CHAPTER 10

ADDITIONAL POTENTIAL CHEMICAL METHODS FOR THE REMOVAL OF MANGANESE OXIDE COATINGS FROM FOSSIL BONES

Methods briefly investigated that are either not suitable due to kinetic constraints or due to inadequate validation have also been investigated and discussed. These methods are presented in this chapter for future research purposes.

10.1 Diethylene triamine pentaacetic acid (DTPA)

This ligand is widely used for complexing certain metals. Although DTPA also complexes with calcium, which is not favourable for this application, the stability constants for the formation of complexes with iron and manganese are larger.

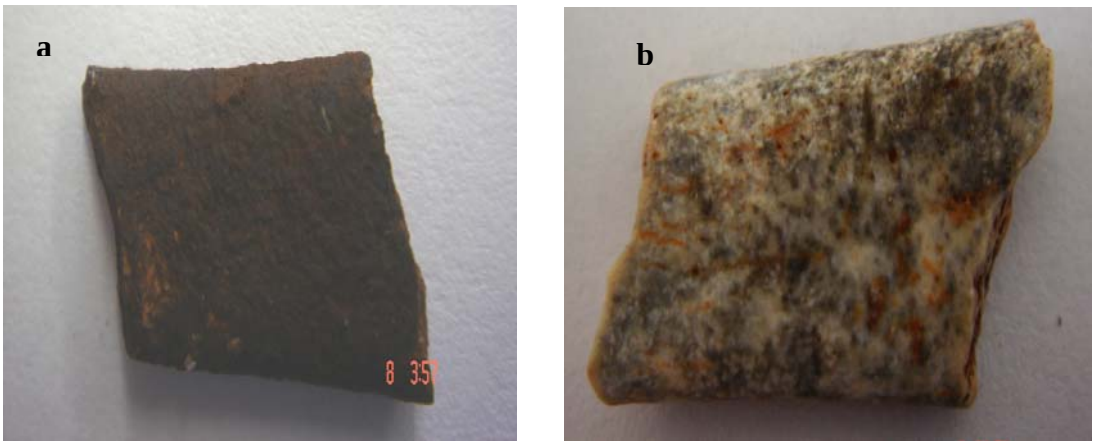

Figure 10.1 Manganese oxide coated fossil bone fragment **a,** before treatment in DTPA **b,** after treatment in a DTPA solution of 0.01 mol L^{-1} at natural pH treatment of manganese oxide coated fossil bone fragment in the DTPA solution.

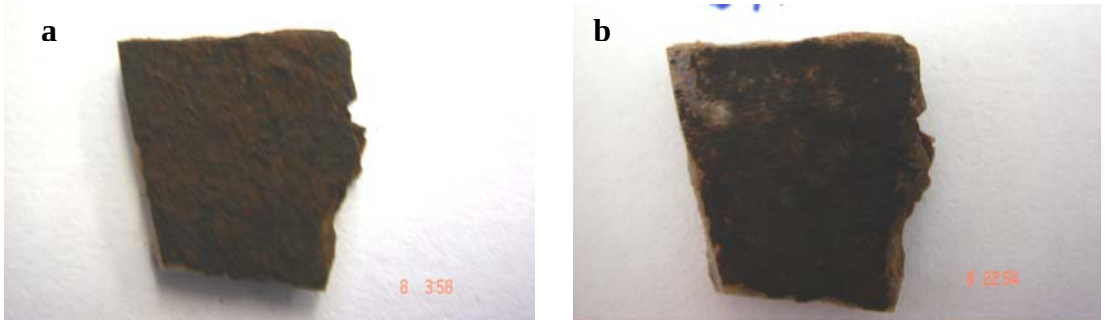

Figure 10.2 Manganese oxide coated fossil bone fragment **a,** before treatment in DTPA **b,** after treatment in a DTPA solution of 0.001 mol L^{-1} of manganese oxide coated fossil bone fragment in the DTPA solution.

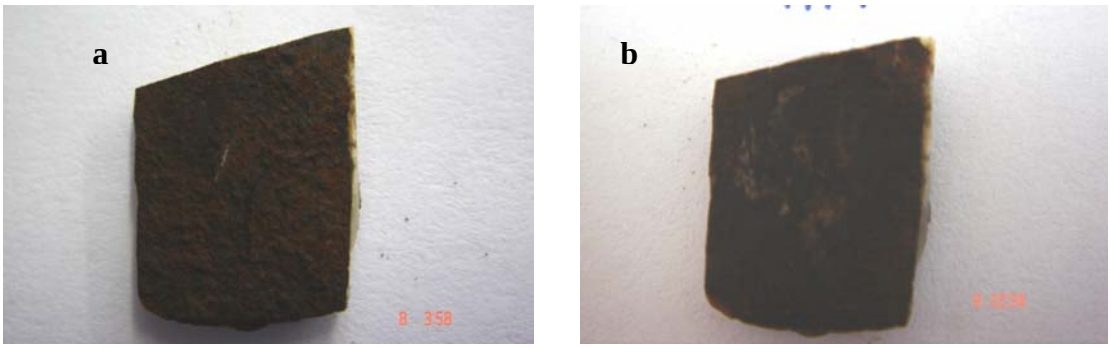

Figure 10.3 Manganese oxide coated fossil bone fragment **a,** before treatment in DTPA **b,** after treatment in a DTPA solution of 0.0001 mol L^{-1} of manganese oxide coated fossil bone fragment in the DTPA solution.

Figure 10.4 Comparative images of three different fossil bone fragments after treatment in 0.01 mol L^{-1}, 0.001 mol L^{-1} and 0.0001 mol L^{-1} DTPA ligand concentrations.

From the before and after images for each bone fragment soaked in DTPA at the natural pH as shown above, it can be observed that the concentration of DTPA used has a direct effect on the degree of removal of manganese oxide coating from the surface of the fossil bone specimen. Although this method has not been validated using SEM and gravimetric analysis, from the overall appearance of the bone surface (colour and texture), it looks as though no obvious dissolution occurs for the highest DTPA concentration employed of 0.01 mol L^{-1}.

10.1.1 Stability constants for DTPA metal-ligand complexes

Stability constants can help to accurately predict and explain the trends observed for the DTPA reaction at the bone (predict bone dissolution) and manganese oxide surface (removal of the dark oxide coating) of the fossil bone and are thus presented in table 10.1 below.

Table 10.1 Stability constants for some DTPA metal complexes of interest (Martell, *et al*., 2004).

Metal.	**Complex**	**LogK**	**Temperature / C^{o}**	**Ionic strength**
Mn^{2+}	ML	15.2	25	0.1
Mn^{2+}	MHL	4.45	25	0.1
Mn^{2+}	M_2L	2.09	20	0.1
Fe^{2+}	ML	16.2	25	0.1
Fe^{2+}	MHL	5.30	25	0.1
Fe^{2+}	M(OH)L	9.41	25	0.1
Fe^{2+}	M_2L	2.98	20	0.1
Ca^{2+}	ML	10.75	25	0.1
Ca^{2+}	MHL	6.11	25	0.1
Ca^{2+}	M_2L	1.6	25	0.1

10.2 Acetic acid treatment

Acetic acid as a fossil bone preparation technique is not new, however it has only previously been used in the removal of calcareous coatings and matrices from fossil specimens vertebrates. The method required the use of dilute solutions of acetic and formic acid (Toombs and Rixon, 1959).

The main problem with the use of acetic acid and why it has been a common yet questionable method used in the preparation of fossil bones is that because it is a weak acid and can remove calcareous deposits from bones it also has in some case been found to cause weakening of the bone matrix itself rendering the fossil specimen more fragile, and damaging vital taphonomic information. In order to try and determine how effective this method of preparation is, two fossil bone fragments were used. Each was broken into two fairly equally sized pieces and each were allowed to soak for 6 hours in a designated concentration of acetic acid. Each specimen was dried and re-aligned with its corresponding fragment.

Figure 10.5 Fossil bone fragment (uncoated) **a,** before treatment in acetic acid solution **b,** after treatment in a 1.0 mol L^{-1} solution of acetic acid. **c,** rejoined fossil bone fragments showing visible signs of bone matrix and surface dissolution after soaking in the acetic acid solution.

The off-white glossy appearance of the fossil bone before treatment is typical and this is a result of diagenisis and other chemical processes.

The loose material visible on the image in fig.10.5 is bone matrix, which is breaking apart due to dissolution at the fissure or joint of the two fragments. The colour of this area is also indicative of bone matrix surface change having occurred, as the image that was taken before soaking appears to be a darker to off-white (cream). The lighter white material is the underlying matrix (chalk like texture) which has not undergone as much diagenisis as the surface layer, thus when it is exposed it resembles the true hydroxyapatite colour.

The 1.0 mol L^{-1} acetic acid solution used appears to alter the texture, colour and surface topography of the fossil specimen which was broken into two fragments and rejoined. The fossil bone fragment on the left side appears to show obvious signs of dissolution as severe smoothing of the surface has occurred even removing hairline

cracks that were previously visible. To the touch, the texture is also similar to chalk or a compacted powder like material, again this shows evidence that severe dissolution of the bone surface has taken place.

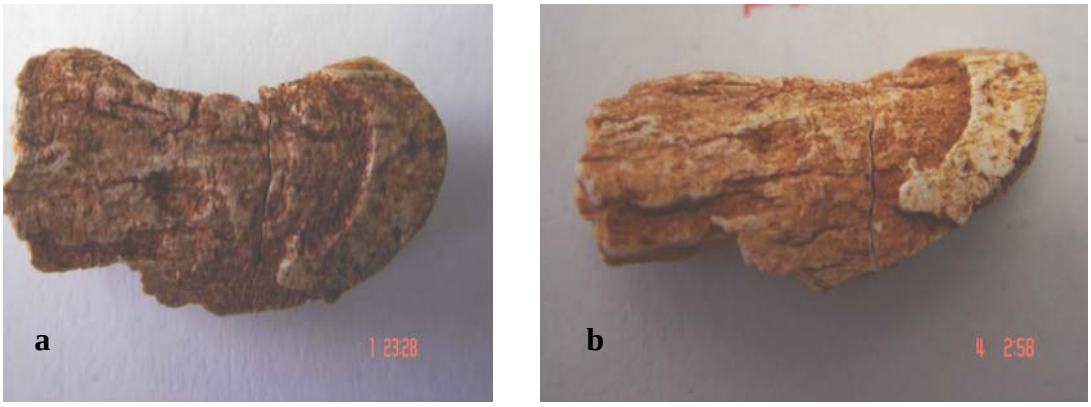

Figure 10.6 Fossil bone fragment (uncoated) broken into two fragments and rejoined **a,** before acetic acid solution treatment **b,** after treatment in a 0.1 mol L^{-1} acetic acid solution.

The lower concentration of acetic acid used in the treatment of the uncoated fossil bone above appears to change the surface of the fossil to a lesser extent than the previous concentration of 1.0 mol L^{-1} used.

The use of acetic acid for the removal of surface coatings on fossil bones could perhaps thus only be employed at extremely low acetic acid concentrations however the time of cleaning would then have to be extended as compensation.

10.3 Thioglycolic acid (TGA) as fossil bone preparation method

The use of thioglycolic acid in the preparation of fossil specimens coated in hematite has been investigated previously by Greenwood and Howe (1975). Previously all hematite matrixes were removed mechanically, however, only with varying degrees of

success. The main problem with the mechanical method was the finer surface features of the bone are often damaged or destroyed (Kummel and Raup, 1965; Rudner, 1972).

Table 10.2 Data pertaining to gravimetric and elemental analysis for three different % concentrations (v/v) of thioglycolic acid (TGA) solutions for three manganese oxide coated fossil bone fragments.

	0.1% TGA	**5% TGA**	**15% TGA**
Initial mass/g	0.3379	0.3032	0.2964
Final mass/g	0.3339	0.2823	0.2790
Change in mass	0.004	0.0209	0.0174
Mass % loss	1	7	6
Fe	0.3	3.1	2.9
Mn	2.1	8.4	9.2
P	3.6	37	44
Ca	6.8	57	70

The elemental concentrations in table 10.2 are final concentrations and therefore only an overall change can be observed, not relative to separate time intervals. This is only useful for the quantification of each element after the total treatment period.

The mass loss % for each different concentration of thioglycolic acid does not vary considerably from the concentration range used in the above gravimetric analysis. It also seems that the overall mass loss % levels off after significantly higher concentration of the thioglycolic acid solution is used. This could imply an eventual

stabilization of the Ca, P, Mn, and Fe evolution concentrations, thus preventing possible bone matrix destruction.

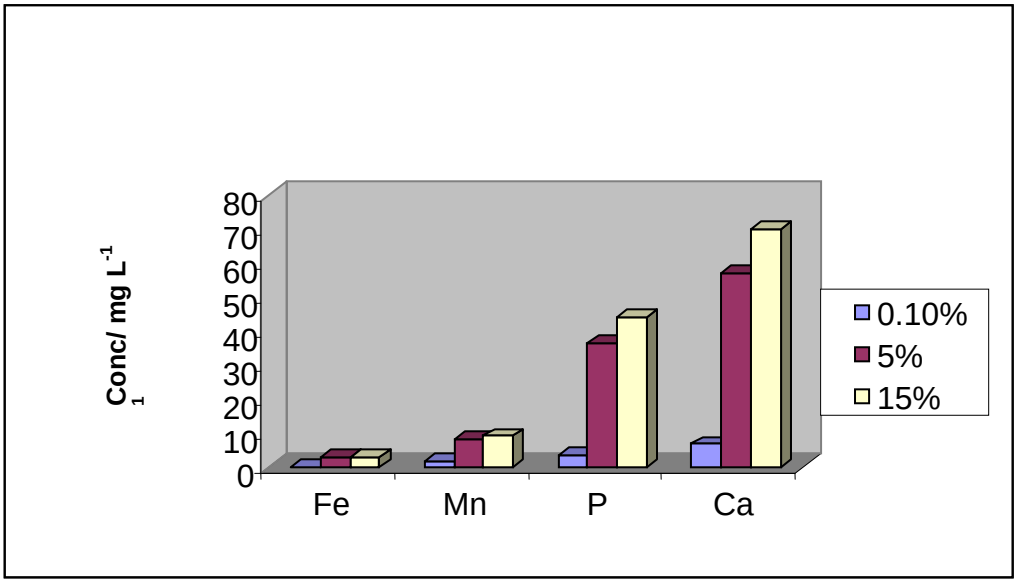

Figure 10.7 Different final elemental concentrations for three manganese oxide lightly coated fossil bone fragments soaked in three different % concentrations (v/v) of thioglycolic acid over an 18 hour period.

An expected trend of increased Fe, Mn, P and Ca concentrations from a thinly manganese oxide-coated surface of a fossil bone was obtained when the concentration of thioglycolic acid was increased, as illustrated in fig. 10.7. The coating itself, which contains fairly high concentrations of Ca and P, contribute to the elevated calcium and phosphorous concentrations evolved. Greenwood and Howes (1975) successfully demonstrated the preparation of hematite coated bones of the cranium of a specimen of a Perciform fish of Miocene age from Zaire, using a dilute aqueous solution of thioglycolic acid.

CHAPTER 11

CONCLUSIONS

11.1 Characterization of fossil bone coatings

Characterization techniques that were successfully used included, powder X-ray diffraction, Raman spectroscopy, IR spectroscopy, Inductively coupled plasma Optical emission spectroscopy, and X-ray microprobe and XRF mapping.

The manganese oxide fossil bone coatings were characterized to be mainly a type of birnesitte with variations also found to contain a number of other mineral compositions also being detected through Powder XRD analysis. Powder XRD analysis of five manganese oxide coating originating from five different fossil bone surfaces showed that the was no significant difference in the XRD pattern produced and hence shows that all the fossils from the site at which they were excavated are covered in a similar chemical composition of mineral coatings. It was established that these fossil bone manganese oxide coating are highly amorphous due to the poor resolution of the XRD pattern and Raman peaks obtained.

11.2 Hydrogen peroxide as a method for the removal of manganese oxide coatings from fossil specimens

Hydrogen peroxide was determined to effectively and efficiently remove manganese oxide coatings from fossil bones and teeth. The optimum concentration was found to be in a range of 7.6 – 9.7 mol L^{-1}. Validation of the method by gravimetric analysis and SEM proved that negligible surface and bone matrix modifications takes place, thus this cleaning method was found to be non-invasive to the fossil specimens. The

hydrogen peroxide was determined to act as a reducing agent when reacted with the manganese oxide coatings. It was also used to partially remove thick hematite coatings from dinosaur fossil bone fragments from the Karoo in South Africa.

The same reduction reaction did not occur when granular manganese oxide was precipitated with calcium carbonate and iron oxide. This indicates that the bone surface forms a key component in catalyzing the manganese oxide reduction reaction.

The main driving force of the reaction was shown to be the continual replacement of the hydrogen peroxide solution, thus removing the Mn(II) produced, and maintaining the proton gradient which drives the reduction process. This replacement is also important because the reducing solution is rapidly neutralized and most of the dissolution of the manganese oxide coatings occurs below neutral pH conditions. It should be noted that this method will also remove all organic residues (on and possibly in) the fossil material (Cukrowska *et al*., 2005).

The evolution of large amounts of oxygen also plays an important role in the removal of the oxide coating as a mechanical force and has to be monitored depending on the size of the bone fragment to avoid any possible mechanical damage of the fossil bone.

A flow through system was developed and implemented in the cleaning of approximately 7000 manganese oxide coated fossil specimens from the Coopers D site located in the Cradle of Humankind.

11.3 Nitrites as a method for the removal of manganese oxide coatings from fossil specimens

The non-invasive removal of manganese oxide coatings from fossil bones was achieved through the use of nitrites as a reducing agent. This cleaning method was optimized and the optimal pH and concentration of the nitrite solution were determined to be 3.50 and 1.8 mol L^{-1} respectively. Validation using SEM and gravimetric analysis proved that the use of nitrites under optimized conditions does not cause damage to the bone. This method proved successful in removing all manganese oxide coated bones that where treated under the conditions optimized.

11.4 Hydroxylamine as a method for the removal of manganese oxide coatings from fossil specimens

Hydroxylamine was shown to efficiently remove all manganese oxide fossil bone coatings for an optimized concentration of 0.14 mol L^{-1} and initial pH value of 3.75. Using the same validation techniques for the hydrogen peroxide and nitrite methods, the hydroxylamine was shown to be non-invasive, yet highly efficient in removing all manganese oxide fossil bone coatings.

The rate of manganese oxide removal was also found to be considerably faster than the other methods developed. From using the optimized conditions, a species distribution plot was generated. It shows that the main mechanism involved in the reaction between the manganese oxide and hydroxylamine is a reductive dissolution reaction rather than a complex formation reaction.

11.5 Chromium(III) as a method for the removal of manganese oxide coatings from fossil specimens

Chromium(III) was used to remove manganese oxide coatings from fossil bone specimens via a reductive oxidation reaction. The method was partially optimized for the initial pH value and Cr(III) concentration. The rate of removal was shown to be extremely slow with discolourations (green) of the cleaned bone surface. One of the products this cleaning method produces is chromium(VI) which is toxic and not environmentally friendly.

11.6 Alternative methods for the removal of manganese oxide coatings from fossil bone specimens

A number of other potential chemical methods for the removal of manganese oxide fossil coatings were investigated. These methods were, however, not optimized or extensively validated using the validation methods of SEM and gravimetric analysis.

Acetic acid, which has been previously used by palaeontology as a fossil bone preparation method was also examined, and shown to cause extensive dissolution and damage to the fossil bone surface.

11.7 Summary and Qualitative fossil preparation method analysis

Even though the three methods developed for removing manganese oxide coatings from fossil bone specimens were optimized and validated, each method has advantages and disadvantages. Selection of one of the developed cleaning methods would, thus depend on the degree of manganese oxide coating, type, size, and age of the specimen.

Table 11.1 Comparison for all developed and one non-optimized or validated method.

	Hydrogen peroxide	**Nitrites**	**Hydroxylamine**	**Chromium(III)**	**DTPA**
Replacement time/ hours	1-2 depending on specimen size	6-12	24	N/A	N/A
Sample size	Smaller samples	Any size	Any size	Any size	Any size
Initial pH	Approx. 2.9	3.54	3.75	Not optimised	Approx. 3.0
Final pH	Approx. 5.5	4.8	4.9	N/A	N/A
Efficiency	Good	Very Good	Very Good	Poor	Good
Disadvantage	Requires solution replacement	Nitrous gases produced	Requires continual monitoring	Slow and toxic Cr(VI) produced	Not validated yet