CHAPTER 3

MATERIALS, SAMPLING, EXPERIMANTAL METHODS AND ANALYTICAL TECHNIQUES

This chapter deals with materials and methods, experimental procedures, sampling techniques and site background.

3.1 General optimisation and validation procedures

For all of the cleaning methods developed, a general optimisation and validation procedure was followed so as to prevent bias and irregularities occurring for any particular method. Furthermore this was also necessary for final comparison of all the methods developed. Emphasis was also placed on validation for each method developed as it is imperative that no damage is incurred to the fossil specimens through cleaning.

3.2 Reagents

3.2.1 Preparation method using hydrogen peroxide

Hydrogen peroxide (analytical grade 30%, obtained from Saarchem) was used. All dilutions were prepared using Milli-Q distilled deionised water.

3.2.2 Preparation method using nitrites

Analytical grade sodium nitrite, purchased from Sigma, was used.

3.2.3 Preparation method using hydroxylamine

All solutions were prepared from 99% assay hydroxylamine chloride obtained from NT Laboratory Supplies (PTY) LTD.

3.2.4 Preparation method using Chromium(III)

Chromium (III) as $Cr(NO_3)_3 \cdot 9H_20$ of purum, p.a. was used for all solutions, and was obtained from Fluka Chemie, AG, CH-9470 Buchs.

3.3 Sampling methods, handling and protocols

Sampling is the process of selecting a representative number from the total (Harris, 1998). The use of new methods of sample preparation and extraction, and the advent of modern technology, makes it possible to measure a broad range of trace elements at concentrations as low as parts per trillion. Unfortunately being able to have such high detection levels comes at a price, as it means sampling and sample storage have to be carried out in such a way that ensures representation of all samples, and no contamination (Horowitz, 1997). This means that a large percentage of analytical errors originate from sampling. These include: contamination, loss, and physical or chemical perturbation (Riley *et al*., 1975). These are all matters that were considered and avoided.

3.4 Fossil sites sampled

Fossil fragments were obtained from Coopers D and Gladysvale open and closed excavated cave sites. These are two of many Plio-Pleistocene sedimentary deposits in the Cradle of Humankind, South Africa. Fossils obtained from Gladysvale were only used in

the early stages of this research, so focus is placed here on those from Coopers D. The site is located approximately 45 km northwest of Johannesburg, between Sterkfontein to the west and Kromdraai to the east (Berger, *et al*., 2003).

3.4.1 Manganese oxide coated fossil specimens

Coopers D: Based upon faunal remains, the site has an age of ~1.6-1.9 million years old. A wide range of mammal and reptile species are represented, including remains of *Paranthropus robustus*, a hominin that lived in South Africa between 1 and 2 million years ago. Fossils occur in calcified breccia deposits now open to the surface (Berger, *et al*., 2003).

Gladysvale: Based upon faunal remains, the site has an age range of 200 kyr to 1.3 million years old. A wide range of mammal species are represented, including the remains of *Australopithecus africanus.* These fossils occur in decalcified deposits now open to the surface (Hall, *et al*., 2006).

3.4.2 Hematite coated fossil specimens

All haematite coated fossils were obtained from the Karoo collections from the Bernard Price Institute for Palaeontological Research at the University of the Witwatersrand, and are much older than the manganese oxide coated fossil specimens, dating in excess of 60 million years old. Many of the specimens include fragments of dinosaurs or early mammal-like reptiles.

3.5 Sampling procedures and methods

Sample specimens collected from the Coopers D deposits were stored in a safe lock up cabinet until experimental work commenced. Rare hominid remains were locked away in a fire resistant safe, and were not used for experimental purposes. A few elements were cleaned once the method was optimised and validated.

Micro sampling techniques included ultra fine drilling of the surface coatings for microwave digestion for elemental analysis. Chiselling or fragmentation of portions or the whole specimen to be used for optimisation and validation experiments using various concentrations and for different durations was performed on different bone fragments performed in duplicate. For the mass cleaning procedure, the entire faunal assemblage from Coopers D was subjected to the optimised and validated hydrogen peroxide cleaning process. In total, on completion of this project, which is ongoing, approximately 7000 specimens would have been chemically cleaned. This is to enable taphonomic study of each bone in the collection, so as to prevent bias, and provide an understanding of what agent(s) modified and accumulated the bones.

3.5.1 Protocols

Selection of fossil sample specimens was carried out by qualified members of the Institute for Human Evolution (School of Geosciences at the University of the Witwatersrand). Any destructive work (dissolution of fossils and any other morphological alterations) was performed on unidentifiable bone fragments with the permission of the permit holder.

3.5.2 Sample selection for optimization and validation experiments

Due to the fact that each bone is unique in size, texture and shape, all bones selected for experimental purposes were of similar size and only the flat and relatively smooth surface bones (cortical) were used initially. Once a method was optimised and validated, other bone types (porous spongy) were treated under the same conditions to ensure the method worked across a broad spectrum of bone types. Fossil teeth all exhibit a flat surface and are of similar size, so sample selection was not as stringent as for bone material.

3.6 Optimisation and validation

All methods developed were optimised and validated using a number of different techniques and methods, including Scanning Electron Microscopy (SEM), gravimetric analysis and elemental evolution trends. Optimisation was performed using matrix experiments with bone specimens of similar sizes and textures (For example, flat, small bones approximately 5 cm in diameter). All experiments and experimental points for each matrix and validation were performed in duplicate and repeated between two and three times to ensure reproducible results.

Validation methods included weighing residues and bone fragments before and after treatment, bone surface examination using SEM and segmentation of bones before treatment followed by edge to edge realignment analysis.

3.7 Analytical techniques and instrumentation

3.7.1 Inductively coupled plasma optical emission spectroscopy (ICP-OES)

Compared to ordinary flames in spectroscopic methods, inductively coupled plasma attains much higher temperatures. In addition, most of the new and advanced analytical techniques are also able to eliminate many of the common interferences caused by the sample or flame itself (Harris, 1999).

For the determination of total metal concentrations in the solutions after and during bone immersion, an inductively coupled plasma optical emission spectrometer (ICP-OES) with a charge coupled device (CCD) detector (Spectro Ciros, Klever, Germany) was used.

All ICP-OES measurements were conducted using a Spectro Genesis Automatic Optical Emission Spectrometer. An inductively-coupled plasma excitation and a semiconductor-based detector system for quantitative and semi-quantitative analysis were employed.

3.7.2 pH meter and electrode

The pH of reagents and reaction products was determined using a multilane P4-SET3 pH meter (WTW, Germany) equipped with a pH combined electrode with integrated temperature probe (SenTix41).

3.7.3 Scanning electron microscope (SEM)

In scanning electron microscopy (SEM), backscattered electron (BSE) imaging is used. An SM-840, JEOL, Tokyo SEM was used to examine high-resolution resin replicas of bone surfaces. The replicas were prepared using standard methods (d'Errico, 1998; Backwell and d'Errico, 2001).

3.7.4 Atomic absorption spectroscopy (AAS)

The major limitation of this method is that it is non-sensitive to non-metals and cannot distinguish different states of metal.

A Varian Spectr AA-10 Atomic absorption spectrometer (using flame atomization) was used, and was fitted with a manganese hollow cathode lamp.

3.7.5 Raman spectroscopy

The raman spectroscopy of bone tissue has a disadvantage as fluorescence interference from collagen occurs if visible or near-UV excitation is applied, and from haem iron if NIR excitation is utilized (Goodship *et al*., 2003).

A majority of all tissue specimens show intense fluorescence when excited with visible laser light, and scatter light at all wavelengths at which silicon photo detector Raman spectroscopy is feasible. Analysts in Raman tissue spectroscopy have therefore generally employed near-infrared lasers to obtain spectra from specimens (Goodship *et al*., 2003).

A state of the art Jobin-Yvon T64000 triple spectrograph was used to obtain all Raman spectroscopy data.

3.7.6 Powder X-Ray diffraction

Powder X-ray diffraction is used to identify the different crystalline phases present in a solid sample. X-ray crystallography relies on the dual wave/particle nature of X-rays to discover information about the structure of crystalline materials. The most well known use of X-ray powder diffraction is for the identification of crystalline compounds by their diffraction pattern (Connolly, 2005). All powder X-ray diffraction measurements were recorded on a Brooker D8 X-ray Powder Diffractometer.

3.7.7 X-ray microprobe analysis

X-ray fluorescence spectroscopy is no longer regarded as a new instrumental technique for elemental analysis; however continuous evolutionary developments continue to redefine the role of this important analytical tool for surface elemental mapping.

The phase analysis of the chemical structure of bone was conducted with the use of tabletop XRD device - Philips X'Pert MPD in the Institute of Physics of Polish Academy of Science in Warsaw, Poland. The data were compared with the JCPDF (International Centre for Diffraction Data) database.

The X-ray fluorescence synchrotron-based measurements were made in HASYLAB, Hamburg, Germany. During the analysis, the sample was exposed to a monochromatized beam composed of photons with mean energies close to 17 keV. Energy of photons was obtained with the use of Si (111) monochromator and focused with a polycapillary

semilens. Mapping of the bone structure was made for selected sample areas with a step of about 10 μm in each direction. Step size was chosen to be comparable to the diameter of the radiation beam, around 11 μm.

The supplementary analyses were done with the use of a scanning electron microscope LEO 1430 VP in the Department of Chemistry, Catholic University of Lublin. The microscope is equipped with an Si(Li) detector (made by RÖNTEC) with a resolving power of 180 eV, measured for MnKα line. A voltage of 20 keV and the beam current of 0.7 nA were applied in all the measurements. Such parameters enabled the detection of a wide range of elements comprising the sample, including very light ones (starting from C). The lightest elements could not be detected in HASYLAB.

All samples were analyzed using a Spectro Midex M Micro-XRF system. This is a powerful XRF instrument as it combines a high voltage capable x-ray tube (up to 50 kV) with the most modern energy dispersive x-ray detection systems (Si drift chamber). This system combines the intense excitation of the 30 W tube and good spatial resolution with the silicon drift chamber detector with a throughput count rate of 100, 000 cps, which allows for fast mapping over large areas.