RADIOCARBON, Vol 49, Nr 2, 2007, p 211–216

REMOVAL OF CONTAMINANTS FROM ORACLE BONES DURING SAMPLE PRETREATMENT

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ABSTRACT. Animal bones and tortoise shells were used for divination by the Chinese royal family during the Shang Dynasty (~16th–11th century BC), and the divination results were recorded as inscriptions on oracle bones and shells, which are very valuable cultural remains and record many important events in the Shang Dynasty period. Thus, radiocarbon dating of oracle bones was used to build a precise chronology of the late Shang Dynasty. Due to their original burial conditions and the fact that in subsequent decades the pieces were traded or archived in museums, oracle bones are expected to be contaminated with exogenous materials from the environment and the conservation process. During dating, we found that some samples were contaminated by conservation chemical reagents. The contaminated samples were purified by removing exogenous chemicals with a series of organic solvents, in a method modified from Bruhn et al. (2001). Both whole bone and gelatin samples were processed with this purification method, resulting in satisfactory improvements in dating results.

INTRODUCTION

Inscriptions on bones and tortoise shells were thought to be the oldest written characters in China, and were used for divination by the Chinese royal family during the Shang period. The animal bones and tortoise shells were called oracle bones and oracle shells, respectively, and are together termed *Jiagu* in Chinese. Some Jiagu were correlated with royal dates of the Shang Dynasty, important historical events, astronomical incidents, etc., and are very valuable artifacts for studying the history of the Shang Dynasty. Oracle bones are also good materials for radiocarbon dating.

The Xia–Shang–Zhou Chronology Project aimed to establish a chronological framework for the 3 earliest dynasties in Chinese history, with a specific sub-project called "Dating and Phasing of Yinxu Oracle Bones." Selected oracle bone ^{14}C ages were determined by accelerator mass spectrometry (AMS), their calibrated ages compared to late Shang Dynasty events, and the results used to model the sequenced phases (Bronk Ramsey 1995, 2001).

During initial chemical treatments and age measurements of the bone samples, we found that a few of the samples were contaminated with conservation chemicals at some point in their archiving.

SAMPLES

A total of 107 bone samples were collected from archives such as the Institute of Archaeology of the Chinese Academy of Social Sciences, the Chinese National Library, and the Shandong Provincial Museum. Because oracle bones are very precious, approval from 4 Chinese ministries had to be obtained, which eventually allowed for the collection of 1- to 2-g samples from each oracle bone.

PROBLEMS USING ROUTINE PRETREATMENT

Considering the amount of time the artifacts had been in various collections or museums and lacking any conservation documents, researchers were very discreet in their treatment of bone samples. We

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Proceedings of the 19th International ^{14}C Conference, edited by C Bronk Ramsey and TFG Higham
RADIOCARBON, Vol 49, Nr 2, 2007, p 211–216

consulted with archaeologists about whether conservation chemicals were ever observed during previous sampling. The condition of the bone samples was also carefully examined in our laboratory. We studied and compared the different pretreatment methods for bone samples (Brown et al. 1988; Stafford et al. 1988; Nelson 1991; Hedges and van Klinken 1992; Arslanov and Svezhentsev 1993), first using a routine process to treat bone samples that were previously successfully dated. After physical examination, the samples were ultrasonically cleaned in deionized water; washed with acid (0.5N HCl), alkali (0.2–0.5N NaOH), and acid (0.5N HCl); hydrolyzed at 90 °C (pH 2–3); then filtered through a glass fiber filter and lyophilized as per routine pretreatment for bone samples (Yuan et al. 2000). In the initial results on 31 samples measured with ENAMS at Peking University, a few samples were found to be obviously older than the ages expected by archaeologists of the Shang period. We thought the most likely explanation for the cause of the anomalous results might relate to the incomplete removal of unknown contaminants by the standard pretreatment procedure. This assessment was confirmed by Fourier transform infrared (FTIR) analysis.

FTIR is a sensitive analytical method that can be used to identify chemical structure and groups of unknown materials, and can also be used to test the extent of the purification of bone protein. We applied FTIR to archived gelatin samples after dating. The results of FTIR showed that, compared with the samples that were in the expected age range and chemical reagent gelatin, those obviously older than expected—such as SA98244, SA98234, SA98197, and SA98198—exhibited an evident absorption peak at 2925–2930 cm^{-1} (Figure 1). The antisymmetric stretching of CH_2 in chain alkanes indicated that the anomalously older gelatin samples were probably contaminated with chain alkanes.

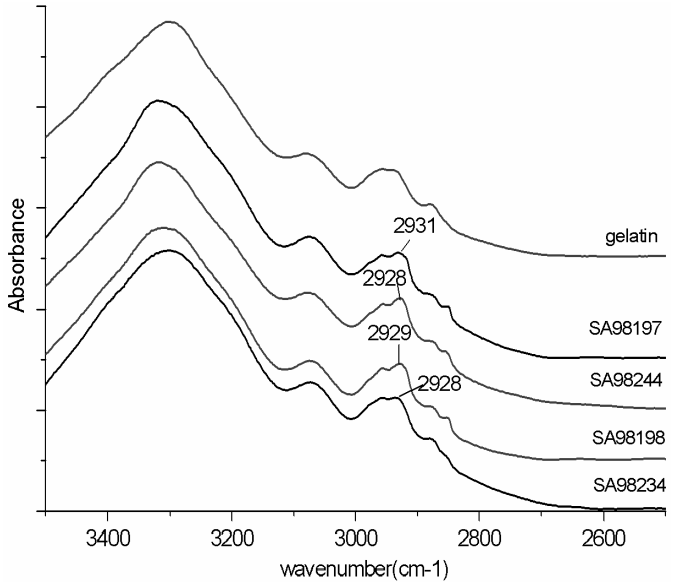

Figure 1 The FTIR spectra of standard gelatin and anomolously aged oracle bone samples, which exhibited an evident absorption peak at 2925–2930 cm^{-1}.

To further clarify the nature of the contaminants in the bone samples and apply appropriate chemical pretreatments, we examined 62 untreated samples with a microscope and selected questionable substances found in cracks or holes on their surfaces. The questionable substances were then analyzed with FTIR. The results indicated that some of bone samples did have conservation chemicals and adhesives, specifically:

- Sample SA98199 had adhesive on its surface, which is the copolymer of tri-polymethacrylic resin according to the FTIR spectrum (Figure 2).
- Samples SA98203, SA98230, and SA98239 had adhesive films on original marks, which were identified as nitrocellulose lacquer according to FTIR spectra (Figure 3).
- For samples SA98168, SA98224, and others, there were peaks at ~2925–2930 cm^{-1}, which indicated the existence of chain alkanes.

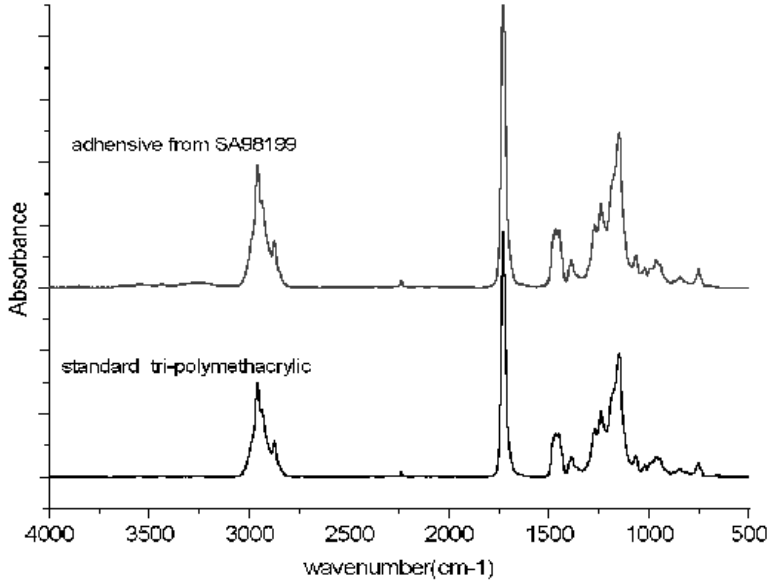

Figure 2 FTIR spectra of adhesive from SA98199 and standard tri-polymethacrylic

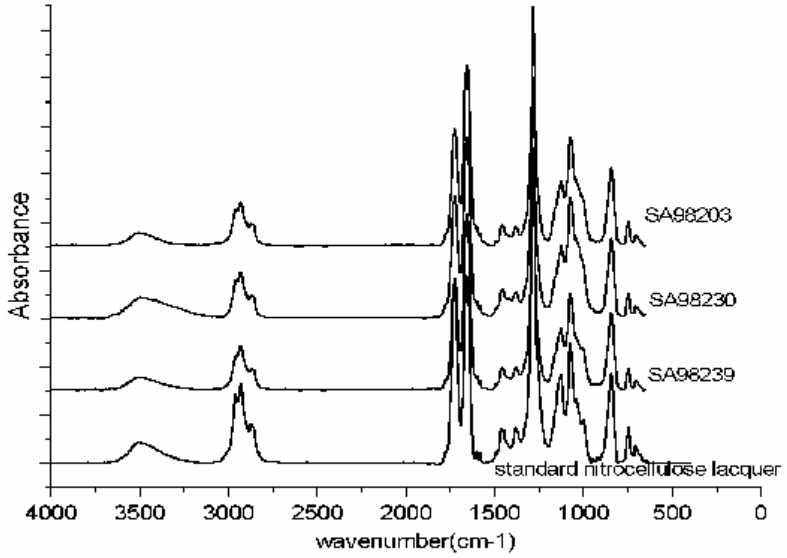

Figure 3 The FTIR spectra of adhesive from SA98203, SA98230, SA98239, and standard nitrocellulose lacquer.

METHODS FOR REMOVING THE CONTAMINANTS

Most existing methods for purifying bone and gelatin samples that are contaminated with conservation chemicals and adhesives are suited to specific substances; it is difficult to purify objects such as oracle bones, which contain unknown contaminants. Bruhn et al. (2001) used a computer-controlled Soxhlet-type extractor to remove deliberately added contaminants on wood pieces of known ages, such as rubber glue, wood glue, epoxy resin, methyl cellulose, Caparol, Klucel, sugar, polyethylene (PEG), paraffin, and beeswax. The solvents used included trichlorethylene or tetrahydrofurane, xylole or trichlormethane, petroleumether, acetone, and methanol. We applied these solvents to purify the gelatin and bone samples. We found that the method of Bruhn et al. (2001) is widely applicable. To increase the versatility of the removal of different contaminants (especially paraffin and beeswax) and to avoid excess heating that would potentially result in a loss of collagen, Bruhn et al. (2001) modified the suite of solvents, using tetrahydrofurane instead of trichlorethylene, and trichlormethane instead of xylole. As far as both the treated objects and contaminants were concerned, this modification was more relevant for our work. Generally, contaminants such as tri-polymethacrylic resin and nitrocellulose lacquer can be dissolved with these organic solvents, and most of our oracle bone samples were purified with tetrahydrofurane, trichlormethane, petroleumether, acetone, and methanol.

Chemical Treatment Modifications for the Purification of Gelatin

Bruhn et al. (2001) presented a method for automated Soxhlet-type extractions for wood, which we took as a starting point and modified for bone protein. Five organic solvents were divided into 2 groups according to their water solubility, with the first group composed of acetone and methanol, and the second composed of trichlorethylene, xylole, and petroleumether. For water-soluble organic solvents, the gelatins were put in columns and eluviated with solvents; for insoluble solvents, the gelatin samples were solved with water and then extracted with organic solvents.

The extraction procedures using water-soluble solvents were as follows. A small amount of quartz wool was placed in the bottom of a glass exchange column with a 2-mm interior diameter and a length of 200 mm. Approximately 20 mg of gelatin for purification was poured into the column, and eluviated with 20 mL of acetone and then 20 mL of methanol. The gelatin in the column was then washed with solvents in deionized water and filtered with filter glass fiber. The filtered gelatin was transferred to a separatory funnel and extracted 3 times with trichlorethylene, xylole, and petroleumether, respectively. The gelatin solution was heated at boiling point to remove residual organic solvents, and then lyophilized. A total of 9 samples were purified with this method (Table 1). Among them, some samples have ages evidently older than their real ages (e.g. SA98234, SA98244, SA98197, and SA98198). To verify the efficiency of the purification method, samples with ages that were considered to be within the expected range were also treated with this method (SA98252, SA98242, SA99094, SA99097, and SA98169), and their results were compared to the original analysis. This comparison showed that the extraction method did not itself add exogenous carbon to the material.

The purified gelatin samples were converted into graphite and measured by the Peking University (PKU) AMS machine. The results of all experimental samples are compiled in Table 1. The results show that the ages of anomalously old samples returned ages within expectation after solvent purification. Additionally, the ages of purified samples at 1 σ that were considered within expectation are in agreement with their original ages at 2 σ before purification. The FTIR spectra of the evidently older samples show that the peaks at 2925–2530 cm^{-1} disappeared, indicating that the contaminants were removed after purification. Taken together, this suggests that the purification method is effective.

Table 1 Purification efficiency of gelatin.

Lab nr[a]	Original ^{14}C age (BP)	$\delta^{13}C$ (‰)	Recovery ratios of gelatin	^{14}C age after purification (BP)	Can ^{14}C ages be included in the calibration model?[b]
SA98169-1	3160 ± 40	–8.06	—	—	No
SA98169-2	3065 ± 35	–7.79	—	—	Yes
SA98169p	—	–7.89	76.5	3075 ± 30	Yes
SA98234-1	3275 ± 45	–8.20	—	—	No
SA98234-2	3230 ± 30	–8.12	—	—	No
SA98234p	—	–8.20	64.6	3040 ± 30	Yes
SA98244-1	3545 ± 40	–12.82	—	—	No
SA98244-2	3650 ± 35	–12.76	—	—	No
SA98244p	—	–11.31	68.9	3065 ± 35	Yes
SA98242	3040 ± 30	–7.36	—	—	Yes
SA98242p	—	–7.29	64.4	3055 ± 35	Yes
SA99097	2980 ± 35	–10.26	—	—	No
SA99097p	—	–10.26	31.2	2925 ± 35	Yes
SA98197p[c]	—	—	7.1	—	—
SA98198p[c]	—	—	6.0	—	—
SA98252p[c]	—	—	3.1	—	—
SA99094p[c]	—	—	18	—	—

[a]The letter p following the lab number indicates a purified gelatin sample.
[b]Indicates whether or not the ^{14}C age can be included in the model of sequenced phases with an agreement index high enough when the model is calibrated with OxCal v 3.9 (Bronk Ramsey 1995, 2001).
[c]Recovery ratio is too low; no sample was prepared and measured.

Table 1 also indicates that the yields of various samples can differ greatly, and some sample yields are too low after purification for further preparation and measurement. Additional experiments showed that low yields mainly resulted from the gelatin dissolving in methanol. To improve the yields, we used ethanol instead of methanol; the gelatin losses were reduced and the purification effect was also evident. We also used 5 solvents successively to try to eluviate the gelatin in the column and also obtained evident purification effects on the protein. This alteration of the method produced a slow flow velocity, which resulted from gelatin swelling in solvents such as methanol. Thus, this modification to the method is unsuitable for routine work.

Purification of Oracle Bone Samples

Usually, collagen is not soluble in methanol, yet we observed some gelatins that were. This probably resulted from the poor preservation of collagen in some bone samples. During gelatinization of collagen, many small peptides are formed that are soluble in methanol. If methanol was applied directly to bone samples before gelatinization, the soluble phenomena could be avoided or reduced greatly. In additional experiments, we used trichlorethylene, xylole, petroleumether, acetone, and methanol to treat 8 likely contaminated bone samples by ultrasonicating physically cleaned bone samples, placing the material in 50-mL ground-glass stopped conical flasks, and rinsing with 30 mL each of trichlorethylene, xylole, petroleumether, acetone, and methanol, respectively. The solutions were vibrated 3 times for 30 min each at middle vibration velocity. The last methanol wash was flushed with deionized water and gelatinized by the routine method, graphitized, and measured by ENAMS at PKU. The yields were normal on the whole, and there was no evident older age in the dating results. The results of these 8 preliminary samples led us to use the tetrahydrofurane,

trichlormethane, petroleumether, acetone, and methanol modification to purify 62 additional bone samples. Half of the 62 bone samples were analyzed by FTIR, and 9 were found to be contaminated. Because all 62 samples were from archives without any records regarding their conservation treatments, we decided, as a precaution, to use the modified procedure as a standard method of treating the bone samples from museums. The results of measurements by the compact AMS (National Electrostatics Corp., Middleton, Wisconsin, USA) at PKU were mostly satisfactory, with the exception of several samples requiring further research. The report on results of these additional experiments and analysis is now in preparation.

CONCLUSION

Our 6-yr investigation on the efficacy of chemical treatments on oracle bones indicates that artifacts such as these, which have come from museums and research institutes, are likely to have been conserved in some manner, and rigorous purification methods should be used to remove contaminants before routine pretreatment. The results of our experiments using a modified organic solvent chemical treatment on oracle bones indicate that the method was effective in bringing most anomalous ages into agreement with expectations, even when the exact nature of the contaminant or conservation material was not known.

This is a significant improvement for the study of these important artifacts, considering the restrictions on access to the samples, combined with the highly variable conditions of storage, lack of conservation records, and likely variable protein survival. The research results reported here also indicate additional improvements that may be made in the future and that are currently under investigation.

ACKNOWLEDGMENTS

This work is supported by the Xia–Shang–Zhou Chronology Project. We are grateful to the following institutions for providing the oracle bone samples: Institute of Archaeology, the Chinese Academy of Social Sciences; National Library of China; Shandong Provincial Museum; Palace Museum; Tianjin Museum; Arthur M Sackler Museum of Art and Archaeology at Peking University; Institute of History, the Chinese Academy of Social Sciences; Museum of Jiling University; and the National Museum of China. We would also like to especially thank Professors Liu Yiman, Cao Dingyun, and Huang Shuze for their help in collection samples, for providing phase information of samples, and for their valuable discussions.

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