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SAMPLE CHEMISTRY FOR THE OXFORD HIGH ENERGY MASS SPECTROMETER

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ABSTRACT. Chemical pretreatment procedures for the decontamination, extraction, and isolation of organic materials for ^{14}C dating using the Oxford accelerator system are described. Specific details are given for the isolation and chromatographic purification of amino acids from bone and tooth collagen, of lipids from sediments, and of cellulose and glucose from wood, paper, and textiles. A description is also given of the apparatus used for the routine preparation of 1 to 5mg graphite samples on tantalum wire, for use in the accelerator ion source.

The high energy mass spectrometer (HEMS) approach to ^{14}C dating allows the use of very small samples in the low milligram range. Sample pretreatment and decontamination procedures can be both more vigorous and more selective than those used by conventional dating laboratories. Specific chemical compounds can be isolated from archaeologic or geologic samples; such compounds may be characteristic of particular source materials and, hence, provide more detailed information than is generally possible using bulk organic samples. The Oxford Radiocarbon Unit has concentrated on three sample types that represent the kind of material we expect to work on initially: bone, lake sediment, and wood.

AMINO ACIDS FROM BONE.

The usual practice of dating the acid insoluble residue ("collagen") fraction from bone is fraught with complications regarding reproducibility and contamination. It is possible to isolate the collagen amino acids and to purify these using ion exchange chromatography. This has been attempted (see, eg, Taylor and Slota, 1979), but not established as a routine procedure. The small samples required for HEMS dating allow high purity amino acids to be separated from bone using conveniently-sized apparatus.

The amino acid content of bone is first estimated using the standard Kjeldahl nitrogen analysis, or, more usefully, from GC to HPLC analyses that can separate enantiomers (see, eg, Hoopes, Peltzer, and Bada, 1978). Bones are cleaned by scraping or grinding off surface debris and sonication in distilled water and/or 0.1M HCl for a few minutes. After crushing to <25 mesh, the inorganic matrix is dissolved in 1M HCl, using sonication and several changes of acid to speed release of CO_2. The acid insoluble residue is treated with alkali to remove humic acids and hydrolyzed in 6M HCl for 8 to 12 hours at 105° in sealed tubes. Finally, the liberated amino acid

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mixture is desalted on Biorad AG50X8 cation exchange resin, using 2 to 3M NH_3 solution for elution.

Wand (ms) developed a method for the isolation of the specific amino acid hydroxyproline from bone hydrolysates. This involves deamination of all amino acids except proline and hydroxyproline with nitrous acid, followed by preparative high pressure liquid chromatography (HPLC) using pH2.5 phosphate buffer on Partisil 10SCX cation exchanger. Modification of this procedure by the addition of a desalting step before HPLC improves the separation of proline from hydroxyproline, and allows UV detection at 200nm to monitor the chromatography. The final amino acid products from these procedures may be assayed for purity using gas chromatography (GC) and HPLC. Such methods are also applicable to tooth, antler, and ivory collagen, and with slight modification, to shell conchiolin amino acids.

LIPIDS FROM SEDIMENTS. Lake sediments are normally dated using the total organic matter, which may not give the actual time of sedimentation because of the mixed origin of the various organic components. Insoluble compounds such as lipids may offer more attractive dating samples, because they can often be related to specific plant sources (aquatic or terrestrial) and should give a more precise date for deposition of the sediments.

A general scheme, based on work by Cranwell (1973), Brooks et al (1976) and Birks (1980), is being developed to isolate n-alkanes and n-carboxylic acids from sediments for dating. Samples are demineralized with 6N HCl/40%HF at room temperature for 2 to 3 days, filtered, and dried under vacuum at 40°. Macrofossils are removed at this stage for separate identification and dating. The dried sediment is then extracted with benzene, using sonication and several changes of solvent. This solvent extract is reduced in volume and chromatographed on a silica column, using hexane to elute hydrocarbons and more polar solvents for elution of fatty acids. Analysis of lipid fractions is carried out by GC on packed OV-1 columns, and selected chain length alkanes or acids can be isolated by preparative GC for dating.

Further fractions that may be significant for dating can be isolated from the solvent-extracted sediments. For example, humic acids can be extracted with alkali and reprecipitated for analysis; amino acids can be obtained by hydrolysis in 6M HCl and subsequent purification as for bone amino acids.

CELLULOSE AND GLUCOSE FROM WOOD. The standard method for preparation of cellulose from wood can be used for the small samples required in HEMS dating. These usually involve solvent extraction of resins, alkali, and acid extraction of other soluble components, followed by oxidation of lignin with

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$NaClO_3/HCl$. It is possible to extend this treatment to complete hydrolysis of the cellulose to glucose, which can be purified by preparative HPLC for dating. This would also allow critical analysis of the final product by GC or HPLC to assess purity. Such a scheme can also be applied to cellulose-based textiles and paper samples.

GRAPHITE TARGET PREPARATION. Initial experiments (Hedges, Wand, and White, 1980) confirm that graphite is the material of choice for ion source targets. Wand (ms) developed a procedure for preparing graphite on tantalum wire supports, which has now been modified for routine production of 1 to 5mg of graphite from 5 to 10mg of sample carbon. The synthesis apparatus is shown diagramatically in figure 1.

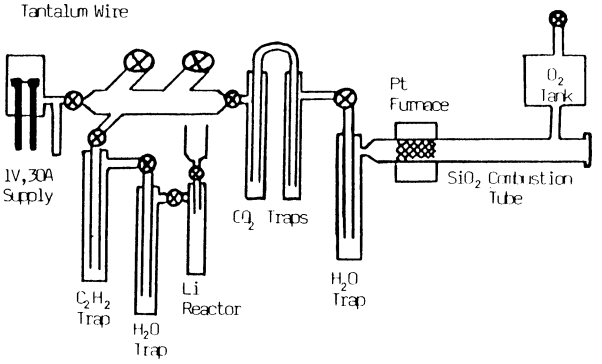

Fig 1. Graphite preparation system

Combustion takes place in a silica tube under 1 atm oxygen, with final oxidation over a supported platinum catalyst at 450°C. CO_2 is dried by passage through a dry ice/methanol trap and collected in liquid N_2 cooled traps. Lithium carbide is prepared by reacting this CO_2 with molten Li metal at 600-700°C for 5 minutes; the CO_2 may either be added to the hot Li or (for small samples) the CO_2 can be frozen into the Li reactor and heated with the lithium. After cooling the Li_2C_2, water is added to produce acetylene, which is dried by passage through a dry ice/methanol trap and collected in a liquid N_2 cooled trap. This acetylene is then expanded in a dry ice/methanol trap and frozen into the cracking vessel with liquid N_2. Routine yields are better than 90%.

The cracking reaction takes place at an acetylene pressure of 10 to 50mbar. A tantalum wire, 0.5mm diameter x 5mm long, is heated resistively between stainless steel electrodes, with a current of 25 to 30amps at 1 volt, to a temperature of ca

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2000°C, measured with an optical pyrometer. Ca 10 minutes is sufficient for this reaction, yields being ca 40 to 50% based on the acetylene.

Because of the gas to solid reaction, changing composition in the gas phase may introduce isotopic fractionation in the graphite product. Even with 100% yeild, it is likely that spatially different isotope ratios will be present. The yields obtained could not be increased by changing acetylene pressure, wire temperature, or time of reaction. Wand (ms) measured stable isotope ratios on graphite prepared by this procedure, and concluded that the bulk sample could have fractionated ^{14}C by ca 2%. Such variation can probably be corrected by the use of standard linear factors during the analysis on the HEMS system.

The graphite, as prepared on tantalum wires, is compact, robust, and easily stored. Wires may be mounted directly into a reflected beam ion source (Hedges et al, 1980), or the graphite can be removed from the wire and pressed into a hole of 1mm diameter for the inverted spherical ionizer sputter source (White, in press). Both techniques have been in use at Oxford and routinely produce adequate C^- ion beams.

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