite, which forms the major crystalline carbonate fraction in most shells, and the subsequent reprecipitation or recrystallisation of the carbonate in a calcite structure. Often this process will alter the isotopic ratio of the carbonate and affect the 'true' age because bicarbonates of different age may be present in the post-depositional matrix. The exchange usually occurs on the exterior shell surfaces in terrestrial environments and is common in samples found below the water table. Recrystallised shells may often show a 'chalky' or powdery exterior (Taylor, 1987; Aitken, 1990). In cases where the suspected recrystallised shell is an aragonitic secreter, x-ray diffraction analysis will reveal the presence or extent of calcite and the sample may rejected or accepted in the light of the result. Where the shell is composed mineralogically of calcite, visual observation is the only method for considering recrystallisation. Submitters should collect hard, non-porous, thick shells with fresh surfaces and preserved textures to minimise the possiblity of post-depositional isotopic exchange. Also, it is a good idea to obtain aragonitic secreting species which inhabit the intertidal zones or a few metres below that, so the age obtained from the shellfish can be reliably compared with the marine shell reservoir corrections commonly available around the world (see Stuiver and Braziunas, 1993).

Shells are pretreated chemically using an acid wash procedure which removes the exterior shell where contamination through isotopic exchange is most likely to have occurred post-depositionally. In some laboratories, the exterior surfaces are ground using drills to remove the outer layer and obtain the fresh fraction for dating.

Summary of Pretreatments by material

INDEX | Introduction | Measurement | Applications | WWW Links | k12 | Publication | Corrections | Age calculation | Calibration | Pretreatment | References | Awards | Credits | What's new? | Email |

HTML DOCUMENT BY T.HIGHAM.