Supercritical Fluid Extraction (SFE) for the Removal of Lipid and Interfering Compounds Prior to Radiocarbon Dating of Archaeological Artifacts

Using Teledyne ISCO Syringe Pumps

Syringe Pump Application Note
AN30

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Introduction

The radiocarbon dating of many archaeological artifacts, such as Egyptian mummies, can be inaccurate due to contamination from soil organic matter. Traditional decontamination methods require the use of harsh acid-base pre-treatment methods, which can be destructive to these delicate aged artifacts [1].

The supercritical fluid extraction (SFE) pretreatment using carbon dioxide with methanol as co-solvent has the potential to replace such methods and, when coupled with a non-destructive argon or oxygen plasma for microgram carbon removal, can serve as a method for sample preparation prior to accelerator mass spectrometry isotope ratio determinations [2]. In this study, a supercritical fluid extraction system consisting of two Teledyne ISCO 100D pumps for pumping solvent (carbon dioxide) and modifier (methanol) was used [see note], in addition to an ISCO SFX-2-10 extraction module. Experimental extraction conditions of 3000 psi and 40 °C using ultra high purity carbon dioxide were applied to fiber, macroflora, charcoal, and wood samples to reduce their lipid and soil humic content. Similar conditions were also used on burial gauzes from Egyptian mummies containing lipid constituents, and the gauzes were sent for plasma oxidation followed by radiocarbon dating.

Experimental Procedure

The current study was performed in two steps: one with unadulterated linen spiked with known organic matter to understand the effectiveness of SFE pre-treatment, and the other with the aged artifacts from Egyptian mummies and Russian textiles.

The spiking experiments were performed by initially mixing the organic matter with the linen in hexane. The linen samples were soaked for 24 hours in supersaturated solutions of the mixture, and then oven-dried before weighing. Smaller pieces of the spiked linen were then cut and placed in the oven in the SFE extraction unit (Model: 2-10).

Two Teledyne ISCO 100DX pumps, controlled by a Series D controller [see note], were employed to supply carbon dioxide and methanol (co-solvent) under pressure to contact the dried samples. A piece of metallic capillary tubing having a very small internal diameter was connected at the far end of the SFX 2-10 unit to serve as a back-pressure regulator. The capillary tubing was placed in a restrictor heater set at 60 °C to prevent precipitation of the extracted contaminants in the capillary column as the temperature fell to ambient conditions. The spiking experiments were done at 40 °C and pressures of 3000 and 6000 psi with 10% (v/v) methanol as a modifier. The spiked contaminants included beeswax, coconut oil, humic acids, and frankincense oil. A control sample consisting of linen saturated in hexane with no contaminant was also extracted using modified SC-CO_2 for comparison purposes. The effectiveness of the SFE pre-treatment was adjudged by comparing the weights of the linen samples before and after the extraction. In order to maintain the purity of the extracts, high grade CO_2 (99.995% pure) and HPLC-grade ultrapure methanol were used for the experiments. The ISCO SFX 2-10 unit was consistently cleaned with the SC-CO_2 – methanol mixture between experiments in order to prevent cross contamination.

In the second set of experiments, the archaeological artifacts were weighed and photographed, then cut into smaller samples of equal dimensions. The weights and photographic evidence before and after extractions were used to indicate the effectiveness of the SFE pre-treatment process. Cleaning experiments were done at 40°C. Each SFE cleaning used 100 mL of CO_2 + 10% (v/v) methanol with variable flow rates and residence times, and at different pressures. The samples were dried by allowing 100 mL of CO_2 to flow through the extractor after treatment at 40°C and 3000 psi. SFE pre-treated extracts were collected for analysis using a Bruker Ultraflex II MALDI-TOF mass spectrometer (MS) in the positive ion mode. Samples after pre-treatment were sent for plasma oxidation followed by radiocarbon dating. A schematic and the picture of the unit used for SFE pre-treatment of the archaeological artifacts are shown in Figure 1 on the following page.

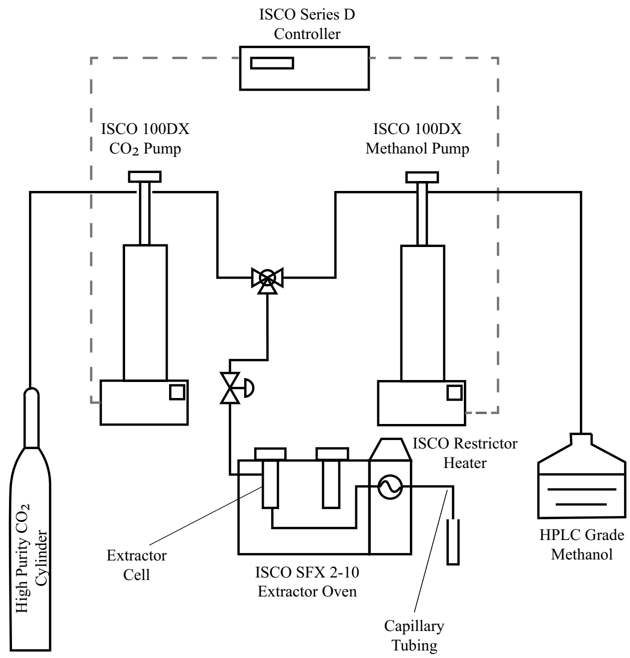

Figure 1: Photo and Schematic of the Supercritical Fluid Cleaning of Archaeological Artifacts

Results and Discussion

As indicated earlier, the spiking experiments were done to study the efficacy of modified supercritical CO_2 in removing certain contaminants from the archaeological artifacts. The spiking experiments indicated that supercritical CO_2 modified with 10% (v/v) methanol removed the beeswax, coconut oil, and humic acids from linen. There was no significant effect of pressure on the removal of coconut oil and beeswax from linen, but more humic acids were removed at a pressure of 6000 psi when compared with that at 3000 psi. This removal of humic acids from linen was not complete at the higher pressure, since there were still traces of humic acid left behind in the cell and in the linen samples after extraction. Gravimetric analysis indicated that at 40°C and 6000 psi, modified SC-CO_2 was capable of removing about 25.2% beeswax, 18.4% coconut oil, 5.80% humic acids, and 0.6% frankincense oil (by weight) from the respective spiked linen samples.

The extracts from SFE cleaning of the Egyptian mummy gauzes and the Russian textiles were characterized with respect to their organic constituents. MALDI-TOF spectra mass spectroscopy study of the Egyptian mummy burial gauzes measured between 500-5000 Da, indicating the presence of a polymer in the collected extracts that contain repeating oligomeric units differing by 74 Da, which is characteristic of the polymer, polyglycerol.

In addition, GC/MS analysis (Table 1) showed the presence of fatty acid moieties as their methyl esters in the Egyptian burial cloths. The fatty acid methyl esters (FAMES) range from C14:0 through C18:3, and arise through the use of vegetable oils and/or wax esters in the preparation of the mummy for burial [3]. The occurrence of FAME derivatives in the SC-CO_2 – methanol extract is indicative of possible in-situ reaction between the extraction solvent mixture and the lipophilic oils in the burial gauze. The presence of odd- carbon numbered acids is also indicative of a ruminant fat being used in burial preparation. One of the authors has in the past reported in-situ methylation of vegetable oils during SFE [4,5]; other investigators such as McDaniel et al [6], Wyatt and Haas [7], and others [8] cite the synthesis of FAMEs under supercritical CO_2 in the presence of methanol. It should be noted that both potash and natron were widely used by the Egyptians—which could serve as a source of alkali metal salts as possible catalysts for producing FAMEs. If our rationale is correct, then a number of materials associated with archeological artifacts could be identified based on their methylated products [9, 10].

Table 1: Database fit from mass spectrophotometric characterization of FAME content in Egyptian mummy gauzes extracted with SC-CO_2 modified with 10% (v/v) methanol at 40°C and 3000 psi. (nd = not defined.)

Compound	T_r (min)	Child Mummy	Child Mummy (2)	Bovine Mummy
C 14:0	20.3	nd	95	nd
C 15:0	22.2	95	97	nd
C 16:0	24.1	98	99	95
C 16:0 (isomer)	24.7	nd	nd	72
C 18:0	27.5	nd	96	nd
C 18:1	28.2	nd	98	nd
C 18:2	29.3	nd	98	nd
C 18:3	30.6	98	91	nd

Similar experiments performed with the Russian textile samples indicated the presence of a polymeric moiety in the MALDI-TOF spectra with repeating oligomeric units differing by 58 Da. The assignment of structure and the origin of this compound are still pending. The Russian textile samples were then treated with modified SC-CO_2 at 40°C under different pressures of 3000, 6000 and 9000 psi, and the effectiveness of SFE cleaning was obtained by gravimetric analysis of the samples. The efficiency of contaminant removal from the Russian textiles using modified SC-CO_2 at 40°C was found to increase linearly as a function of pressure (Figure 2).

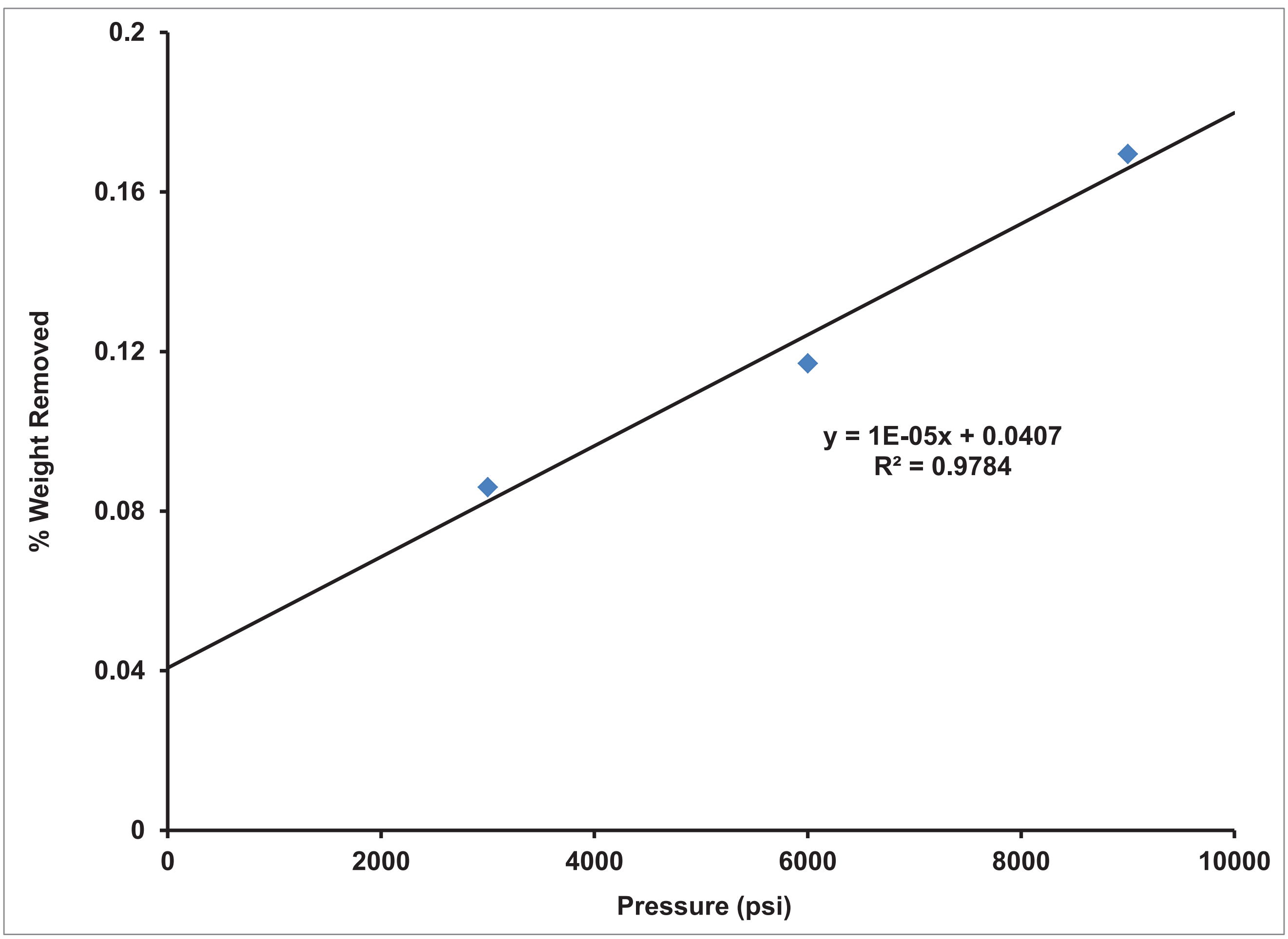

Figure 2: Weight percent of organic matter extracted from the Russian mummy sample at 3000, 6000 and 9000 psi and 40°C using SC-CO_2 modified with 10% (v/v) methanol

The aforementioned study indicates that using a mixture of supercritical carbon dioxide and methanol is effective in the removal of selected organic matter from various archaeological artifacts, thereby suggesting that supercritical carbon dioxide, modified with an alcoholic solvent such as methanol, is a viable non-destructive pretreatment prior to plasma oxidation and radiocarbon dating for an accurate estimation of the age of the artifacts. Excellent agreement has been recorded between the current pretreatment method (boxes) and those pretreated using strong acid or base (circles) applied to various archeological artifacts as shown in Figure 3.

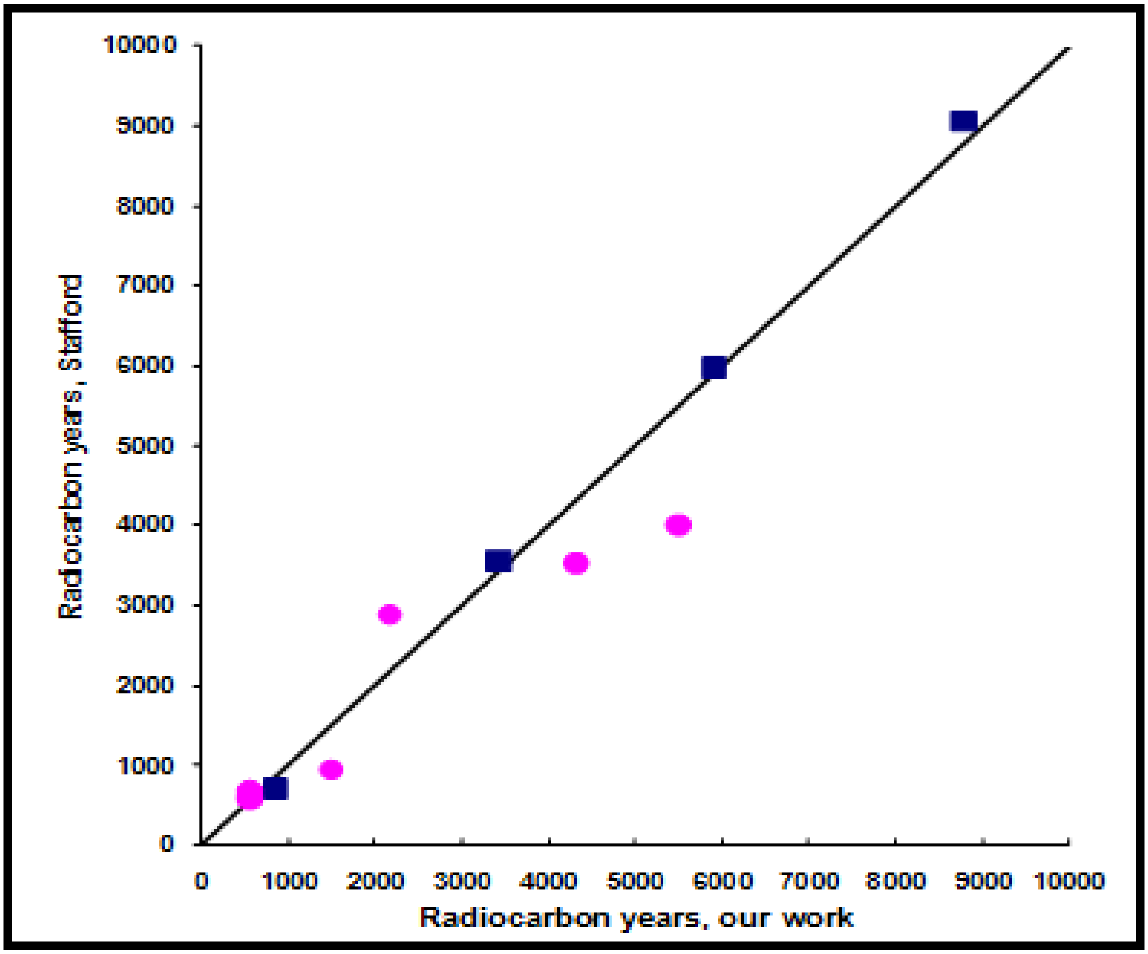

Figure 3: Radiocarbon dates by pre-treatment with strong acid or base (circles) vs. supercritical pretreatment (boxes)

References

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Note

The 100DX model pump and Series D controller, which were used during the original experiment, are discontinued. The current model 260x pump and the SyriXus controller are the recommended replacements for the older 100DX and Series D models.

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Product model names have been updated in this document to reflect current pump offerings.

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