The effects of cellulose nitrate treatment and organic solvent removal on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of collagen and bioapatite in modern mammal bone

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ABSTRACT

This study examines the effects of cellulose nitrate application and subsequent removal on stable isotope values in modern mammal bone which may be altered by addition of the consolidant in older museum archaeological and paleontological collections. Cellulose nitrate in the form of Duco cement was applied to modern whale and seal bones. Both treated bone and untreated controls were soaked in 100% acetone to remove cellulose nitrate and test effects of acetone on stable isotope values. Stable isotope values were measured in bone collagen ($\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$) and bioapatite ($\delta^{13}C_{structural\ carbonate}$, $\delta^{18}O_{structural\ carbonate}$, $\delta^{18}O_{phosphate}$). The $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$, $\delta^{13}C_{structural\ carbonate}$, and $\delta^{18}O_{phosphate}$ values were unaltered by application of cellulose nitrate or exposure to acetone. The $\delta^{18}O_{structural\ carbonate}$ values were altered by exposure to cellulose nitrate in an unpredictable manner, most likely due to exchange of hydroxyl groups with differing isotope values. Care should be taken when using $\delta^{18}O_{structural\ carbonate}$ values from cellulose nitrate-treated bones as they may not represent an original isotope signature. Fourier transform infrared (FTIR) spectroscopy detected cellulose nitrate in all samples treated with the consolidant, including traces in bones soaked in 100% acetone (48 hours) to remove it. This indicates that our procedure was not entirely adequate to fully remove cellulose nitrate. Although remnants of cellulose nitrate in treated bones apparently did not alter the isotope values, it is hereby suggested that future attempts to remove cellulose nitrate from bone include additional soaks or possibly sonication.

1. Introduction

Chemical analysis of archaeological and paleontological bones has become ubiquitous in recent years including DNA sequencing, proteomics, carbon dating, Fourier-transform infrared spectroscopy (FTIR), gas chromatography mass spectrometry (GC-MS), scanning electron microscopy (SEM), X-ray diffraction (XRD), X-ray fluorescence (XRF), inductively coupled plasma mass spectrometry (ICP-MS), stable isotope mass spectrometry, and other analytical methods. In order to apply data from these methods to questions about an animal's biology, ecology, or evolution, one requires bone unaltered by post-mortem diagenesis or museum conservation practices. This study focuses on the effects of applying and removing a particular bone consolidant, cellulose nitrate, on the stable isotope values in a modern bone. Stable isotopes are widely-used and useful indicators for human and animal diet, ecological niche, provenance, metabolism, and paleoclimate (Ambrose 1993, DeNiro and Epstein 1978, DeNiro and Epstein 1981, Fogel et al. 1997, Kelly 2000, Koch 1998, Koch et al. 1994, Makarewicz and Sealy 2015, Peterson and Fry 1987). The application of cellulose nitrate on bones may alter the stable isotopes' original values and result in faulty interpretations. This has been observed after application of other consolidants on bone (France et al. 2011, France et al. 2015) and has also been problematic for radiocarbon isotope measurements (Brock et al. 2017). This study examines the direct effects of cellulose nitrate application and removal on stable isotope values, uses the stable isotope results to interpret possible pathways of alteration that may be relevant to other chemical analyses, and recommends best practices for the safe removal of cellulose nitrate prior to analyses.

Cellulose nitrate is a polynitrate ester of cellulose with variable degrees of nitrate substitutions resulting in subtle formula variations [$C_6H_9(NO_2)O_5$, $C_6H_8(NO_2)_2O_5$, $C_6H_7(NO_2)_3O_5$]. Cellulose nitrate is often stabilized with camphor or other solvents (Horie 2010, Koob 1982, Selwitz 1988) and can be purchased under several brand names including Duco cement, Ambroid, Celluloid, Durofix, and others. Its beneficial bonding properties, ease of use, low cost, and commercial availability contributed to its common use as a consolidant and coating for ceramics, glass, and metals for many years (Horie 2010, Johnson 1994, Selwitz 1988). It was not used widely for bone consolidation (Johnson 1994), but personal communication with fossil and archaeological preparators indicates that often the easiest consolidant at hand, including cellulose nitrate, may have been used in specimens acquired decades ago. After application, cellulose nitrate consolidants tend to cleave and lose volatile plasticizers or solvents, causing them to crack, peel, and discolor over time (Horie 2010, Koob 1982, Quye 2011, Selwitz 1988, Shashoua et al. 1992). They have been replaced by other more stable resins in recent decades, but older museum collections can still be found with cellulose nitrate consolidant. The application of cellulose nitrate and its concurrent stabilizing solvents on bone may interact with the latter's molecular structure and alter the original stable isotope values or render the bone unviable for other chemical analyses as well.

Bone is comprised of organic components and inorganic mineral. The primary organic component is collagen, from which both carbon and nitrogen stable isotope values are typically measured. Bioapatite [general formula $Ca_5(PO_4)_3(OH)$] is the mineral component containing both phosphate ($-PO_4$) and structural carbonate (CO_3) ions, the latter of which substitutes in both

the $–PO_4$ and –OH sites (Elliot 2002). Carbon stable isotope values are measured in the structural carbonates, while oxygen isotope values are measured in both phosphates and structural carbonates.

Previous studies have observed varying effects on stable isotopes during the application and removal of common consolidants such as polyvinyl acetal/acetate/alcohol (PVAc, PVOH, "Alvar", "Elmer's glue", "AYAx" series), polyvinyl butyral ("Butvar"), methyl methacrylate/ethyl methacrylate ("Paraloid", "Acryloid"), acrylic emulsions ("Rhoplex"), cellulose nitrate, shellac, and collagen-derived glue ("hide glue"). Collagen carbon and nitrogen isotopes are generally unaffected by PVAc/PVOH, Butvar, Paraloid, Rhoplex, or hide glue (France et al. 2011, France et al. 2015, Moore et al. 1989, Tuross and Fogel 1992, Takahashi et al. 2002). Phosphate oxygen isotopes are unaffected by PVAc, Paraloid, Butvar, and shellac (France et al. 2011, France et al. 2015, Stephan 2000). Structural carbonate carbon isotopes are also unaffected by PVAc, Paraloid, and Butvar (France et al. 2011, France et al. 2015). However, structural carbonate oxygen isotopes do experience alteration during the application and removal of PVAc, Paraloid, and Butvar (France et al. 2011, France et al. 2015).

Only one study has examined effects of cellulose nitrate (Stephan 2000) wherein it was determined that oxygen isotopes in phosphates are unaffected by the consolidant. The effects on collagen and structural carbonates is currently unknown, and the results for phosphates require verification. Unlike many other consolidants, cellulose nitrate contains nitrogen which presents the potential for exchange with collagen. It also contains nitrate ($-NO_2$) and hydroxyl (-OH) ions which are functionally similar to the $–PO_4$, $-CO_3$, and –OH ions in bioapatite and may exchange with these ions in situ or during extraction chemistry (see Methods and Materials).

2. Materials and Methods

Sections of a modern whale rib were used for all experiments. Sections of a seal femur were subsequently used exclusively for collagen experiments when coincidentally identical nitrogen isotope values were observed in the whale rib and the cellulose nitrate (see results section), thereby rendering it impossible to observe nitrogen isotope differences between whale bone collagen treated with cellulose nitrate versus untreated bone. The high porosity and minimal outer layer of compact bone (~2mm) in both specimens represented a good proxy for the most susceptible archaeological and paleontological bones that have experienced extensive dissolution and loss of material during burial. Consolidant penetration was significant and large surface areas of bone were exposed to potential alteration.

Samples were treated with cellulose nitrate in a block design reproduced in triplicate (Table 1). Cellulose nitrate used in the experiments was the common consolidant Duco® cement (ITW Devcon). Duco cement contains cellulose nitrate (10-20% by weight), acetone (70-80%), isopropanol (1-10%), 1-methoxy-2-propanol acetate (<5%), and camphor (<5%), although exact proportions are proprietary information (MSDS). Duco cement was thinned to ~40% solution (v/v) using 100% acetone; the resulting solution is estimated to contain ~4-10% cellulose nitrate by weight. Half of the bone disks were fully submerged (~2-3 minutes) and agitated to facilitate full coverage of bone surface and internal pores; half remained untreated as controls. After

drying for 24 hours, treated whale rib disks were ~17% consolidant by weight; seal femur disks were ~3% consolidant by weight. Both treated and control disks were sectioned into wedges. Half of the treated and control wedges were submerged in two rinses of 100% acetone for 48 hours to remove cellulose nitrate if present and test effects of the solvent. The approximate time between initial application of cellulose nitrate and subsequent removal was 3-5 days. Whole intact whale rib and seal femur wedges were used for collagen analyses; whale rib and seal femur wedges powdered with mortar and pestle were used for Fourier transform infrared analyses (FTIR); powdered whale rib was also used for phosphate and structural carbonate analyses. A thin film of undiluted Duco cement was allowed to dry on a clean glass plate and analyzed for its isotope values and via FTIR.

Collagen was extracted from bone wedges using a common acid-base-acid method after Longin (1971). Approximately 200-500mg of solid bone was sonicated for 20 minutes in ultra-pure water to remove remaining salts or loose clinging particles. Samples were decalcified in 0.6M hydrochloric acid at 4°C, changing the acid daily, until reaction ceased. After rinsing to neutrality with ultra-pure water, samples were soaked for 18 hours in 0.125M sodium hydroxide. This step mimics extractions performed on paleontological and archaeological bones which may require removal of humic and fulvic acids formed during post-mortem organic diagenesis. Samples were rinsed to neutrality and the crude collagen remaining was then hydrolyzed in 0.03M HCl at 95°C for 18 hours and freeze-dried.

Structural carbonates were isolated using methods of Bryant et al. (1996). Approximately 50mg of powdered sample was soaked in 2.5% sodium hypochlorite for 18 hours to remove organic material then rinsed in ultra-pure water. Samples were soaked in 1M acetic acid buffered with 1M calcium acetate for 4 hours to mimic removal of secondary carbonates that may form in archaeological or paleontological bones during burial, while limiting potential for recrystallization of secondary carbonates (Garvie-Lok et al. 2004). Samples were rinsed and dried at 60°C.

Phosphates were isolated using methods of Dettman et al. (2001). Approximately 10-20mg of bone powder was soaked in 2M hydrofluoric acid for 18 hours to dissolve the mineral and liberate phosphate ions. The acid solution was diluted and buffered with 20% ammonium hydroxide. A solution of 2M silver nitrate was added and the resulting silver phosphate precipitate was collected, rinsed copiously with ultra-pure water, and dried at 60°C.

All samples were analyzed for stable isotope values using Thermo Delta V Advantage mass spectrometers at the Smithsonian MCI Stable Isotope Mass Spectrometry Laboratory. Collagen samples (~0.5mg) were combusted in a Costech 4010 Elemental Analyzer (EA) coupled to the mass spectrometer via a Conflo IV interface; purified N_2 and CO_2 was analyzed for nitrogen and carbon isotope ratios. Structural carbonates (~4.0mg) were acidified in concentrated phosphoric acid (SG≥1.92) at 25°C for 18 hours in a Gas Bench II system; purified CO_2 was analyzed for carbon and oxygen isotope ratios. Phosphates (~0.5mg) were thermally decomposed at 1450°C in a Thermo temperature conversion elemental analyzer (TCEA) coupled to the mass spectrometer via a Conflo IV interface; purified CO was analyzed for oxygen isotope ratios. A sample of pure cellulose nitrate film was included in EA and TCEA runs to determine its carbon, nitrogen, and oxygen isotope values. A sample was included in Gas Bench runs to

determine the ability, if any, of phosphoric acid to release CO_2 from lingering cellulose nitrate in bone samples.

All isotope values are reported in standard delta notation:

$\delta = [(R_{sample} - R_{standard}) / R_{standard}] * 1000$

where δ represents the isotope system (i.e. $\delta^{15}N$, $\delta^{13}C$, or $\delta^{18}O$), R is heavy:light isotope ratio (i.e. ^{15}N:^{14}N, ^{13}C:^{12}C, or ^{18}O:^{16}O), units are permil (‰), and the standards are atmospheric N_2, V-PDB, and V-SMOW for nitrogen, carbon, and oxygen respectively. Raw values are corrected using a 2-point linear calibration based on associated standards. The $\delta^{15}N_{collagen}$ and $\delta^{13}C_{collagen}$ values (AIR and VPDB-LSVEC scales, respectively) were corrected against Urea_UIN3 and an acetanilide calibrated to USGS 40 and USGS 41 amino acids (Schimmelmann et al. 2009). The $\delta^{13}C_{structural\ carbonate}$ and $\delta^{18}O_{structural\ carbonate}$ values (VPDB-LSVEC scale) were corrected against LSVEC and NBS19 carbonates, and subsequently converted to VSMOW values for direct comparison to $\delta^{18}O_{phosphate}$ values using Coplen et al. (2002). The $\delta^{18}O_{phosphate}$ values (VSMOW-SLAP scale) were corrected against USGS 34 and USGS 35 nitrates. Precision based on replicate analysis and reported errors within the international standards are ±0.2‰ (1σ) for $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$, $\delta^{13}C_{structural\ carbonate}$, and $\delta^{18}O_{structural\ carbonate}$ values; the error is ±0.4‰ (1σ) for $\delta^{18}O_{phosphate}$ values.

The efficacy of the cellulose nitrate removal was tested using Fourier transform infrared (FTIR) spectroscopy. Powdered samples were analyzed by attenuated total reflectance (ATR) FTIR using a Thermo Nicolet 6700 FTIR with Golden Gate ATR (diamond crystal, single bounce, 45º) and equipped with a DTGS detector. Each spectrum was collected from 450 to 4,000 cm^{-1} for 64 scans at a resolution of 4 cm^{-1}. The following samples and treatments were analyzed: reference sample of Duco cement (containing cellulose nitrate), control bone with no application of cellulose nitrate, treated bone with Duco cement applied and not removed, and treated bone with Duco cement applied and removed with 100% acetone and oven drying at 80ºC. FTIR spectra of cellulose nitrate are characterized by nitrate group absorption bands at 1637 cm^{-1} (asymmetric) and 1274 cm^{-1} (symmetric stretch), and a characteristic peak at 825 cm^{-1} (N-O stretch) (Noake et al. 2017). The presence of the absorption band (symmetric NO_2 stretch) at 1274 cm^{-1} was the primary used in identifying cellulose nitrate presence or absence in the infrared spectra from the bone samples.

3. Results

Cellulose nitrate was detected in all bone samples where it was applied. Indeed, the FTIR spectrum from samples treated with 100% acetone solvent and oven drying at 80ºC show a small spectroscopic feature at 1280 cm^{-1}, which suggests some cellulose nitrate remains (Figure 1) and the treatment method is only partially successful in removing the Duco cement.

Results from the whale rib show no statistical differences in any measured isotopes between control samples (i.e. no Duco cement applied) exposed to acetone versus those not exposed to acetone (Mann-Whitney U test, $p>0.1$ in all cases). For subsequent testing, all control samples (exposed and not exposed to acetone) are lumped together for comparison with Duco cement-treated samples.

The $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ seal femur values show a tight clustering of data (Table 1, Figure 2). The $\delta^{13}C_{collagen}$ show a range of -12.1 to -13.0‰ (average = -12.4‰, $1\sigma = 0.3$); the Duco cement itself produced a $\delta^{13}C$ value of -26.3‰. The $\delta^{15}N_{collagen}$ show a range of +18.0 to +18.7‰ (average = +18.3‰, $1\sigma = 0.2$); the Duco cement produced a $\delta^{15}N$ value of +12.0‰. Using a Mann-Whitney U test, there is no statistical difference (i.e. $\alpha \leq 0.05$) between samples treated with Duco cement versus controls for either $\delta^{13}C_{collagen}$ ($p = 0.748$) or $\delta^{15}N_{collagen}$ ($p = 0.470$). Whale rib $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ values are also presented in Table 1. However, as noted previously, the similar values between untreated whale rib $\delta^{15}N_{collagen}$ (average = +11.5‰) and the Duco cement itself (+12.0‰) precluded use of this bone for comparing treated and untreated experimental groups.

The $\delta^{18}O_{phosphate}$ whale rib values also show a relatively tight clustering of data considering the inherent error of ±0.4‰ in each value (Table 1, Figure 3). These values show a range from +18.6 to +20.8‰ (average = +19.8, $1\sigma = 0.7$); the Duco cement produced a $\delta^{18}O$ value of +26.5‰. Using a Mann-Whitney U test, there is no statistical difference (i.e. $\alpha \leq 0.05$) between samples treated with Duco cement versus controls for $\delta^{18}O_{phosphate}$ ($p = 0.873$).

The $\delta^{13}C_{structural\ carbonate}$ whale rib values show a tight clustering of data, while the $\delta^{18}O_{structural\ carbonate}$ whale rib values show relatively greater variability considering the inherent error of ±0.2‰ in each value (Table 1, Figure 4). The $\delta^{13}C_{structural\ carbonate}$ show a range of -15.5 to -14.4‰ (average = -14.8, $1\sigma = 0.3$). The $\delta^{18}O_{structural\ carbonate}$ show a range of +26.5 to +28.2‰ (average = +27.3‰, $1\sigma = 0.7$). The pure Duco cement itself produced no measurable peaks when run on the Gas Bench II using the same methods as sample runs. Using a Mann-Whitney U test, there is no statistical difference (i.e. $\alpha \leq 0.05$) between samples treated with Duco cement versus controls for either $\delta^{13}C_{structural\ carbonate}$ ($p = 0.261$) or $\delta^{18}O_{structural\ carbonate}$ ($p = 0.109$).

It is should be noted that the Mann-Whitney U test produces a test statistic for the $\delta^{18}O_{structural\ carbonate}$ that is almost deemed significant (i.e. $p \sim 0.1$), whereas all other isotope systems show clear statistical similarity between treated and untreated samples (i.e. $p > 0.26$). The choice to use non-parametric tests was made based on small sample sizes within experimental groups, with the understanding that this reduces the statistical power of any comparisons. A more powerful parametric one-tailed t-test does show a statistical difference between $\delta^{18}O_{structural\ carbonate}$ values of treated versus untreated samples ($p = 0.0281$), but must be applied with caution due to small sample sizes and possibly non-Gaussian distributions. Similar t-tests for the remaining isotope systems show no significant differences ($p > 0.24$ in all cases).

4. Discussion

The application of two acetone rinses for 24 hours each did not completely remove cellulose nitrate from bone material, although it removed a significant amount. It is therefore suggested that one add additional rinses to the removal chemistry, or possibly add sonication to facilitate solvent penetration into all pore spaces. Harsher solvents may also be an option, but previous studies suggest that acetone has no effect on isotope values and generally does not damage the bone material (France et al. 2011, France et al. 2015, Moore et al. 1989). These

authors recommend acetone as the solvent of choice to remove cellulose nitrate with the understanding that traces of the consolidant may be left behind. However, as discussed below, traces of cellulose nitrate are typically removed during further extractions and do not directly interfere with isotope value measurements.

The application of Duco cement and the exposure to acetone solvent have no apparent effects on the $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$, $\delta^{13}C_{structural\ carbonate}$, or $\delta^{18}O_{phosphate}$ values in bone. The $\delta^{18}O_{structural\ carbonate}$ shows some apparent alteration in the presence of Duco cement. Although the low power of the non-parametric statistical tests only shows significant $\delta^{18}O_{structural\ carbonate}$ differences between treated and untreated samples at $p = \sim 0.1$, the differences between these two groups is clearly outside the bounds of the ±0.2‰ (1σ) analytical error (Figure 4). The most likely explanation for the observed pattern is exchange of relatively labile hydroxide (-OH) ions between the bioapatite and the isopropanol or the cellulose nitrate in the Duco cement mixture. The oxygen in the bioapatite –OH groups is incorporated into the mass spectrometer measurement of the $\delta^{18}O_{structural\ carbonate}$ value. The –OH groups are released during acidification in the form of water which isotopically equilibrates with released CO_2 in the sample vial. Any –OH exchange would therefore be reflected as a difference in $\delta^{18}O_{structural\ carbonate}$ values between treated and untreated samples. The idea that –OH exchange during consolidant application is responsible for isotope alteration is supported by previous work examining effects of consolidants on bone isotopes (France et al. 2011, France et al. 2015) where –OH exchange appeared to be the mechanism most likely affecting observed $\delta^{18}O_{structural\ carbonate}$ differences. It should be noted that some research suggests that sample pre-treatment with sodium hypochlorite and buffered acetic acid, as used in this experiment, can slightly alter bone carbonate isotope values (Garvie-Lok et al. 2004, Pellegrini and Snoeck 2016, Pestle et al. 2014). This study has employed the best practices from this research to minimize these effects, but cannot completely eliminate the possibility that the observed oxygen isotope alteration may be due in small part to sample pre-treatment methods in conjunction with effects from cellulose nitrate exposure.

Given the theoretical and experimental evidence, one can reasonably rule out other possibilities for the observed $\delta^{18}O_{structural\ carbonate}$ differences including direct atomic exchange, complete ion group exchange, and failure to remove the consolidant. In order for any atoms within the collagen or bioapatite to directly exchange with Duco cement, the backbone structure of the molecules would have to cleave and reform. This is energetically demanding and highly unlikely, especially for the strong bonds within collagen, phosphate ions, and carbonate ions. Recent work by Gao et al. (2015) suggests that pure carbonates, such as aragonite, with no –OH groups show no effects of acetone solvents on $\delta^{18}O$ values. This further supports that isotope exchange will not occur between the backbone structures of the bioapatite, but rather requires labile functional groups to facilitate such alterations.

Complete exchange of bioapatite ionic groups (i.e. $–CO_3$ and $–PO_4$) with cellulose nitrate $–NO_3$ groups is also unlikely. While the weakest bonds within cellulose nitrate are those binding the $–NO_2$ groups to the ring oxygens, cleavage requires >100°C temperatures (Selwitz 1988, Shashoua et al. 1992). Cleavage below these temperatures requires considerable time and would not be appreciable over the duration of this experiment. Furthermore, if entire bioapatite $–CO_3$ were exchanging with other ionic groups, one would expect to see differences in the $\delta^{13}C_{structural}$

$_{carbonate}$ values as well. Since no $\delta^{13}C_{structural\ carbonate}$ differences are observed, it is unlikely that the bioapatite $–CO_3$ groups are affected by the consolidant.

Finally, there may be traces of cellulose nitrate lingering in the bone if the acetone rinses did not successfully remove it, as was the case in this study. After treatment with Duco cement, the collagen and phosphate extraction chemistry rely on acidic liberation of the compounds of interest (see Materials and Methods). As cellulose nitrate is not soluble in acid, it should be eliminated with the residual solids that are separated from the respective supernatants containing the collagen or phosphate ions. The carbonate extraction chemistry lacks this separation mechanism, but pure Duco cement did not produce any peaks in the mass spectrometry analysis. Any lingering cellulose nitrate in the carbonate samples will not acidify in phosphoric acid to liberate CO_2 with a unique $\delta^{18}O$ value which will contaminate the $\delta^{18}O_{structural\ carbonate}$ values. The Duco cement will only affect $\delta^{18}O_{structural\ carbonate}$ so long as –OH exchange is facilitated in solution via acetone solvent.

Although this experiment did not specifically test analyses besides stable isotopes, it is worth considering potential effects of cellulose nitrate consolidants on other techniques. Given that the organic protein does not appear to experience any cleavage or atomic exchange with cellulose nitrate, it is likely that other organic molecules of a similar structure would be unaffected by this consolidant. DNA, osteocalcin, or other proteinaceous materials would likely remain intact and unaffected, thereby allowing reliable sequencing, carbon dating, FTIR, GC-MS, or other organic techniques on original material. While labile inorganic groups may exchange with the consolidant, the backbone bioapatite remains intact as well. Inorganic analyses, such as XRF, SEM, XRD, and ICP-MS that rely on a preserved mineral structure (and the ions therein) are likely to remain. However, it is worth noting that incomplete removal of cellulose nitrate could be problematic for these and other analyses, such as radiocarbon dating (Brock et al. 2017), that would incorporate the lingering consolidant into measurements.

Cellulose nitrate is no longer a commonly used consolidant due to its propensity to degrade with time. Over the course of several decades, -OH, $–NO_2$, and other secondary products will cleave off the cellulose ring structure either spontaneously or facilitated by UV radiation (Selwitz 1988). The subsequent long-term effects of these breakdown mechanisms on stable isotope values is completely unknown, but worthy of further study since most actual cellulose nitrate-treated samples are likely to have been collected decades ago. To that end, several bone disks from this experiment were treated and placed in long-term storage to be reexamined in 10+ years.

5. Conclusion

After exposing modern bones to cellulose nitrate consolidant in the form of Duco cement and acetone solvent, the following conclusions and recommendations can be drawn:

(1) Rinse bones treated with cellulose nitrate several times (at least 3x) in 100% acetone. Consider sonicating samples to facilitate penetration of the solvent into all pore spaces.

(2) The $\delta^{13}C$ and $\delta^{15}N$ from isolated bone collagen are unaffected by addition of cellulose nitrate or exposure to acetone.
(3) The $\delta^{18}O$ from bioapatite phosphate is unaffected by addition of cellulose nitrate or exposure to acetone.
(4) The $\delta^{13}C$ from bioapatite carbonate is unaffected by addition of cellulose nitrate or exposure to acetone.
(5) The $\delta^{18}O$ from bioapatite carbonate is altered by exposure to cellulose nitrate in an unpredictable manner. Care should be taken when using $\delta^{18}O_{\text{structural carbonate}}$ values from cellulose nitrate-treated bones as they may not represent an original isotope signature.

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Table and Figure Captions

Table 1 – Data

Figure 1 – FTIR spectra showing the removal of cellulose nitrate (CN) from bone samples. The presence of CN in bone samples is indicated by the absorption band (symmetric NO_2 stretch) at 1274 cm^{-1}. The figure shows spectra from Duco cement reference sample (solid line), control bone with no Duco cement (WR-60), treated bone with Duco cement applied and not removed (WR-61), and treated bone with Duco cement applied and removed with 100% acetone (WR-61-2Ao).

Figure 2 – Average collagen carbon and nitrogen isotope values for seal femur samples according to treatment categories. Error bars represent 1σ standard deviation of replicate analyses.

Figure 3 – Average phosphate oxygen isotope values (plotted against structural carbonate carbon values for comparison) for whale rib samples according to sample treatment. Error bars represent 1σ standard deviation of replicate analyses.

Figure 4 – Average structural carbonate oxygen and carbon isotope values for whale rib samples according to sample treatment. Error bars represent 1σ standard deviation of replicate analyses.