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Standardizing Infra-red Measures of Bone Mineral Crystallinity: an Experimental Approach

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Three experiments demonstrate that infra-red spectroscopic measures of bone mineral crystallinity are partially dependent upon sample preparation methods. Intensive grinding of bone samples for Fourier transform infra-red (FTIR) spectroscopic analysis results in a net decrease in splitting factor (SF), a common measure of apatite crystallinity. If grinding is overly intensive, SF measurement may respond more to variation in sample preparation than to the differences in bone mineral crystallinity that it is intended to monitor. Because even slight differences in sample preparation can affect SF values, a set of procedures and standards are proposed as a means of calibrating infra-red crystallinity measures.

Keywords: FOURIER-TRANSFORM INFRA-RED SPECTROSCOPY, FTIR, BONE HYDROXYAPATITE CRYSTALLINITY, RECRYSTALLIZATION, SPLITTING FACTOR, SAMPLE PREPARATION, ARCHAEOMETRY.

Introduction

This paper reports the results of experiments designed to investigate the effects of variation in potassium bromide pellet preparation on splitting factor, an infra-red spectroscopic measure of bone apatite crystallinity. Although this research grew out of the larger study of zooarchaeology and vertebrate taphonomy of the Middle Paleolithic levels of Hayonim cave (Stiner *et al.*, 2001), the results are relevant to a broader range of applications in archaeology.

There are many means of sample preparation, but most infra-red spectroscopic applications in archaeology use the potassium bromide pelleting technique. A transparent pellet is formed by compressing a mixture of powdered bone and anhydrous KBr at high pressure. In the sample chamber of the spectrometer, a beam of infra-red light is passed through the pellet. Photons of infra-red radiation interact with molecules in the bone. Some wavelengths of lights are absorbed promoting atomic bonds to excited vibrational states. Other wavelengths pass through the bone and have no effect. Molecular structure determines which wavelengths will be transmitted and which will be absorbed. The relative abundance or transmittance of light across a range of infra-red wavelengths therefore makes it possible to detect diagenetic changes in bone apatite structure at the molecular level, which can be measured semi-quantitatively using the relative concentrations of infra-red-active groups based on peak height or peak area ratios (Smith, 1996). The major absorption bands in bone mineral can be attributed to PO_4, CO_3, OH, and H_2O groups.

Infra-red spectroscopy has become an important tool for diagenesis research, particularly for the analysis of skeletal materials (Lee-Thorp & van der Merwe, 1991; Rink & Schwarcz, 1995; Sillen & Sealy, 1995; Sillen & Parkington, 1996; Shahack-Gross, Bar-Yosef & Weiner, 1997; Stiner *et al.*, 1995; Weiner & Bar-Yosef, 1990; Weiner, Goldberg & Bar-Yosef, 1993; Wright & Schwarcz, 1996). Applications of IR spectroscopy have expanded rapidly, due partly to the recent availability of Fourier transform infra-red spectrometers (FTIR) which offer many advantages over older dispersive machines. Relatively inexpensive to operate, the newer instruments permit fast, minimally destructive analysis of skeletal and other materials. They are portable and thus can be used in a variety of protected field conditions, making it possible to generate results on diagenesis in ''real time'', as excavations proceed rather than as post-hoc observations (Weiner & Bar-Yosef, 1990; Weiner, Goldberg & Bar-Yosef, 1993). It now is possible to assess the preservation status of bone mineral within minutes, limited only by the contingencies of sample preparation and the types of data sought from these samples.

FTIR spectrometers have been used to assess the fluorine content of bone, the relative proportion of carbonate substituted for phosphate and hydroxyl ions in enamel apatite, to differentiate burned from mineral-stained bones, the diagenetic and/or pyrolytic status of bone proteins, and mineralogical changes in bone

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resulting from chemical pretreatments (DeNiro & Weiner, 1988; Lee-Thorp & van der Merwe, 1991; Stiner *et al.*, 1995; Shahack-Gross, Bar-Yosef & Weiner, 1997; Sponheimer & Lee-Thorp, 1999; Wright & Schwarcz, 1997). Many recent infra-red studies focus on two related aspects of bone diagenesis, bone mineral "crystallinity" and carbonate content (Lee-Thorp & van der Merwe, 1991; Rink & Schwarcz, 1995; Sillen & Sealy, 1995; Stiner *et al.*, 1995; Weiner & Bar-Yosef, 1990; Wright & Schwarcz, 1997). "Crystallinity" refers to the degree of order within the crystal lattice. Substances of high crystallinity are characterized by large crystal sizes and low strain, whereas substances of low crystallinity, such as fresh bone mineral, are characterized by small crystal sizes and high strain resulting from irregularities in the lattice.

With so many new research programmes using FTIR, replicability and its relation to sample preparation technique are major concerns, particularly for studies that rely on quantitative analyses of crystallinity (as opposed to identifying mineral presence/absence). Taphonomic questions, particularly those directed to site formation processes, require that many samples be processed, ideally during excavations (e.g. Stiner *et al.*, n.d.). These research conditions set a premium on reliability, portability, and ease of sample preparation. Sample preparation was greatly refined in our case with the help of three experiments that control for the effects of grinding technique on crystallinity measurement. The results of these experiments and their implications for sample preparation and standards are presented below.

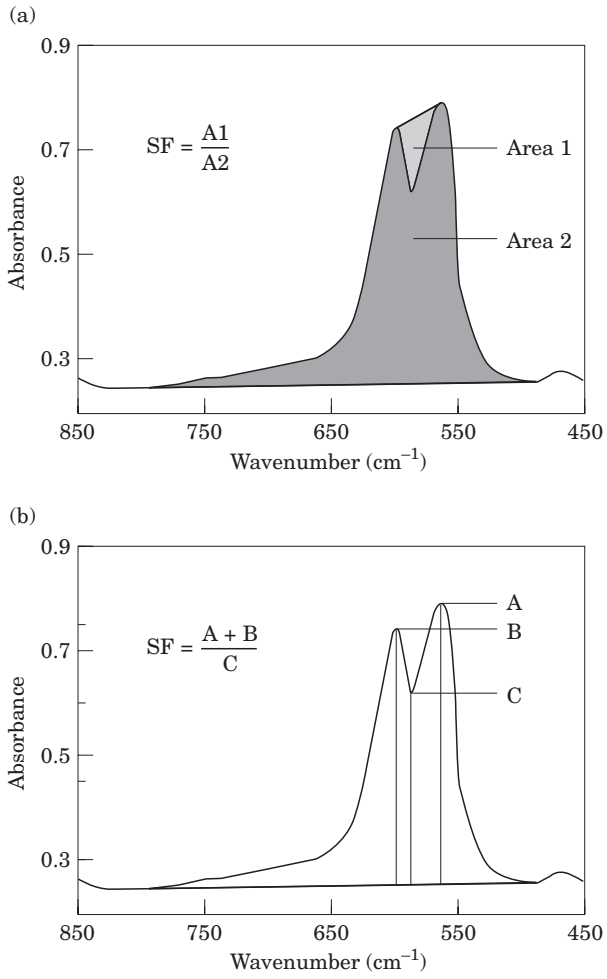

Figure 1. (a) Splitting function (SF) of Termine & Posner (1966). SF is measured as the ratio of the area above and below the phosphate double peak at 603 and 563 cm^{-1}. (b) The splitting factor (SF) of Weiner & Bar-Yosef (1990). SF is measured as the sum of the heights of the 603 and 563 cm^{-1} phosphate peaks divided by the height of trough between them. All heights are measured above a baseline drawn from approximately 780 to 495 cm^{-1}.

Crystallinity measurement

Fresh bone mineral is a poorly crystalline non-stoichiometric carbonate hydroxyapatite (dahllite) composed of minute needle- or plate-like crystals, each only a few hundred angstroms in length (Brown & Chow, 1976; Weiner & Traub, 1992; Ziv & Weiner, 1994). Diagenesis—post-burial processes that affect the physical and chemical properties of bone—often results in a net increase in apatite crystallinity. This process is broadly known as recrystallization and likely involves selective dissolution of the smallest, least crystalline apatite crystals, which may in turn be reprecipitated as more crystalline, thermodynamically stable forms (Hedges & Millard, 1995; Hedges, Millard & Pike, 1995; Wright & Schwarcz, 1997). Bone apatite recrystallization seems to relate to collagen decomposition, carbonate loss, and possibly fluorine uptake (Person *et al.*, 1995, 1996; Sillen & Sealy, 1996; Wright & Schwarcz, 1997).

Crystallinity is of interest to archaeologists as a rough quantitative measure or index of bone mineral preservation. Not only does crystallinity provide an independent assessment of the suitability of skeletal samples for isotopic analyses, it may eventually provide information on how zooarchaeological assemblages have been biased compositionally by preservation conditions. Bone mineral crystallinity can be assessed by infra-red spectroscopy or X-ray diffraction (XRD). In XRD, crystallinity usually is measured as the full width of the 002 reflection at half-height (Sillen, 1989; Tuross, Behrensmeyer & Eanes, 1989; Hedges *et al.*, 1995), but more complex crystallinity indices have also been developed (e.g. Bartsiokas & Middleton, 1992; Person *et al.*, 1995, 1996). Infra-red studies of bone apatite crystallinity derive from the work of Termine & Posner (1966), who measured what they called "percent crystallinity" in known concentrations of synthetic amorphous and crystalline apatites. "Percent crystallinity" is measured as a peak-splitting function calculated as a ratio of the areas above and below the double peak of the phosphate anti-symmetric bending frequency (ν_4) at approximately 550 to 650 cm^{-1} (Figure 1(a)). The double

peak becomes increasingly separated, or split, as crystallinity increases. At least three other indices of apatite crystallinity have been developed subsequently and are known as *crystallinity index* or *splitting factor* (Pleshko, Boskey & Mendelsohn, 1991; Sillen, 1989; Shemesh, 1990; Weiner & Bar-Yosef, 1990; Wright & Schwarcz, 1996). The splitting factor (SF) of Weiner & Bar-Yosef (1990) has become the dominant crystallinity measure in archaeology and is among the infra-red measurements used in our taphonomic research at Hayonim cave (Stiner *et al.*, 2001). SF is calculated by summing the heights of the 563 and 603 cm^{-1} peaks and dividing this value by the height of the trough between them (Figure 1(b)). All heights are measured from a common baseline drawn from approximately 495 to 780 cm^{-1} (Weiner & Bar-Yosef, 1990: Fig. 2).

In archaeology, a standardized means of assessing bone mineral crystallinity is particularly important for comparisons of bone mineral preservation between and among excavation units, sites, and geochemical contexts. Based on results of the experiments presented below, we developed a standard method of KBr pellet manufacture that greatly improves replicability and comparability of SF measurement with a minimum of laboratory equipment. We also suggest a series of standards to be used in future research incorporating the SF measurement, allowing direct comparison of SF values between studies.

Experiment I: Systematic Variation Attributed to Sample Preparer

Preliminary results from Hayonim suggested there might be systematic bias caused by sample preparer behaviour, despite the fact that all preparers were instructed by the first author (following Stiner *et al.*, 1995). A simple experiment was designed to test this hypothesis.

Methods

A small compact bone fragment of Middle Paleolithic age from Hayonim Cave was ground into a homogenous powder with an agate mortar and pestle. This was used as stock powder for making multiple KBr pellets. A few tens of milligrams of this bone were added to a clean mortar and pestle and ground to a fine powder. Excess bone was removed with a Kimwipe lab wipe. The sample was ground once more and excess bone powder was wiped away again, leaving about 0·2 to 0·7 mg. Approximately 50 mg of KBr were then added to the mortar and mixed with the remaining bone powder by a light grinding motion; pellet concentration ranged from about 0·4 to 1·4%. This powder was then pressed into a 7 mm diameter pellet using a Spectratech mini-press (International Crystal Laboratories). Three people were each responsible for the manufacture and analysis of 10 KBr pellets.

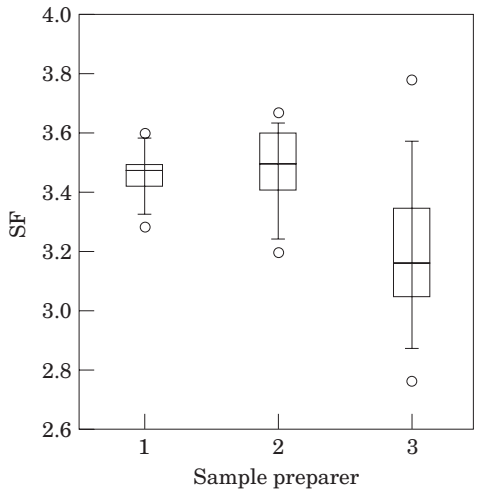

Figure 2. Variation in SF among three sample preparers. The midline denotes the median value, the boxes quartiles, and the whiskers the 75th percentile. The sample-preparers are in order of relative experience. Two-tailed *t*-tests were performed for each pair of preparers: 1 × 2, $P=0{\cdot}759$; 2 × 3, $P=0{\cdot}016$; 1 × 3, $P=0{\cdot}016$.

All infra-red analyses were performed using a Midac Corp. Prospect-IR Fourier transform infra-red spectrometer. The spectral analyses were performed using Grams 386 software (Galactic Industries Corp., Salem, NH, U.S.A.). Instrument parameters were as follows: *scans*=32; *resolution*=4 cm^{-1}; *gain*=auto; *range*=4000–400 cm^{-1}; *mode*=absorbance. The empty sample chamber was used to obtain the background spectrum.

Results

The results confirmed a bias in crystallinity measures relating to individual sample preparer. Sample preparer 3 tended to produce pellets with SFs significantly lower ($P=0{\cdot}016$, two-tailed *t*-test) than those of preparers 1 and 2 (Figure 2). Individual error was proportional to the relative experience in preparing KBr pellets (Figure 2). From a single bone fragment, it was possible to obtain SFs ranging between 2·78 and 3·78, when reported errors in this measurement do not exceed $\pm 0{\cdot}15$ (Sillen & Parkington, 1996).

The exact mechanisms responsible for individual bias in SF measurements were unclear. Because individual error also varied with experience, it seemed that this variability was likely related to some aspect of routenization. Since manual grinding was the least controlled aspect of sample preparation, the remaining experiments focused on this variable.

Experiment II: The Effects of Grinding Method on SF

This experiment addresses the effects of relatively standardized and unstandardized sample grinding on SF measurement across a broad range of crystallinities.

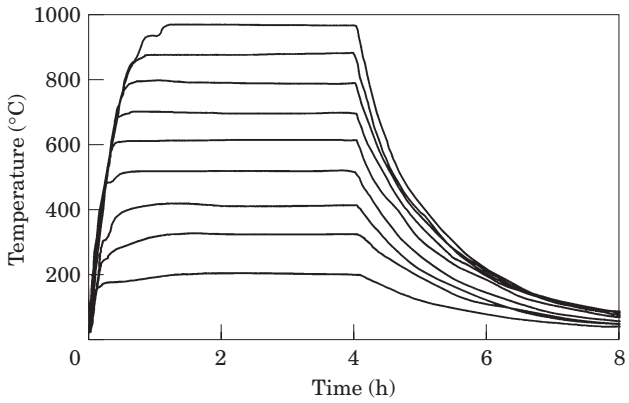

Figure 3. Heating temperature versus time for the experimental bone samples.

Two grinding methods were used for KBr pellet preparation, one in which grinding was done by hand with an agate mortar and pestle, and a more standardized one in which samples were pulverized for a set time period using a Wig-L-Bug ball mill (Crescent Dental Co., Chicago, IL, U.S.A.), in which the sample is crushed by a stainless steel ball bearing in a rapidly vibrating stainless steel vial.

Methods

Because it was not possible to independently measure the crystallinity of archaeological bone samples, a series of experimental samples were created by heating compact cow bone in a small electric kiln over a range of temperature settings. Bone apatite crystallinity is known to increase the temperature (Person *et al.*, 1996; Shipman, Foster & Schoeninger, 1984; Stiner *et al.*, 1995), and thus it was assumed that bones heated at higher temperatures were more crystalline. Fresh cow humeri and femora were cut with a hacksaw into approximately 3×3 cm rectangular fragments; only diaphyseal compact bone was used in the experiment. The heating procedure followed that described by Shipman, Foster & Schoeninger (1984). Thermostat settings ranged from 200 to 1000°C in 100°C increments, but actual temperatures ranged from 206 to 974°C (Table 1). Temperature was monitored by thermocouple and recorded frequently (Figure 3). For each temperature, a bone fragment was placed into the kiln at room temperature. The kiln was switched on for 4 h. This allowed for at least 3 h at the desired temperature. The kiln was then switched off and allowed to cool for 4 h.

As noted above, two methods of grinding were used for KBr pellet production for every sample generated. The first pellet production technique was identical to that described for Experiment I, except that a single individual prepared all of them; these samples will be referred to as grinding-uncontrolled (GU). Fifteen samples were run for each temperature increment. A second set KBr pellets were prepared controlling grinding and particle size. This was accomplished by lightly crushing a few grams of bone in an agate mortar, then transferring it to a stainless steel vial with ball bearing for pulverization for 1 min in the Wig-L-bug mill. Next, the bone powder was passed through a #325 45-μm sieve, and the fraction passing through the sieve was used as a stock powder. KBr was added to create a pellet concentration of 0·5–1·4%, and again placed in a stainless steel vial with ball bearing and shaken in the Wig-L-Bug for 30 s to thoroughly mix the sample. A 50-mg aliquot of this mixture was pressed into a KBr pellet and run in the FTIR as described above (except at 2-cm^{-1} resolution). Eight samples were run for each temperature interval. These samples will be referred to as grinding–controlled (GC).

Table 1. Thermostat setting versus actual temperature for experimentally heated compact cow bone

Thermostat setting (°C)	Actual temp. (°C)
200	206
300	325
400	415
500	522
600	617
700	703
800	793
900	880
1000	974

Results

Splitting factor did not uniformly increase with temperature as expected. This was especially true for the GU samples (Table 2, Figure 4); average SF increased slightly from 206°C (SF=2·90) to 325°C (SF=3·05), but at 415°C it dropped to its lowest value (SF=2·79). Between 415 and 703°C, SF increased rapidly to 6·33. At 703°C, SF again dropped to 5·87 and then rose steadily to 6·26 at 974°C. By contrast, SF did vary according to expectations in the GC samples heated between 206 and 703°C. Average values increased with temperature, though not in a linear fashion: SF rose slowly from 206 (SF=2·90) to 522°C (SF=3·43), then quickly from 617 to 703°C to a maximum SF value of 7·59. At 793, 880, and 974°C, SF fluctuated from 6·32 to 5·83 to 6·02, respectively.

In both sets of samples, precision in the SF measurement, calculated as standard deviation, was positively correlated ($P<0{\cdot}01$) with temperature (Figure 5). At high temperatures, and therefore crystallinities, SF precision tends to drop substantially. Standard deviations varied from $\pm 0{\cdot}05$ at low temperatures to between $\pm 0{\cdot}3$ and $\pm 0{\cdot}4$ at high temperatures.

Discussion

The difference in SF values observed using the GU and GC methods of pellet preparation suggested that

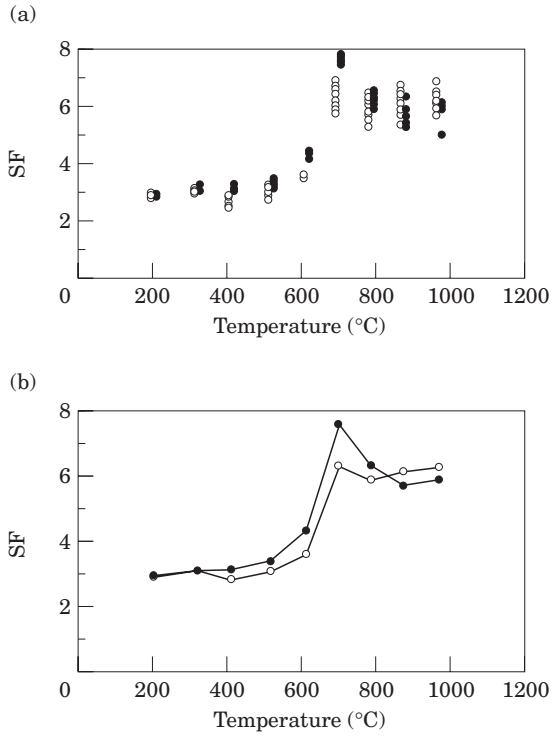

Figure 4. (a) SF versus temperature for GU and GC sample series. Samples for each temperature interval are depicted slightly offset of actual temperature for illustrative purposes; (b) average SF versus temperature. ○=GU; ●=GC.

Table 2. Mean SF and standard deviations by temperature interval and grinding method. Abbreviations: GU, grinding uncontrolled; GC, grinding controlled. Sample sizes per temperature interval: GU, N=*15; GC,* N=*8*

Temperature (°C)	Mean SF		Std Dev SF	
	GU	GC	GU	GC
206	2·93	2·90	0·05	0·06
325	3·05	3·09	0·05	0·08
415	2·79	3·13	0·13	0·09
522	3·08	3·37	0·17	0·12
617	3·57	4·34	0·04	0·11
703	6·33	7·59	0·34	0·13
793	5·87	6·31	0·34	0·22
880	6·16	5·72	0·39	0·34
974	6·26	5·88	0·28	0·36

grinding may have influenced the crystallinity measurement. Interestingly, error in SF in the grinding–controlled samples exceeded that of the grinding–uncontrolled samples in four of the nine temperature intervals, indicating that grinding was somehow standardized within each method of sample preparation, but not between them. This implied that, if differences in grinding were affecting SF measurement directly, it is due to the intensity or type of grinding.

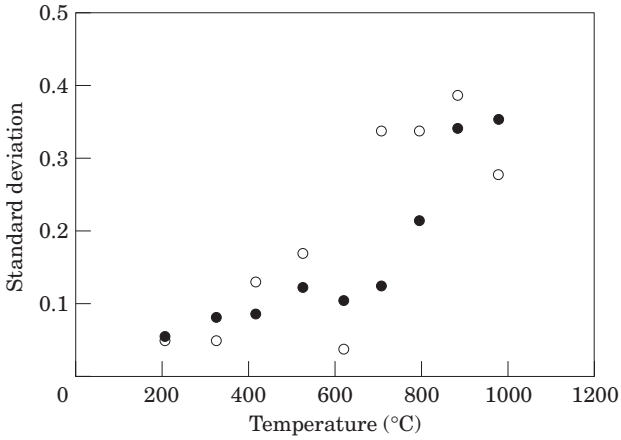

Figure 5. Standard deviation versus temperature for GC and GU sample series. Correlation coefficients: GC (r=0·82, P=0·0075); GU (r=0·898, P=0·001). ○=GU; ●=GC.

If variation in grinding technique affects the measurement of SF, does this arise from particle separation that naturally occurs during the grinding process, or does grinding actually alter the crystalline structure of the bone enough to change its infra-red spectrum? Although the latter seemed more likely, one difference in the two preparation methods related to mechanics of grinding. In both procedures, grinding results in a loose powder and a finely ground powder adhering to the mortar. The loose powder normally was used to make the pellet if a mechanical grinder is used. In the agate mortar and pestle technique, powder adhering to the mortar was used. If the mechanical separation of these fractions was related to variation in crystallinity within the bone mineral, this could also explain the observed differences in SF. These two possibilities were explored in Experiment 3.

The observed relationship between splitting factor and heating temperature was surprising. SF did not uniformly increase with temperature in either sample set, nor therefore with crystallinity. This begs the question of whether SF is a particularly useful measure of crystallinity. Using the GU method of sample preparation, it would be difficult to distinguish relative levels of crystallinity in bones heated between 206 and 522°C and between 700 to 1000°C. Similarly, relative SFs did not reflect crystallinity with the GC method when samples had very high crystallinities. The highest resolutions obtained for SF using either technique fell within a narrow range of temperatures, spanning approximately 522–703°C. The drop in SF seen above 703°C will be discussed further in the next section, but in practice, this phenomenon may not be of consequence in that diagenesis seems rarely, if ever, to push a bone to this level of crystallinity, but campfires may reach these temperatures (Stiner *et al.*, 1995). Clearly, the success of SF as a measure of bone mineral crystallinity is dependent on sample preparation and the level of apatite crystallinity. Although it is likely

that any sample preparation method will yield SF values that do reflect relative levels of bone mineral crystallinity, this is only true within certain ranges of crystallinity.

While error in SF measurement is correlated with heating temperature and therefore bone mineral crystallinity, some problems relating to measurement precision may be overcome with large sample sizes. When only small numbers of samples are used, measurement error can easily swamp variability in SF resulting from diagenesis. Also, some researchers have suggested that an SF value of 2·8 measured on fresh bone suggests standardization of crystallinity measurement (Sillen & Parkington, 1989). Our results indicate that sample preparation should have little effect on SF values at low crystallinities, and that this alone is not sufficient to demonstrate comparability of measurements. As is discussed below, a series of standards spanning a broad range of crystallinities is needed.

Experiment III: The Effect of Grinding Intensity on SF

This experiment was designed to test two hypotheses concerning the effects of grinding on splitting factor values. Although they partly overlap, the hypotheses can be usefully summarized as follows:

Separation Hypothesis—Grinding affects SF by separating fractions of bone mineral of differing crystallinity into loose powder and powder adhering to the inner surface of the mortar, an artifact of how the preparer collects the powder after grinding.

Destruction Hypothesis—Grinding affects SF by altering the crystalline structure of bone mineral.

Experiment III began with grinding a bone fragment, separating the residue adhering to the mortar and loose powder, regrinding both fractions, separating the two fractions again, regrinding both fractions, and so on for a total of nine cycles (Figure 6). If the intensity of grinding affects SF values, SF should show a directional change as the number of grinding cycles increases. If separation of loose powder and adherent fractions is responsible (the separation hypothesis), the SF values will differ consistently, and changes in SF should be bi-directional. For instance, if SF values increase following the powder lineage, they should decrease following the residue lineage, or vice versa (Figure 7(a)). If grinding alters the molecular structure of the bone mineral crystals (the destruction hypothesis), change in SF values should be unidirectional, following both the residue and powder lineages (Figure 7(b)). It should be noted that the separation and destruction hypotheses are not mutually exclusive; both could occur concomitantly. If so, some combination of the traits seen in Figure 7(a) and (b) would be expected.

Methods

Approximately 1·5 g of a bone fragment heated to 617°C from the previous experiment were used. This temperature was chosen because SF values showed considerable variability at this level of crystallinity in Experiment II, so the effects of grinding should be most evident. The bone was placed into a clean stainless steel vial with ball bearing and ground for 1 min in the Wig-L-Bug ball mill. The loose powder fraction (PF) was removed by inverting the open vial and gently tapping it on the lab bench. The residue fraction (RF) was removed by scraping the internal surface of the vial with a laboratory micro-spatula. A few tens of milligrams of each fraction were saved for FTIR analysis. Next, each fraction was reground as described above, and the PF and RF were separated again. Due to the limited quantity of bone powder collected in the RF, it was only possible to regrind each residue fraction from the powder lineage once. Eight pairs of PFs and RFs were produced in this way, illustrated as a flow chart in Figure 6.

For each fraction, three KBr pellets were made by placing 1–3 mg of bone powder into a clean stainless steel vial. Between 200 and 600 mg KBr were added to create a concentration of 0·5%. No ball bearing was used in this mixing stage, to minimize further grinding and residue separation. The mixture was shaken for 30 s, and a 50-mg aliquot was removed and pressed into a pellet, as in previous experiments. FTIR settings and analyses were identical to those used in the previous experiment.

Results

Results demonstrate the intensity of grinding directly influences SF values (Figure 8). Measured SFs ranged between 3·34 and 4·33. In 7 of 8 fraction pairs, the SF of the residue exceeded that of the powder fraction. Regrinding a residue fraction invariably resulted in a large decline in SF, though the difference between the values for powder and adherent fractions diminished with each regrinding cycle. That is, regrinding RF1 caused a greater net drop in SF than did regrinding RF2; regrinding RF2 caused a greater net drop in SF than did regrinding RF3, and so on to RF4. Regrinding powder fractions usually resulted in a small decline in SF, but in two cases (PF1→RF2, PF2→RF3), resulted in a slight, but statistically insignificant, rise in SF.

Discussion

The data clearly demonstrate that grinding can have major effects on SF. There is some support, however, for both the separation and the destruction hypotheses. Because SF declined or remained unchanged following all grinding episodes, the destruction hypothesis is confirmed. The separation hypothesis is supported by the consistency in the difference between powder and

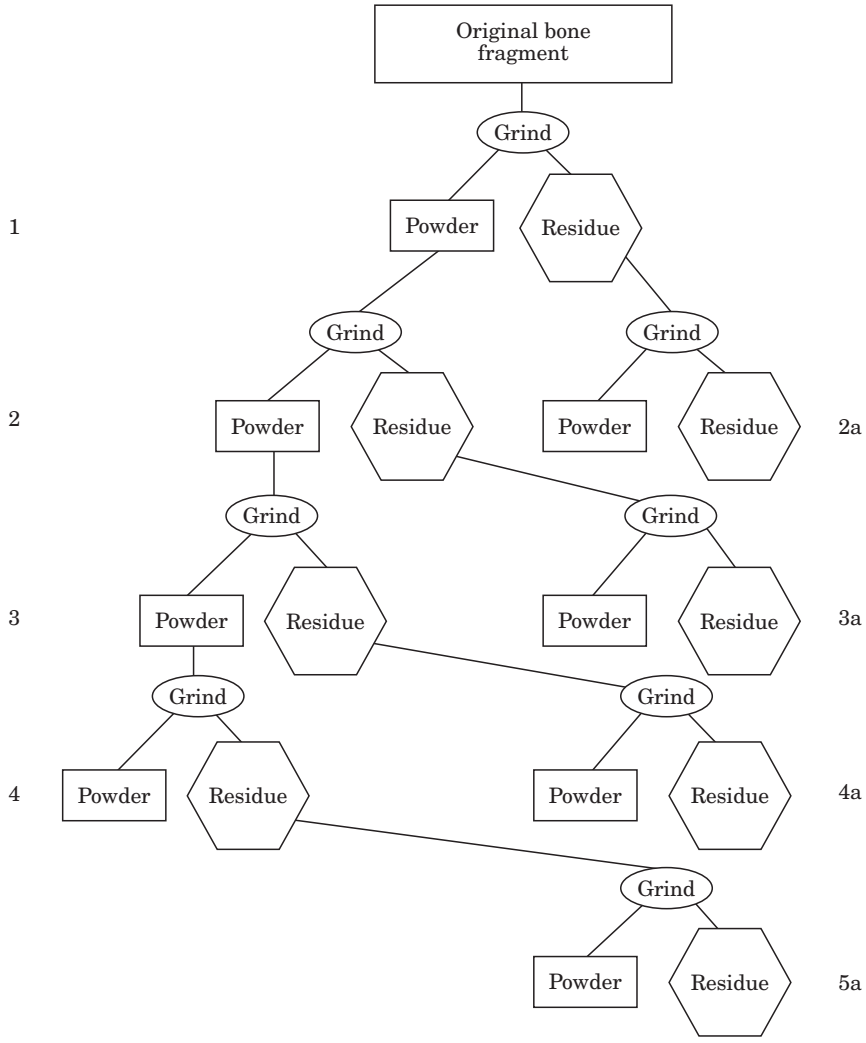

Figure 6. Flow chart of experimental procedures for Experiment III. The experiment begins by grinding a single bone fragment. The resulting powder is divided into two fractions, residue and powder. Each of these is reground, residue and powder are separated again, and further regrinding proceeds as above. Numerals refer to pairs of residue and powder fractions of the same generation, also reflecting the number of grinding episodes they have undergone, and whether they are derived from a residue or powder lineage.

residue fraction pairs. This may be explained by differences in the intensity of grinding of the two fractions. Although both fractions were ground for the same length of time, the residue fraction may have been buffered from further grinding because it attaches itself to the walls of the vial.

If the destruction hypothesis is correct, patterning in the data can be explained in two ways: (1) The greater the quantity of bone ground, the less grinding alters the crystalline structure of that bone sample; and (2) The greater the crystallinity of bone mineral at the outset, the greater its susceptibility to physical degradation from grinding. The first postulate explains the difference in residue and powder fraction SFs and the magnitude of SF decline for the reground powder and residue fractions—there is far more bone in the powder fraction than in the reground residue fraction. Regrinding a residue fraction therefore should have a much greater effect on SF than regrinding a powder fraction. The second postulate explains the successive decrease in net SF difference seen in regrinding RF1, 2, 3, 4, and it can explain two patterns seen in Experiment 2. The increase in error related to temperature (Figure 4) could have resulted from the enhanced effects of grinding at higher levels of crystallinity. Second, the drop in SF values seen above 703°C could be explained by the extreme susceptibility of apatite crystals to deformation from grinding at high levels of crystallinity.

These results further refine the observations from Experiment 2. The difference in SF values between the two methods of KBr pellet preparation from Experiment 2 might seem to relate to particle separation, but if so, mortar and pestle grinding (which uses the residue fraction) would be expected to produce greater SFs than mechanical grinding (which uses the

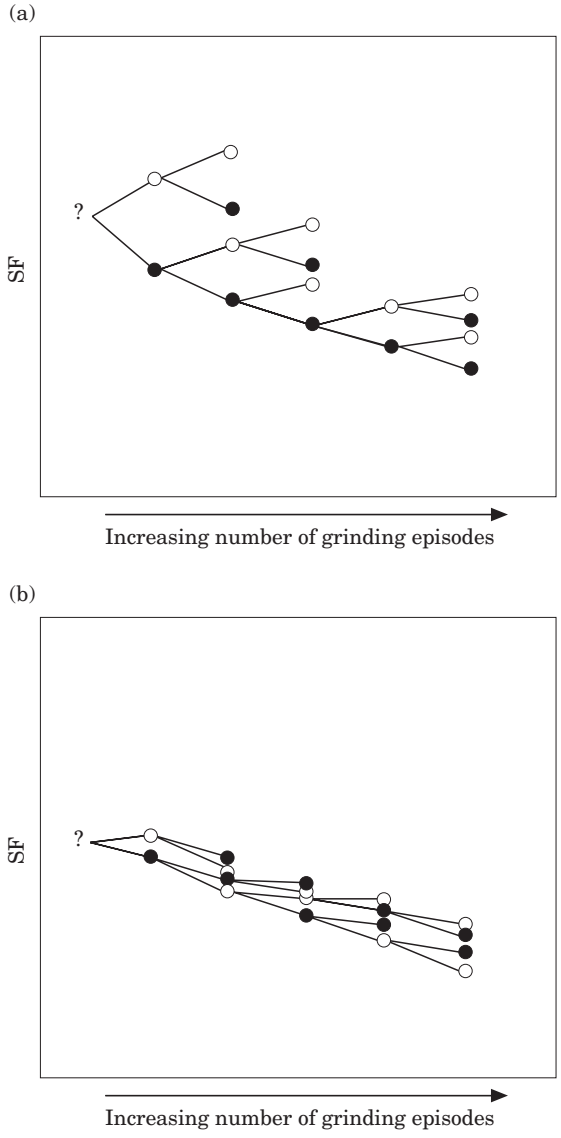

Figure 7. (a) Predicted outcome of the Separation Hypothesis—the relationship between the residue and powder fraction is consistent. Also, there is a decrease in SF value down any powder lineage, and down any residue lineage, there is an increase in SF value, or *vice versa*. (b) Predicted outcome of the Destruction Hypothesis—grinding always results in a unidirectional change in SF. ?=original bone fragment; ○=residue; ●=powder.

powder fraction). In fact, just the opposite is true, contradicting the separation hypothesis. The difference in SF values therefore is best explained by differences in the intensity of grinding in each experiment. Hand grinding in an agate mortar generally was more destructive to apatite crystals than mechanical grinding.

Conclusions from the Three Experiments

The following conclusions may be drawn from this research:

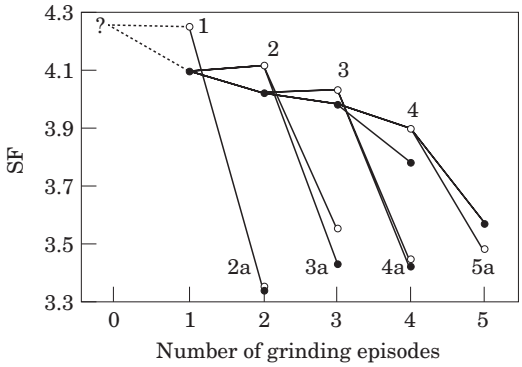

Figure 8. Number of grinding episodes versus average SF for residue and powder fractions. Lines denote grinding lineage. ?=original bone fragment; ○=residue; ●=powder. Numerals correspond to sample pair numbers from Figure 5.

- Intensive grinding of bone samples for FTIR analysis results in a net decrease in splitting factor.
- When grinding is poorly controlled or excessive, it is difficult to distinguish variation in actual bone crystallinity from that introduced by sample preparation.
- Susceptibility to physical alteration of mineral structure from grinding is correlated with crystallinity, and therefore, measurement precision may vary systematically as a function of crystallinity.
- The comparison of experimental results between independent analyses will require the use of a series of standards across a broad range of crystallinity.

These findings may be applicable to XRD crystallinity indices as well. In fact, Hedges, Millard & Pike (1995) report a systematic relationship between grain size and peak width in XRD data. Since SF is affected by crystal size, it is likely that any grinding will cause some reduction in SF, particularly in highly crystalline bone. For this reason, grinding should be kept to a minimum. Mortar and pestle grinding is difficult to standardize, apparently very destructive, and should be avoided, unless grain size is controlled.

To allow calibration of SF measurements between studies, a series of standards should be created by controlled heating at approximately 200, 400, and 600°C, as in Experiment II above (following Shipman, Foster & Schoeninger, 1984). These temperatures were chosen because they cover a broad range of crystallinity, including those in which the detrimental effects of sample preparation are substantial. Both mean values and standard deviations should be reported. If these standards can be correlated between studies, then we will be able to compare the crystallinity of bones between studies, sites, and geochemical context, but until this is done, all studies using measures of bone mineral crystallinity will have to stand alone. These issues refer specifically to the goals of semi-quantitative comparisons of SF and possibly other FTIR semi-quantitative indices. Obviously, they are not particularly relevant to studies employing

Table 3. Mean SF and standard deviations for the proposed bone standards for the recommended sample preparation method (Hayonim Method) in comparison to results for the same specimens using two sample preparation techniques from Experiment 2

Temperature (°C)	Sample preparation technique		
	Hayonim method	GU from Experiment 2	GC from Experiment 2
206	2·77 ± 0·05	2·93 ± 0·05	2·90 ± 0·06
415	3·05 ± 0·04	2·79 ± 0·13	3·13 ± 0·09
617	4·25 ± 0·04	3·57 ± 0·04	4·34 ± 0·11

qualitative analyses of mineral presence–absence (e.g. Weiner, Goldberg & Bar-Yosef, 1993).

Proposed Sample Preparation Method and Standards

The following method of sample preparation was adopted for the Hayonim study of bone mineral diagenesis (Stiner *et al.*, 2001) because it was found to produce highly replicable results, independent of sample preparer, and with relatively low and consistent levels of error. A few grams of bone were mechanically ground for 15 s in the Wig-L-bug ball mill. The resulting powder was sifted through nested 63- and 45-μm sieves. Approximately 3 mg of the 45–63-μm fraction of bone powder was combined with KBr to produce a mixture of 1% bone and 99% KBr. This mixture was reground in the Wig-L-bug for 30 additional seconds for homogenization. A 50-mg aliquot of the resulting homogenized powder was then pressed into a pellet. Although it is suggested that other laboratories also adopt this technique for future research, doing so will not necessarily ensure comparability between studies because a number of additional factors not considered here may also contribute to measurement error (both accuracy and precision). These include crystallinity variation within individual samples, sintering pressures and times, solid-state reactions between the sample and matrix material (KBr), pellet concentration, and spectral resolution, and potential systematic differences between individual FTIR's (Fridmann, 1967; Smith, 1996).

Comparability can be achieved, however, through calibration against a widely available set of standards. Ideally calibration could be achieved through standard apatite samples that could be distributed among laboratories. Since none currently exist, the standards proposed above can be easily replicated with minimal equipment needs. For each standard, eight samples were run using the Hayonim method of sample preparation. Means and standard deviations for the bone standards are presented in Table 3 for the recommended Hayonim method along with the GC and GU methods from Experiment 2. Each method produces statistically different SF values for the same specimens, particularly at higher levels of crystallinity. This further highlights the need for standardization in FTIR measures of bone mineral crystallinity.

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