CHAPTER 7

REMOVAL OF MANGANESE OXIDE FOSSIL BONE COATINGS USING HYDROXYLAMINE

Natural manganese oxides (lithiophorite, todorokite and birnessite) have been known to dissolve in hydroxylamine at concentrations and pH similar to those employed in this method of optimisation. (0.25 mol L^{-1} hydroxylamine ($NH_2OH{\cdot}HCl$), pH 4.7 in 0.1 mol L^{-1} HNO_3 (Chao, 1972)).

$$2MnO_2(s) + H_4ClNO \rightarrow 2Mn^{2+}(aq) + NO_3^{-} + 2H_2O(l) + Cl^{-} \qquad 7.1$$

7.1 Procedure

Fresh solutions were prepared for each procedure from a stock solution. The pH of each standard solution was adjusted and then subdivided into replicate experiments for the purpose of reproducibility.

7.2 Optimization

A set of matrix optimization experiments were performed using a wide range of pH values and concentrations. Thereafter, a smaller optimized field range was selected. The optimum parameters were selected based on observed cleaning efficiency and minimum dissolution of the bone. Due to hydroxylamine having strong reducing and acid properties, all optimization carried out had to also focus on the minimum time required

for treatment of the fossil specimen, so as to prevent dissolution of the bone. Particular attention was paid to surface detail.

7.2.1 Initial optimization

An initial optimization was performed to determine an approximate set of optimal conditions for the concentration and initial pH of the hydroxylamine solution.

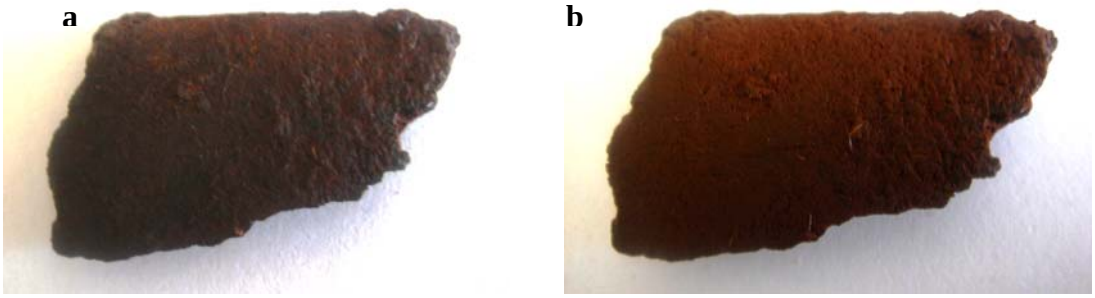

Figure 7.1 Thickly coated manganese oxide coated fossil bone fragment (1a) **a,** before treatment; **b,** after treatment in 0.0014 mol L^{-1} at pH: 3.10 hydroxylamine solution.

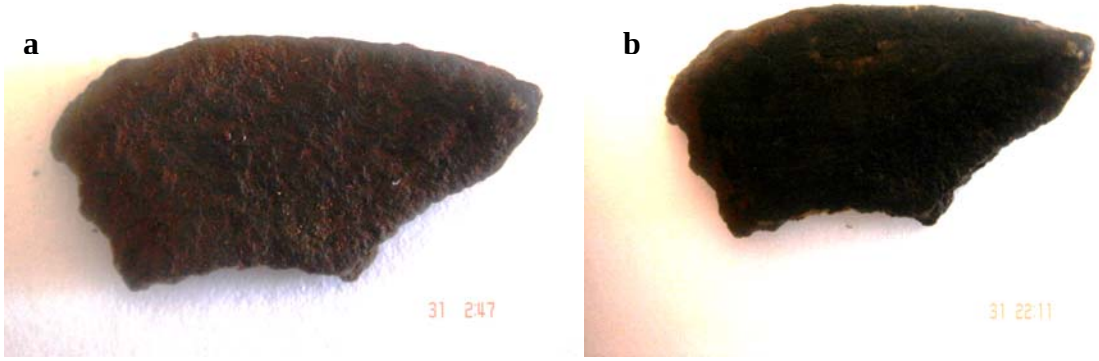

Figure 7.2 Manganese oxide coated fossil fragment (1b) **a,** before treatment; **b,** fossil fragment still heavily coated with manganese oxide after treatment in 0.0712 mol L^{-1} pH: 3.10 hydroxylamine solution.

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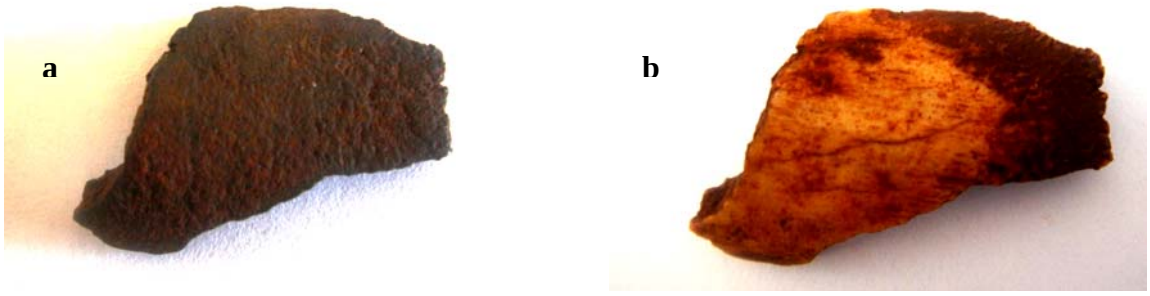

Figure 7.3 Fossil bone fragment (1c) **a,** before treatment in a hydroxylamine solution; **b,** after a hydroxylamine solution of 0.141 mol L^{-1} at initial pH: 3.10 is applied shows good removal of the surface coating.

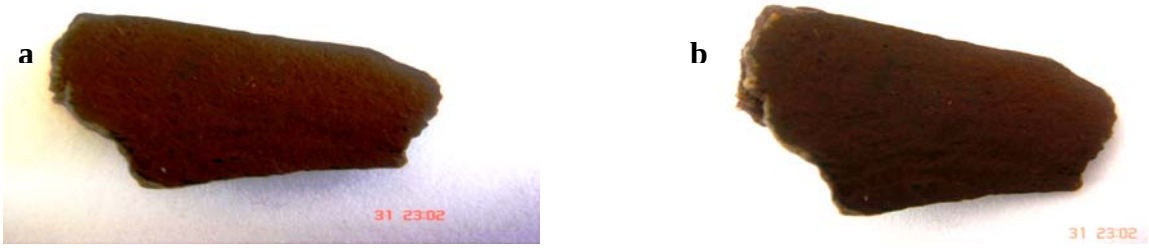

Figure 7.4 Manganese oxide coated fossil bone fragment (2a) **a,** before treatment with hydroxylamine; **b,** after treatment in 0.0014 mol L^{-1} hydroxylamine at initial pH: 3.68 only a small improvement around the edges can be noticed.

The conditions used in fig. 7.4 show almost no manganese oxide coating removal. This could be due to the extremely low concentration employed, limiting the amount of surface manganese that is reduced, and not enabling reduction of the manganese directly bonded to the bone surface.

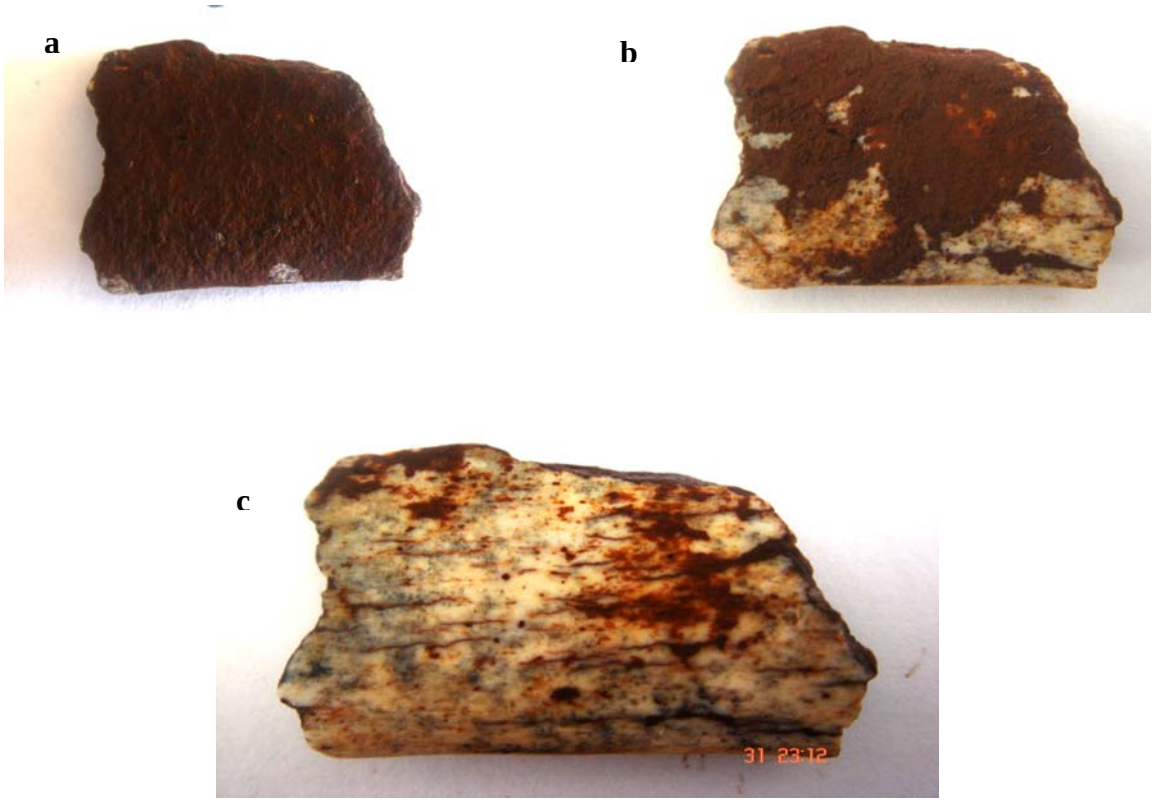

Figure 7.5 Heavily coated manganese oxide fossil bone fragment (2b) **a,** before treatment in hydroxylamine; **b,** after treatment in 0.0712 mol L^{-1} hydroxylamine, at initial pH: 3.68 some of the coating is removed **c,** after light sponging the remaining manganese oxide coating is removed leaving an undamaged, clean bone surface.

Almost complete surface cleaning and removal of the thick dark manganese oxide coating obscuring the bone surface is achieved for the conditions used in fig. 7.5 above.

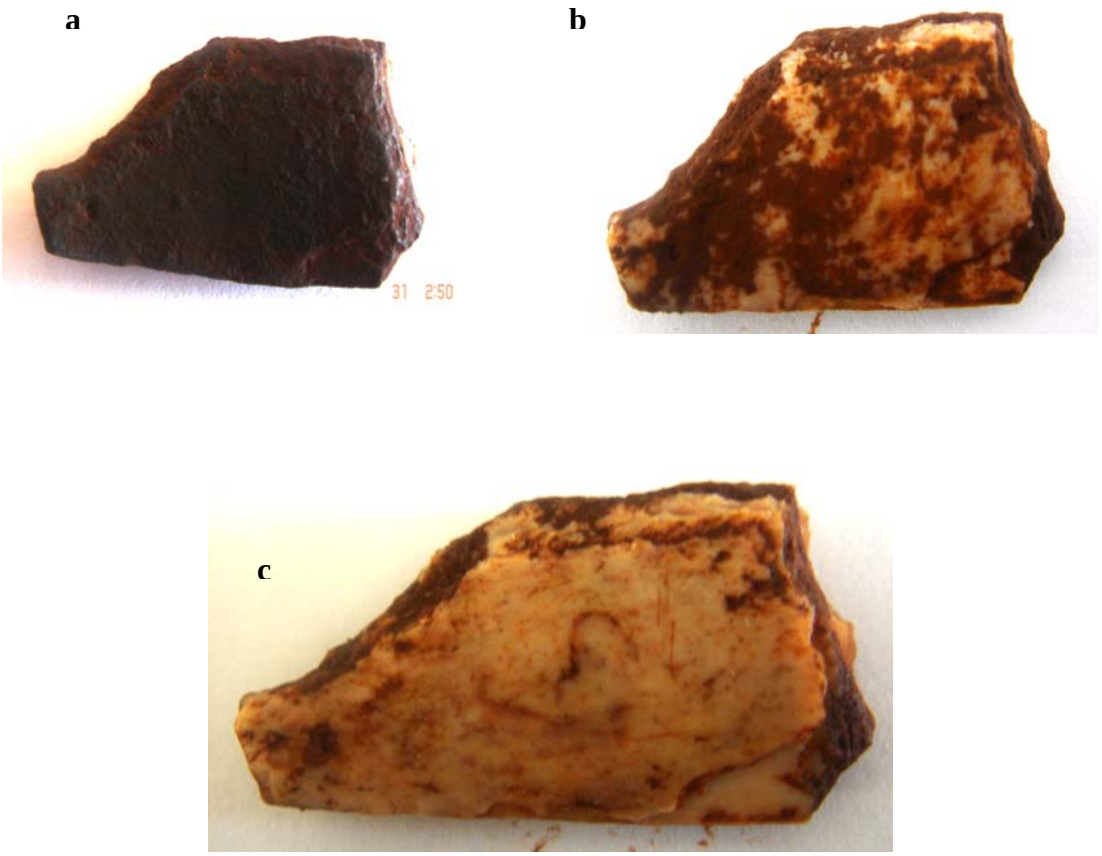

Figure 7.6 Manganese oxide coated fossil bone fragment (2c) **a,** before treatment in a hydroxylamine solution; **b,** after treatment in a 0.141 mol L^{-1} hydroxylamine at initial pH: 3.68 **c,** after further treatment in hydroxylamine and sponging most of the oxide coating is removed with no visible damage to the bone surface. Note how surface modifications are revealed after cleaning.

.Total removal of the manganese oxide coating is achieved from the smooth surface of the fossil specimen. The underlying surface appears to also be unaltered or etched by the conditions used.

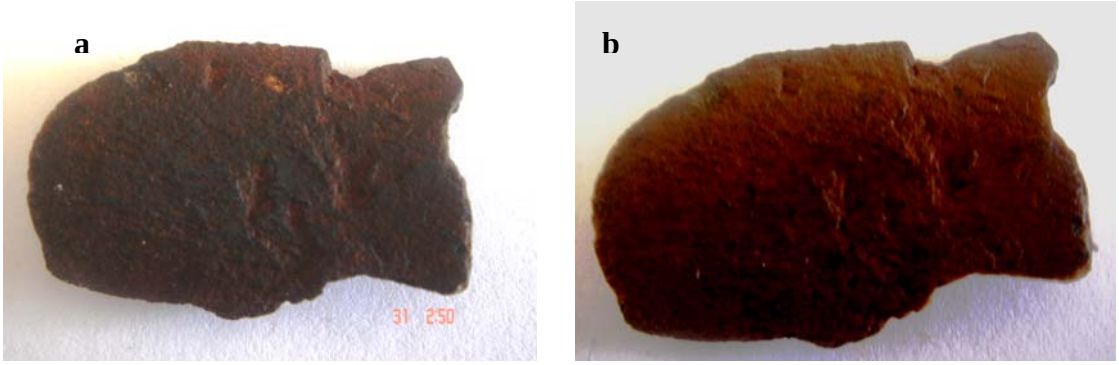

Figure 7.7 Thick manganese oxide coated fossil bone fragment (3a) **a,** before treatment a thick oxide coating covers the bone fragment surface; **b,** after treatment in 0.0014 mol L^{-1} hydroxylamine at initial pH: 4.25 the coating has become lighter in colour, but is not removed without sponging.

Although the colour of the fossil bone coating is changed to a lighter brown, the overall removal efficiency using the conditions employed for the above specimen is inadequate, as the surface of the bone is still obscured. The colour change is thought to be a result of the selective reduction of the manganese component in the oxide coating, thus leaving behind the reddish hematite iron fraction (Neaman, *et al*., 2004)**.**

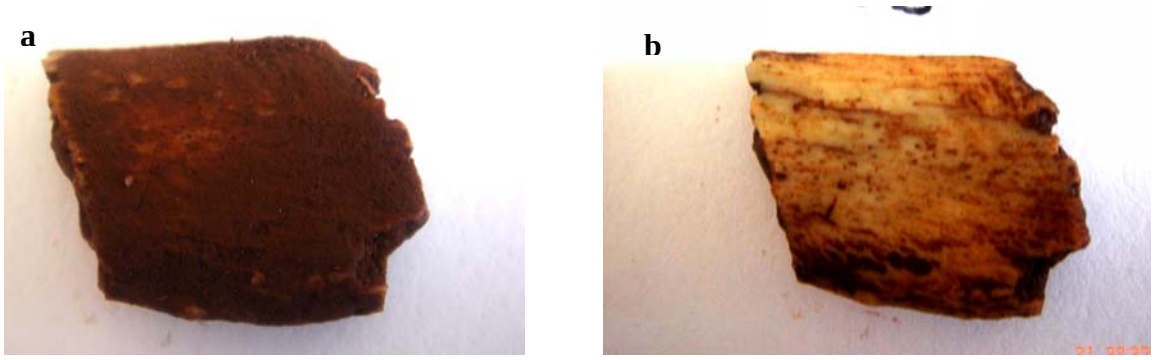

Figure 7.8 Manganese oxide coated bone fragment (3b) **a,** before treatment in hydroxylamine; **b,** after treatment in 0.0712 mol L^{-1} hydroxylamine at initial pH: 4.25, a cleaned undamaged surface is produced.

Good removal of the surface oxide coating is achieved with no visible macroscopic evidence of bone destruction.

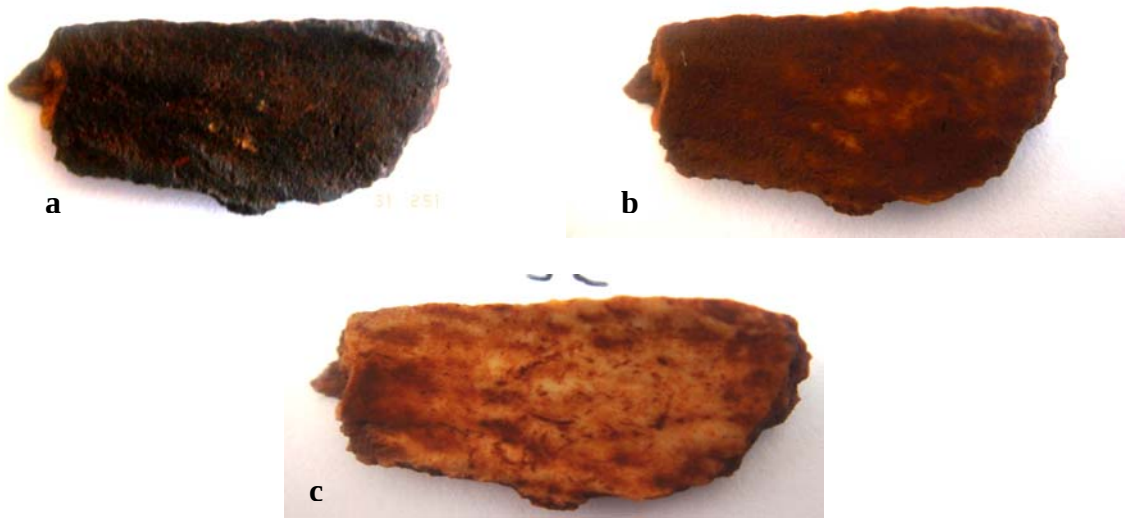

Figure 7.9 Manganese oxide coated bone fragment (3c) **a,** before treatment; **b,** after treatment with 0.141 mol L^{-1} hydroxylamine. At initial pH: 4.25 removal of the oxide coating is apparent with the coating becoming lighter in colour **c,** after sponging, the remaining oxide coating is removed.

Although sponging had to be employed to remove the majority of the coating, the reduction process had weakened the coating to bone bonding sufficiently to prevent damage to the bone surface. Mechanical removal is not always favoured because it can detach and remove loose bone matrix from the surface. However, if the removal is carried out after treatment with the hydroxylamine it may be acceptable.

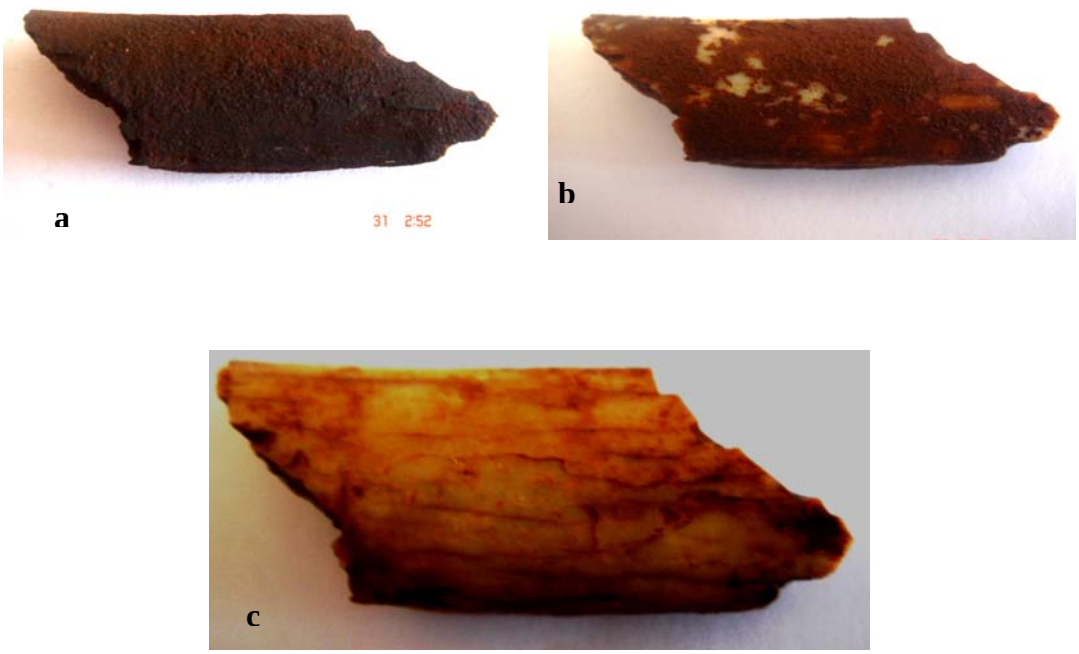

Figure 7.10 Manganese oxide coated bone fragment (4a) **a,** before treatment in hydroxylamine solution; **b,** after treatment with 0.0712 mol L^{-1} hydroxylamine at initial pH: 3.68; **c,** after gentle sponging all the manganese oxide is removed with no visible damage incurred to the bone surface.

A soft non-abrasive sponge was used for gentle cleaning of the fossil specimen after treatment, and proved to be the most effective and efficient way of removing the manganese oxide coatings. When the sample is treated the initial appearance changes from dark brown /black to a lighter brown to red colour. Depending upon the thickness of

the coating this process of soaking followed by wiping can occur in a stepwise manner. The lighter colour formed on the surface is thought to be a result of selective dissolution of manganese, leaving behind the red-brown iron-rich minerals (Neaman, *et al*., 2004). Due to the reaction with the metal or metal oxide surface, ammonium forms as a hydroxylamine decomposition product according to the following equation (Small, 1999):

$$NH_3OH^+ + H_2 \rightarrow NH_4^+ + H_2O \qquad 7.2$$

Another important side competing reaction (7.2) also needs to be considered, which can also influence the concentration of the hydroxylamine solution reacting with the metal (Chambers and Holliday, 1975):

$$NH_3 \cdot H_2O \leftrightarrow NH_4^+ + OH^- \qquad 7.3$$

The iron and manganese oxides were studied in Fe-Mn-nodules in soil from Serra do Navio (Northern Brazil). The method of selective dissolution by hydroxylamine hydrochloride had been studied and applied to selectively dissolving manganese oxides from manganese-iron oxide-rich nodules (Neaman, *et al*., 2004).

This could perhaps suggest the reason for the stepwise colour change from dark black to light brown that occurs during the surface reaction involving all the oxide coated fossil bones. As the surface is exposed to the hydroxylamine treatment solution, the manganese oxide which is responsible for the dark black colour is selectively reduced and leached out, leaving behind only iron oxide or hematite, which has a light brown to red appearance. This remaining hematite coating is then easily removed via gentle sponging

as it no longer bonds directly to the bone surface. It also appears as if this process is surface controlled as the removal and colour change process occurs in stages as opposed to all at once. The cleaning solution is thus required to be reapplied each time the lighter iron oxide layer is mechanically removed.

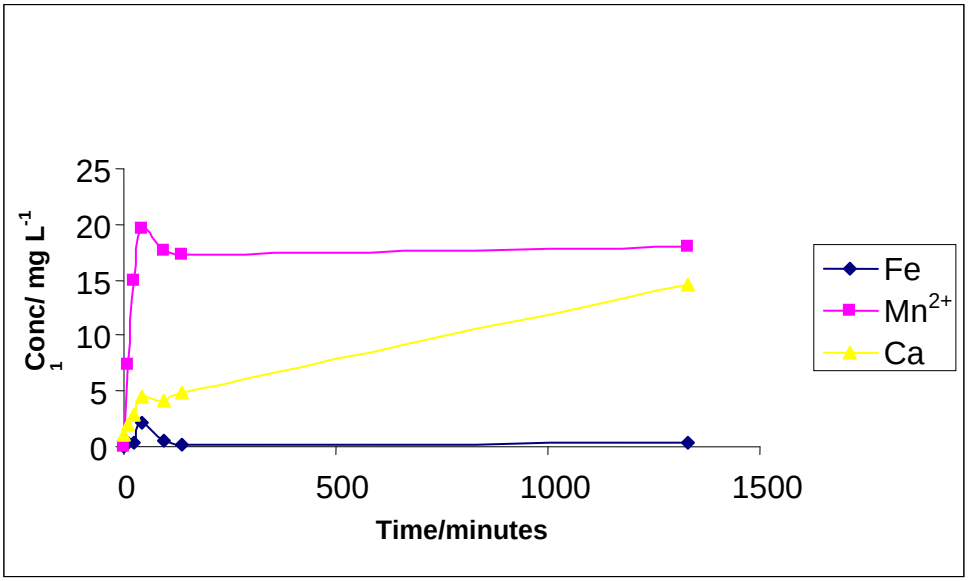

Figure 7.11 Relationship between iron, calcium and manganese evolution trends for coated fossil bones treated with hydroxylamine.

As mentioned above the reduction of the manganese oxide coating using hydroxylamine is suggested to be selective to the reduction of the manganese fraction of the oxide. The iron which is also contained in fairly high concentrations in the coatings is thus left on the surface of the fossil bone, and hence the lighter colour of the coating once soaked in the solution. The above plot (fig. 7.11) also substantiates this as the concentration of the iron

in solution after a substantially long time remains very low and stays constant. This proves that the iron fraction is not being reduced into solution during the cleaning process, but remains weakly bonded to the fossil bone surface after the reaction.

In an investigation carried out by Ramesh (2007), the sinterability of manganese oxide doped hydroxyapatite was investigated, focusing on phase stability, grain size, bulk density, hardness and fracture toughness, all properties related to the interaction between manganese oxide and hydroxyapatite. It was determined that the manganese doped hydroxyapatite exhibited higher mechanical properties than undoped hydroxyapatite (Ramesh *et al*., 2007). This could also suggest that the main strength and attachment of the coating to the bone surface occurs via the manganese and oxygen bond.

It has been shown that in sedimentary materials, manganese often has a high correlation with other elements (Cronan, 1969; Spencer *et al*., 1972). Furthermore it has been demonstrated by (Goldberg, 1954; Krauskopf, 1956) that adsorption onto the manganese oxide layers plays a major role in the associative behaviour (Murray, 1973).

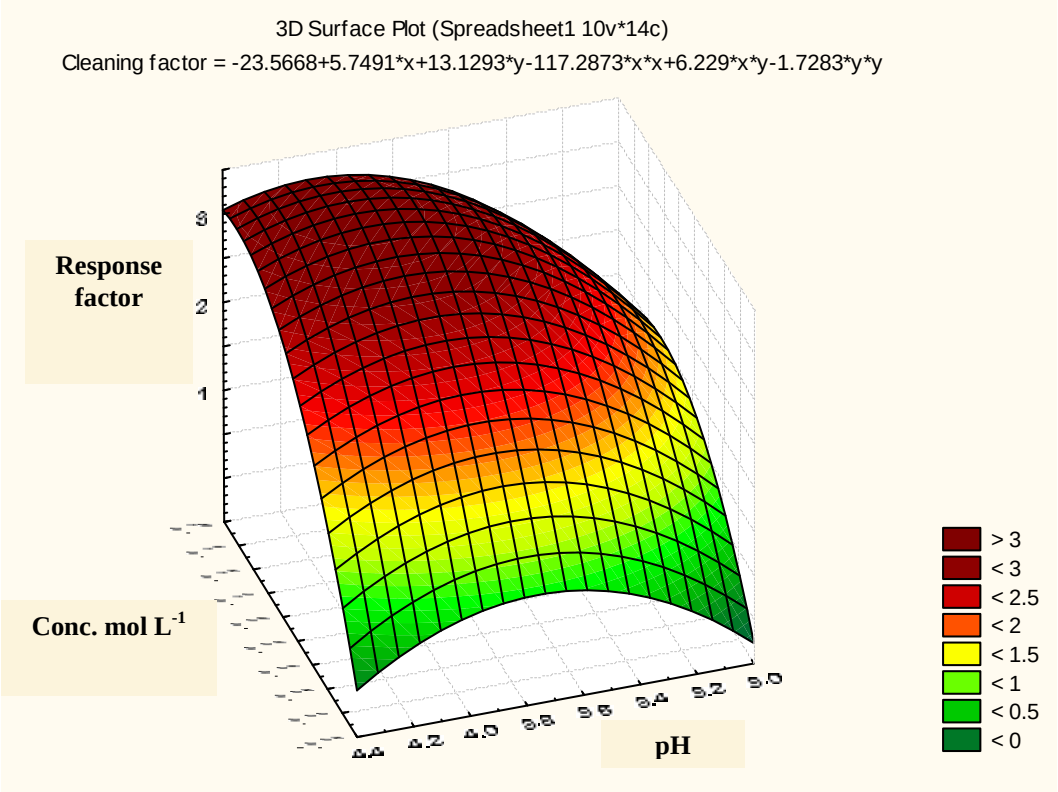

Figure 7.12 Surface matrix plot showing the optimum concentration and pH for different experimental concentrations, and initial pH values used related to the efficiency of manganese oxide removal (response factor).

The surface plot in fig. 7.12 above was obtained from a matrix experiment used in the optimization of the hydroxylamine concentration and pH for manganese oxide coated fossil specimens. The main response factor is the degree of manganese oxide removal with and without wiping after treatment. The response factor is directly related to the degree of removal of manganese oxide coating and visible damage to the bone surface from the cleaning process. The highest degree of manganese oxide removal without any visible damage was assigned a response factor value of 3.0 while a lowest factor value of 0 was assigned for the least amount of manganese oxide removed or visible damage of

the bone fragment. The optimum concentration and pH was found to be 0.14 mol L^{-1} and 3.75 respectively. Although it appears from the plot above that the optimum has not been reached yet, due to the appearance of bone destruction and weakening of the matrix, the above maxima was where the optimal non-invasive conditions were located.

7.2.2 Influence of pH

There is a slight decrease in the efficiency of the reduction of manganese oxide from the surface for a pH of above 4. Although the main reason for this is the forward reaction is driven by the proton concentration, the hydroxylamine also has a natural decomposition process and this is more kinetically favourable at more alkaline pH values. For example, in a study carried out to investigate the relationship between pH of hydroxylamine solutions and the time required for the % loss of NH_2OH, (Corbet, 1935) determined that for solutions of pH range 5-9 there was a 46% loss of NH_2OH after a 24 hour period, as opposed to a 1% loss for a pH range of 4-6 over the same time period.

The above natural alkaline degradation of the hydroxylamine does not appear to have a major influence on the overall efficiency as most of the manganese oxide is removed within 2 hours, thus not allowing the degradation to have an effect on the efficiency, so that by the time the bone was removed from the solution after a longer time period the reaction was complete. Furthermore, other factors such as iron concentration in the coating and the concentration of the hydroxylamine solution could also outweigh the pH degradation effect.

7.2.3 Further optimization

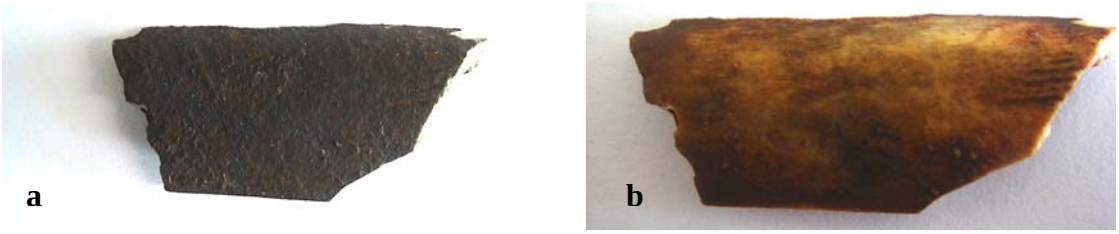

Figure 7.13 Manganse oxide coated fossil bone fragment (1a) **a,** before treatment; **b,** after treatment in 0.03 mol L^{-1} hydroxylamine solution at initial pH: 3.40.

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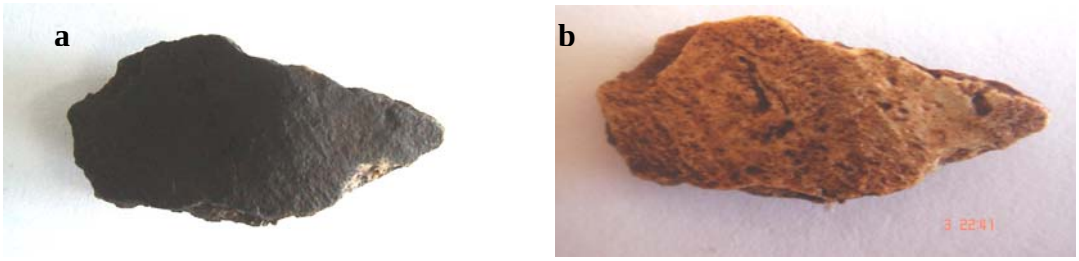

Figure 7.14 Manganse oxide coated fossil bone fragment (1b) **a,** before treatment; **b,** after treatment in 0.03 mol L^{-1} hydroxylamine solution at initial pH: 4.90.

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Figure 7.15 Heavily coated manganese oxide fossil bone fragment (1c) **a,** before treatment; **b,** after treatment in 0.03 mol L^{-1} hydroxylamine solution at initial pH: 6.40 most of the manganese oxide coating is removed.

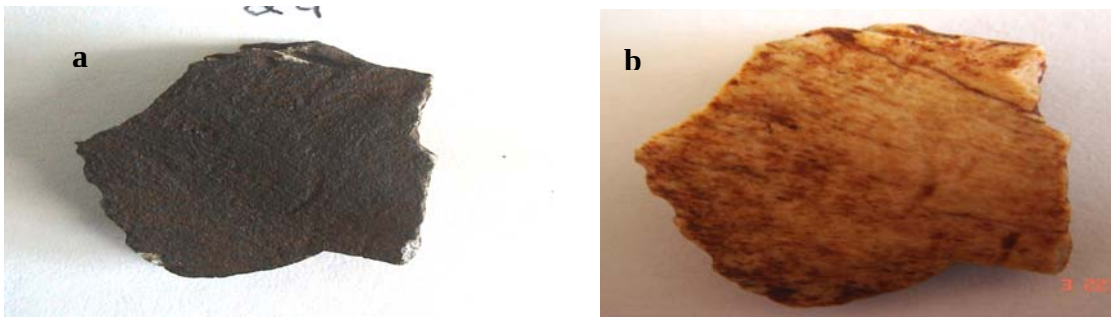

Figure 7.16 Manganese oxide coated fossil bone fragment (2a) **a,** before treatment; **b,** after treatment in 0.085 mol L^{-1} hydroxylamine solution at initial pH: 3.40 all the manganese oxide is removed leaving an undamaged unobscured surface.

The conditions used for the treatment of the manganese oxide coated fossil bone in fig.7.16 show highly efficient removal of the surface coating with no macroscopic evidence of surface alteration or degradation. When compared to the conditions employed for the specimens shown in fig 7.15, the concentration and pH of this solution were considerably different. The lower pH and higher concentrate solution show high dissolution efficiency as seen in fig. 7.16 above.

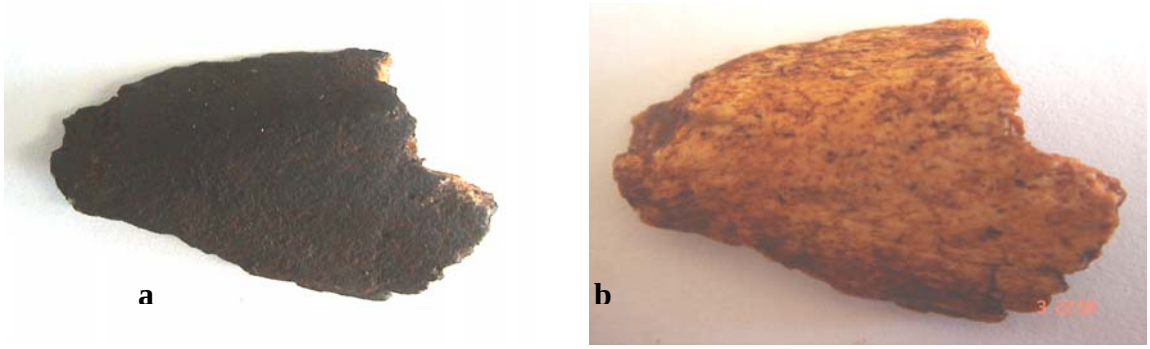

Figure 7.17 Manganese oxide coated fossil bone fragment (2b) **a,** before treatment; **b,** after treatment in 0.085 mol L^{-1} hydroxylamine solution at initial pH: 4.90.

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Although the efficiency of removal is still high, the surface still has grains of the coating imbedded. This could be a result of the pH which is more alkaline than that used in the previous solution where maximum removal was achieved.

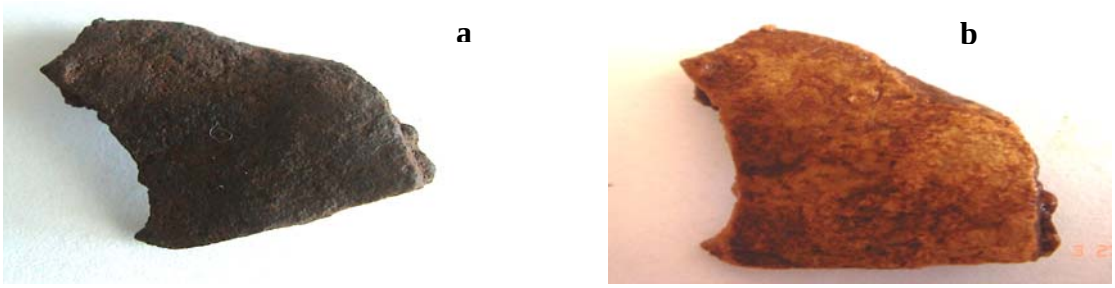

Figure 7.18 Manganese oxide coated fossil bone fragment (2c) **a,** before treatment; **b,** after treatment in 0.085 mol L^{-1} hydroxylamine solution at initial pH: 6.40.

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The surface coating is removed, however, there are still traces of the coating as a result of the pH having been increased to a higher value. These two samples above also vary slightly in their surface texture, which has been previously observed to affect the removal of the oxide coating.

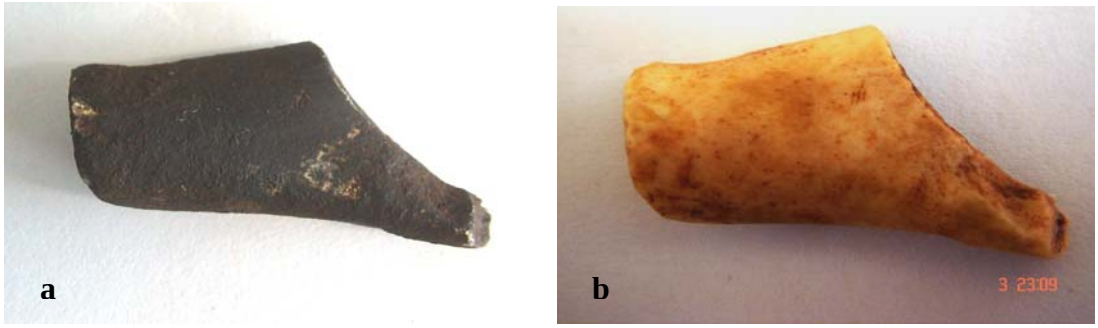

Figure 7.19 Manganese oxide coated fossil bone fragment (3a) **a,** before treatment; **b,** after treatment in 0.14 mol L^{-1} hydroxylamine solution at initial pH: 3.40

The above conditions show good removal of the manganese oxide coating with no wiping required. The highest concentration and lowest pH were used in the conditions above which could thus illustrate that both pH and concentration of the hydroxylamine solution are equally important. This also, this could be due to the extremely smooth texture of this bone surface.

This is also a good illustration of the underlying surface modifications hidden or obscured before cleaning. This feature can be seen as an indentation in the narrow bottom right end section of the fossil specimen in fig. 7.19b.

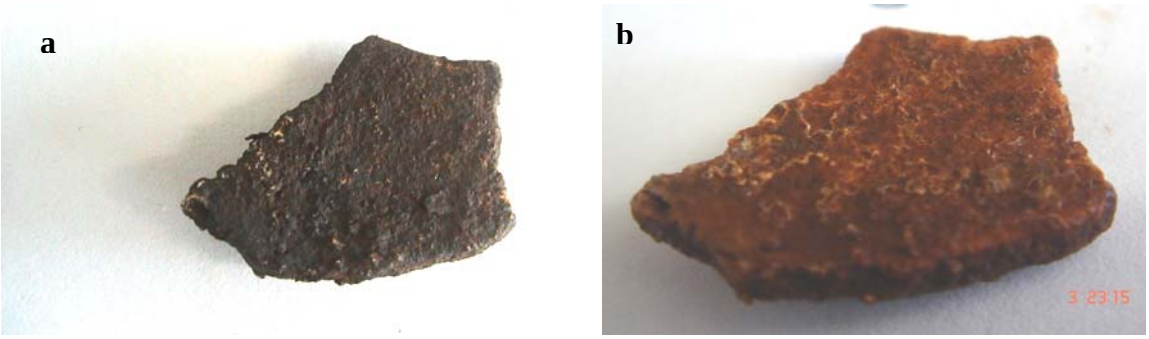

Figure 7.20 Manganese oxide coated fossil Bone fragment (3b) **a,** before treatment; **b,** after treatment in 0.14 mol L^{-1} hydroxylamine solution at initial pH: 4.90.

Less efficient removal is achieved without sponging. This could possibly be due to the rougher underlying surface of the bone, and an increase in pH.

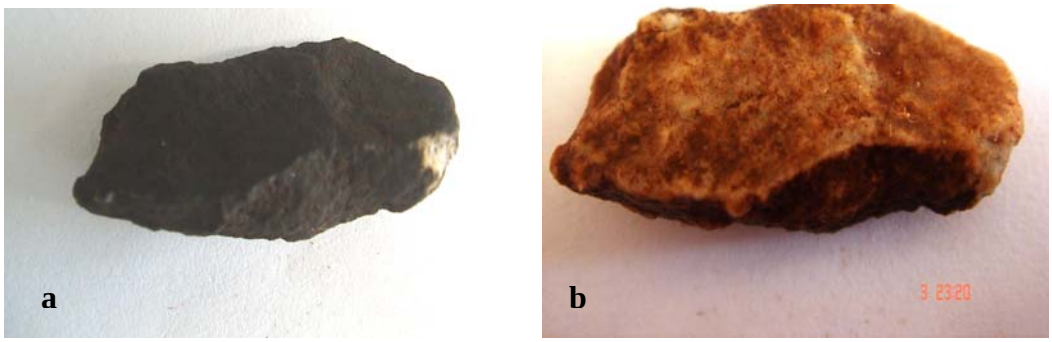

Figure 7.21 Manganese oxide coated fossil Bone fragment (3c) **a,** before treatment; **b,** after treatment in 0.14 mol L^{-1} hydroxylamine solution at initial pH: 6.4

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Efficient dissolution of the dark oxide coating is achieved, however, there are still traces of the lighter brown iron oxide on the surface. Although this lighter material does not obscure the surface modifications, it still does not compare well to the other pH and hydroxylamine concentration combinations.

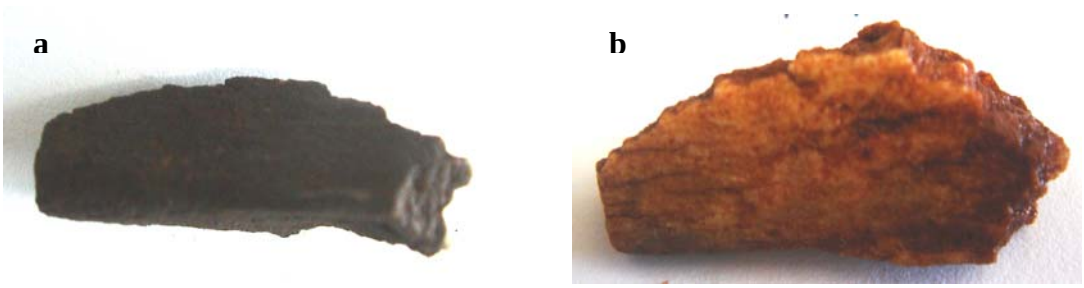

Figure 7.22 Manganese oxide coated fossil Bone fragment (4a) **a,** before treatment; **b,** after treatment in 0.085 mol L^{-1} hydroxylamine solution at initial pH: 4.90.

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Efficient removal of the surface coating is achieved, making the surface of the bone visible.

7.2.4 Relationship between pH and hydroxylamine hydrochloride concentration

From all the above macroscopic optimization matrix experiments performed in triplicate it can be concluded that the concentration and pH work in tandem as removal factors of the manganese oxide coatings, although pH may play a more important role in the overall dissolution process.

The initial and final pH values for the optimization matrix experiment were recorded as shown in table 7.1 below. As hydroxylamine is a strong reducing agent, it is expected that the concentration used has an effect on the change in pH of the system. For the experiment below, the manganese oxide coated fossil bones were left in solution for approximately 20 hours.

Table 7.1 Change in pH for different concentrations of hydroxylamine treatment solutions.

Sample identity	Concentration /mol L^{-1} HA	Initial pH	Final pH
1a	0.03	3.45	5.15
1b	0.03	4.86	5.47
1c	0.03	6.38	6.57
2a	0.085	3.39	5.14
2b	0.085	4.87	5.14
2c	0.085	6.38	6.45
3a	0.14	3.37	4.77
3b	0.14	4.88	4.95
3c	0.14	6.37	6.41
4a	0.085	4.87	5.04

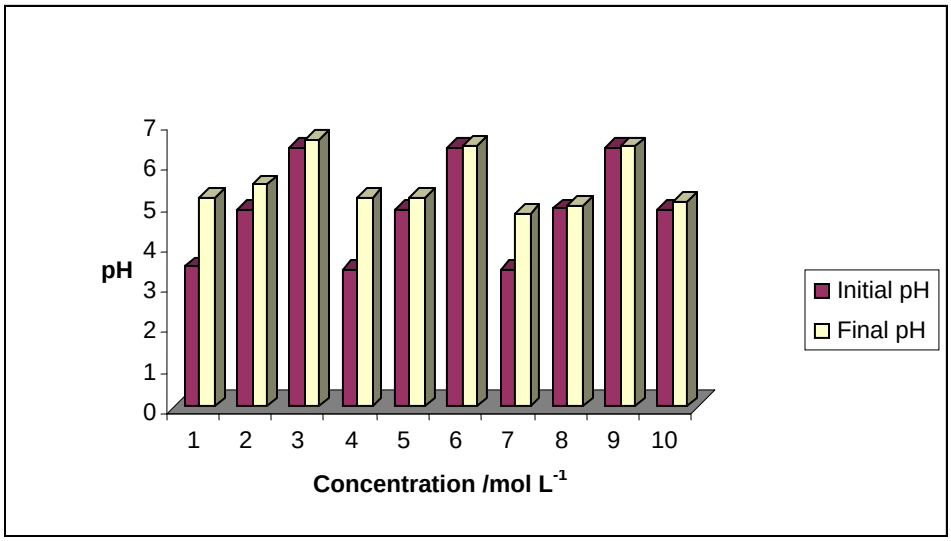

Figure 7.23 Initial and final pH values for three different hydroxylamine concentrations used in the treatment of manganese oxide coated fossil bone fragments.

The change in pH values for different concentrations shown in fig. 7.23 follows a relatively linear trend. The change in pH decreases as the initial pH value used is increased for each concentration. When comparing the same initial pH values and the subsequent change in pH for the different hydroxylamine concentrations, there is minimal variation. This indicates that the concentration of the hydroxylamine (providing it is not a limiting reagent) is not as significant a factor as the pH of the cleaning solution with respect to the pH change of the hydroxylamine solution.

As previously mentioned, providing the concentration of the hydroxylamine ligand is not a limiting reagent with respect to the surface manganese oxide concentration, the pH value of the solution is most likely to have a greater influence when determining the optimum conditions for manganese oxide removal from fossil bone specimens.

7.3 Validation

Validation was performed using multiple fossil bone fragments to establish if the fossil bone undergoes any physical and chemical changes and to quantify the magnitude of these changes.

Validation for three different concentrations used, 0.14 mol L^{-1}, 0.075 mol L^{-1}, 1.0 mol L^{-1} at pH = 4.00.

Performed in duplicate for each experiment. All manganese oxide coated fossil bone specimens were broken into two pieces in order to determine the extent of cleaning and if any surface alteration occurs.

Figure 7.24 Manganese oxide coated fossil bone broken into two fragments and treated in a 0.14 mol L^{-1} hydroxylamine solution **a,** before treatment; **b** and **c** after application of a 0.14 mol L^{-1} hydroxylamine solution at pH: 4.00.

The two fossil bone fragments fit back together edge to edge thus showing that no visible bone surface alteration occurred for this concentration of hydroxylamine.

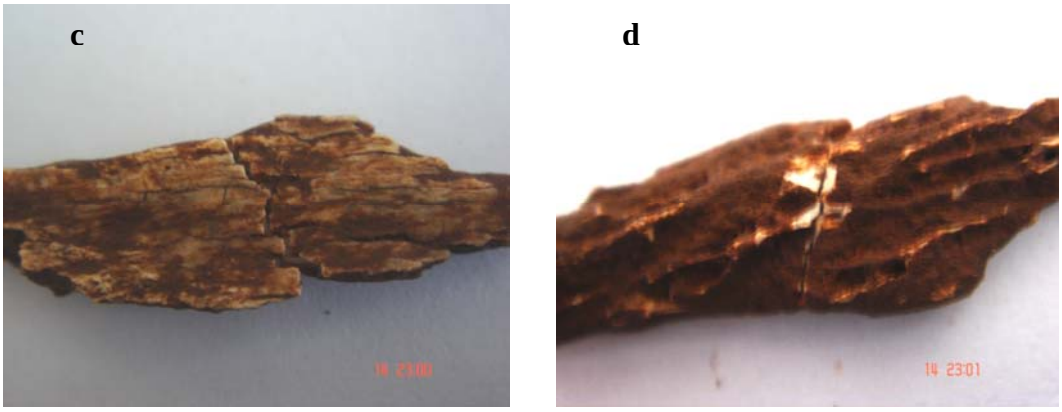

Figure 7.25 Manganese oxide-coated fossil bone broken into two fragments and treated in a. hydroxylamine solution, **a** and **b** before treatment; **c** and **d**, after treatment in a 1.0 mol L^{-1} hydroxylamine solution at initial pH: 5.00.

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Back to back alignment of all the fossil bone fragments is achieved and there is no macroscopic evidence of flaking or obvious surface alteration.

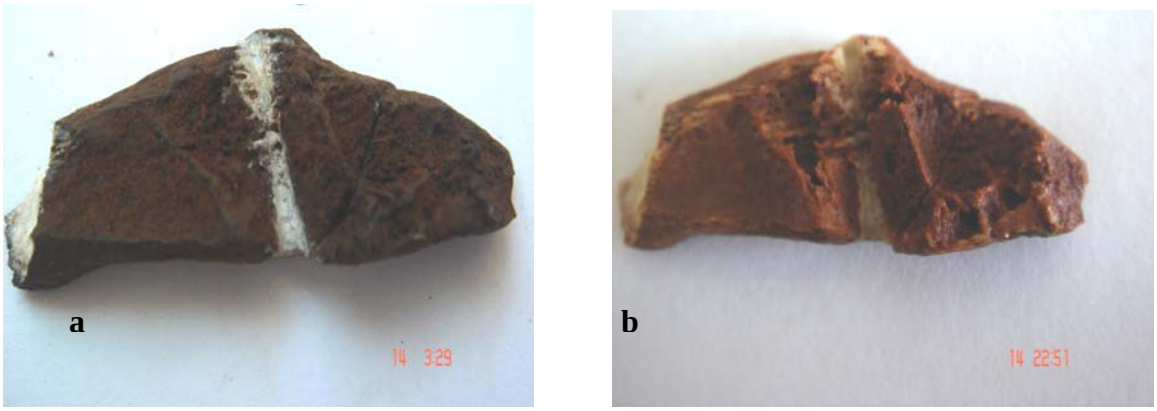

Figure 7.26 Manganese oxide-coated fossil bone broken into two fragments, marked to monitor any possible inner matrix dissolution and treated in a. hydroxylamine solution. **a,** before treatment; **b,** after treatment in a 1.0 mol L^{-1} hydroxylamine solution at pH: 5.00

The remaining bone coating appears lighter in colour with the bone surface stratigraphy more visible after treatment. The other fossil bone fragments treated in another two different hydroxylamine concentrations at the same initial pH also fitted back together edge to edge showing that no visible bone surface alteration occurred for this concentration of hydroxylamine. Although the main aim of this experiment was to validate that bone surface alteration is negligible for all three concentrations, the efficiency of the coating's removal with respect to each bone differs perhaps due to the surface and size differences of each fossil bone specimen and not necessarily the change in concentration or pH of the cleaning solution.

7.3.1 Microscopic validation (Scanning electron microscopy)

In order to validate that the bone surface is not significantly damaged by this cleaning method, scanning electron microscopy was used to examine the surface of an uncoated fossil specimen before and after treatment in the hydroxylamine solution.

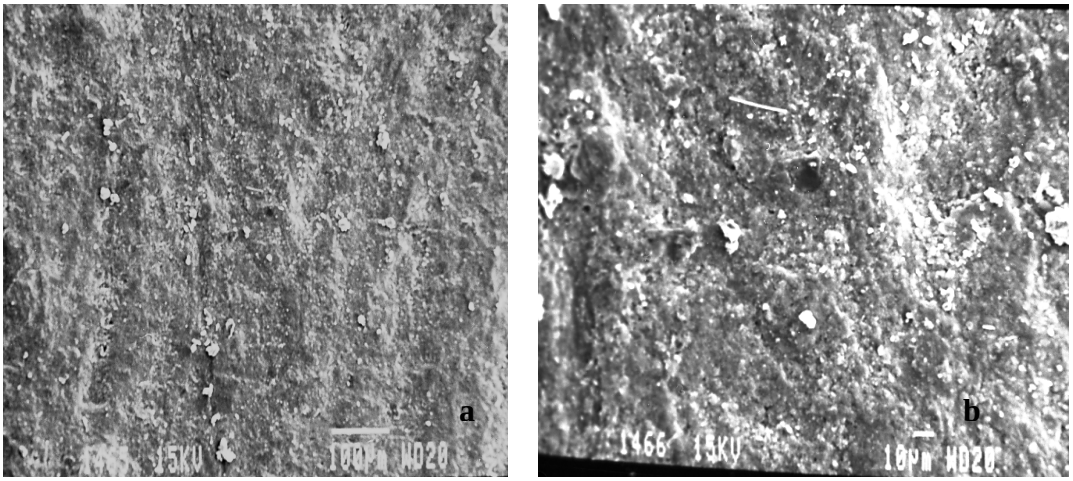

Figure 7.27 Scanning electron microscope image (150X magnification) for an uncoated fossil bone **a,** before treatment in a hydroxylamine solution. **b,** after treatment in 0.14 mol L^{-1} hydroxylamine solution at initial pH: 3.75.

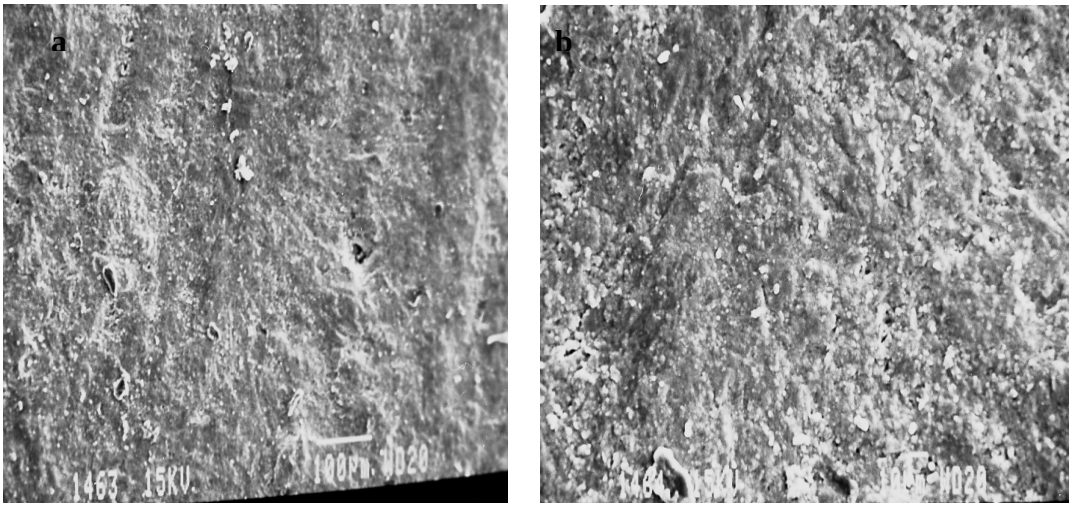

Figure 7.28 Scanning electron microscope image (500X magnification) for an uncoated fossil bone **a,** before treatment in a hydroxylamine solution. **b,** after treatment in 0.14 mol L^{-1} hydroxylamine solution at initial pH: 3.75.

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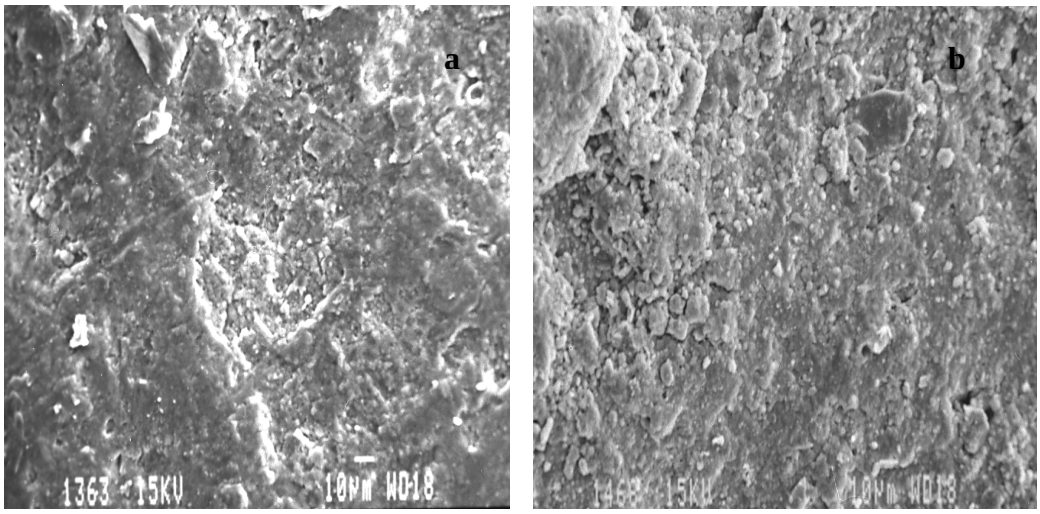

Figure 7.29 Scanning electron microscope image (500X magnification) for an uncoated fossil bone **a,** before treatment in a hydroxylamine solution. **b,** after treatment in 0.14 mol L^{-1} hydroxylamine solution at initial pH: 3.75.

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There is little evidence of bone dissolution occurring from the two sets of micrographs presented above. Both the before and after images of each set (150X and 500X magnification) seem to have the same characteristic surface patterns with no visible evidence of bone dissolution.

7.3.2 Further optimization and validation of two different pH values after 24 hours

Duplicate coated fossil bone fragments were selected in order to confirm the optimal pH value at which the reduction reaction occurs is correct and further to validate that no visible bone surface and matrix damage is occurring.

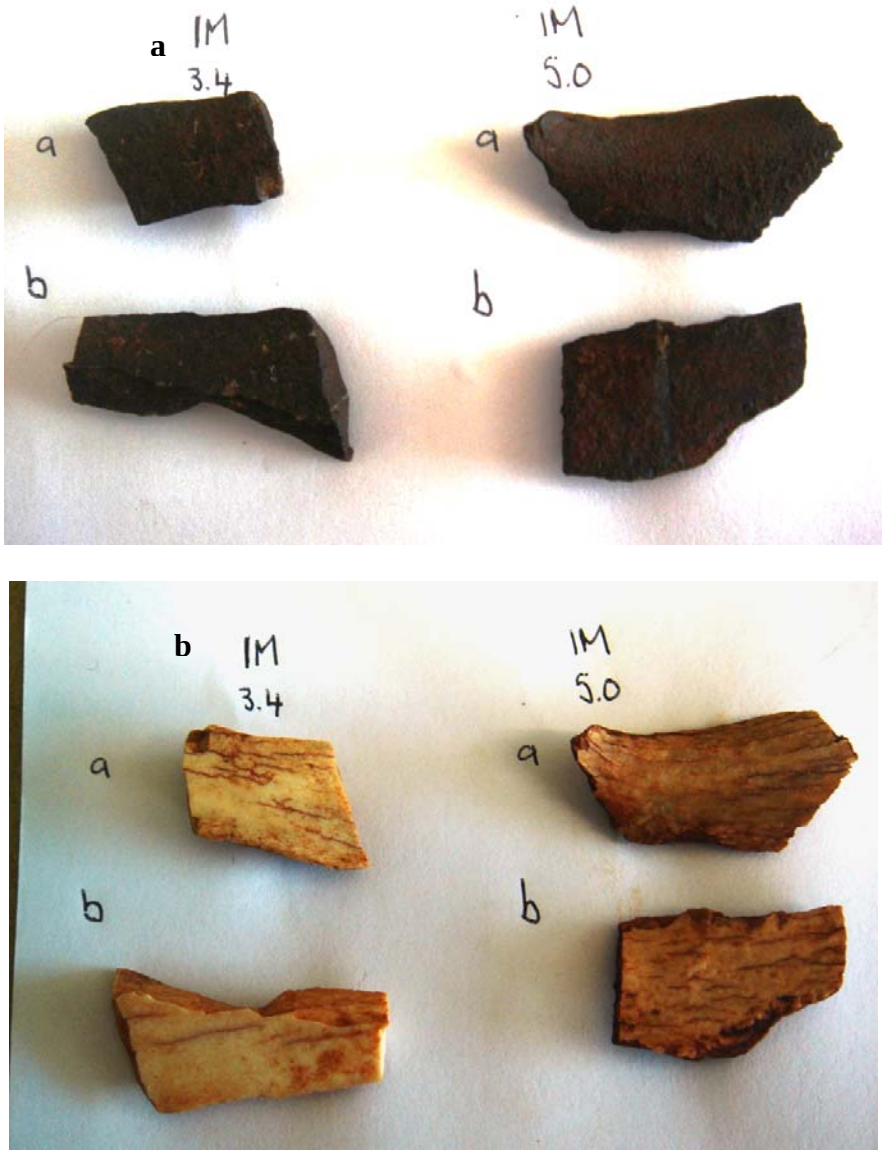


Figure 7.30 pH validation and optimization performed in duplicate on four separate manganese oxide coated fossil bone fragments for pH 3.4 and 5.0: **a,** before treatment in a 1.0 mol L^{-1} hydroxylamine solution; **b,** after the hydroxylamine treatment process.

The fossil bones above that were treated in 1 mol L^{-1} solution of hydroxylamine at an initial pH of 3.4 and 5.0 show no signs of surface etching or alteration. However as shown in table 7.1, the degree of manganese oxide removal does show a dependence on the initial pH of the hydroxylamine solution.

7.3.3 Gravimetric analysis for two different hydroxylamine concentrations

Gravimetric analysis was performed to quantify the residual amount of elements of interest such as Mn, Fe and Ca that are dissolved from the bone or bone surface. While this form of validation can be useful, it does not indicate the exact origin of the mineral that is detected and measured. This implies that calcium evolution cannot be directly linked to bone surface dissolution, because soil and manganese oxide coatings not visible to the naked eye and/or trapped in the subsurface of the bone could also be sources of these elements.

Gravimetric data

Experimental conditions: 1.0 mol L^{-1} and 0.14 mol L^{-1} at pH: 3.4.

Masses before and after treatment of the manganese oxide coated bone fragments performed in duplicate for each concentration.

Table 7.2 Concentration and mass values for two different hydroxylamine concentrations performed at pH 3.4 in duplicate.

Sample Concentration/ mol L^{-1}	Concentration/ mg L^{-1}			Initial mass/g	Final mass/g	Mass loss %
	Fe	Mn	Ca			
1.0 a	0.9	31.8	67.2	1.2554	1.2440	0.91
1.0 b	2.6	47.2	45.1	0.5284	0.4780	9.5
0.14 a	1.4	63.9	52.3	0.9217	0.8739	5.2
0.14 b	0.6	41.9	33.9	1.7981	1.7721	1.4

Although the overall mass loss percentage is higher for the higher hydroxylamine concentration, the additive difference for both duplicate specimens is only approximately 4%. This change relative to the difference in concentrations is negligible. The variation in % loss within each duplicate can be attributed to the variation in size, texture and surface area of each bone fragment.

7.4 Reaction time and cleaning efficiency

Even though this method has been shown and proved to be non-invasive for bone fragments soaked in the hydroxylamine solution at optimal pH and concentration, a qualitative kinetic evaluation of the removal process has also been performed. Further aspects of the overall reaction mechanism were also focused on, based on the images below.

Before treatment

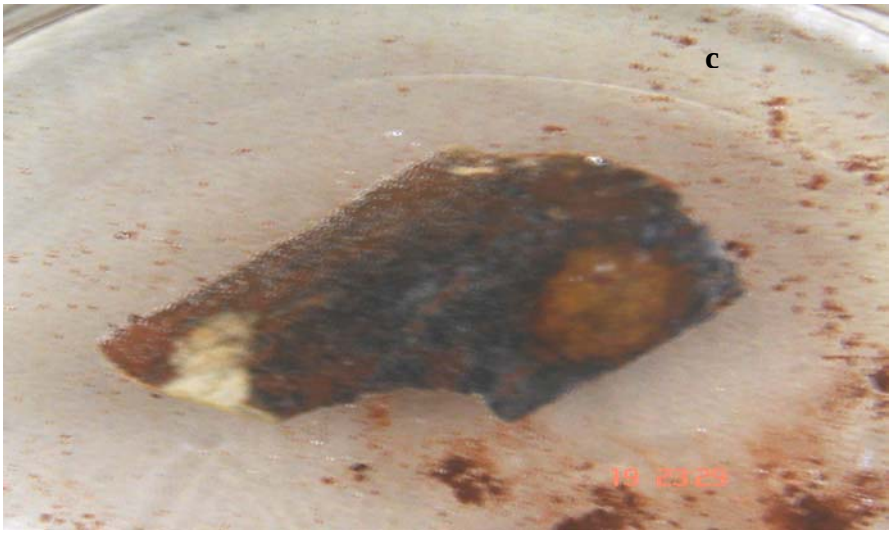
a

9 minutes

b

19 minutes

c



74 minutes

Figure 7.31 Sequential illustration of the hydroxylamine surface reduction of the manganese oxide coated fossil bone fragment soaked in 0.14 mol L^{-1} hydroxylamine solution at different time intervals after **a,** before the treatment process ; **b,** 9 minutes; **c,** 19 minutes; **d,** 74 minutes.

Before treatment

a

9 minutes

b

c

19 minutes

d

e

74 minutes

Figure 7.32 Sequential illustration of the hydroxylamine surface reduction of the manganese oxide coated fossil bone fragment soaked in 0.14 mol L^{-1} hydroxylamine solution as a function of time after **a,** before treatment process; **b** and **c,** 9 minutes; **d** and **e,** 19 minutes and **f,** after being soaked for 74 minutes.

As the manganese oxide coating is removed by the hydroxylamine solution, the coating turns a light brown colour and flakes off the bone surface. It appears that the dissolution occurs in stages. While the top layer of the coating is reduced and removed using light wiping, the underlying manganese oxide coating once again remains intact. The procedure was thus then repeated several times to remove each underlying layer.

The underlying layer is a dark black colour similar to the original top coating prior to treatment. Furthermore it was found that if the coated fossil bone fragment was not removed after a short time period, the penetration depth of the hydroxylamine increased thus causing dissolution of more manganese.

The above images of a manganese-coated fossil bone treated in 0.14 mol L^{-1} hydroxylamine, sponged and rinsed using deionised water at different time intervals, shows a remarkable difference in the colour of the coating before and after rinsing. The initial colours of the untreated manganese coating range from dark brown to black. After treatment the upper layer of the coating becomes a light brown colour and the texture also changes. The coating seems to form flake-like structures that are easily removed by rinsing and gentle wiping using a wet sponge.

7.4.1 Species distribution

Hydroxylamine can act as a complexing agent and or reducing agent depending on the metal species it is reacted with. In order to try and determine which role the hydroxylamine performs at the manganese oxide interface on the fossil bone coating, a species distribution model is presented below. All conditions for the optimized hydroxylamine method of removing manganese oxide fossil bone coatings were used.

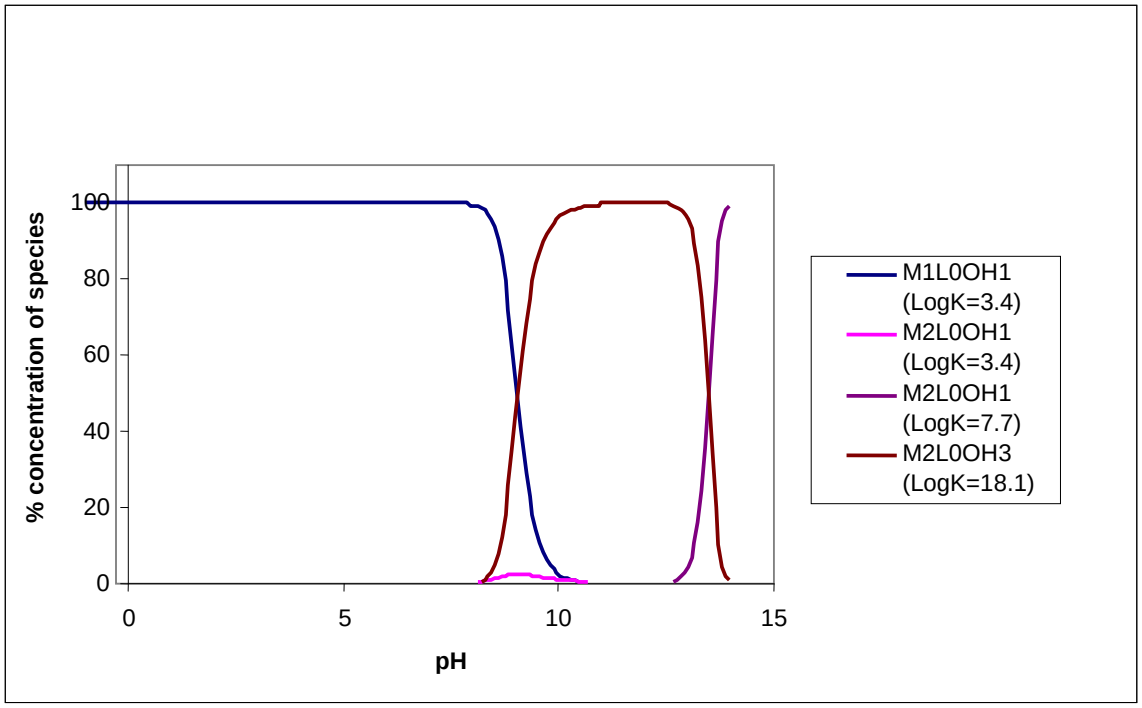


Figure 7.33 Species distribution of manganese(II) and hydroxylamine complexes.

The species distribution diagram in fig. 7.33 above is important as it shows that complex formation, whereby the hydroxylamine and Mn(II) complex is not favored under the conditions used as log K value for the Mn(II) : Hydroxylamine is 0.54 at 20°C, u=0.5 (Martell, *et al*., 2004). This demonstrates the mechanism by which the manganese oxide is removed using hydroxylamine proceeds mainly through a reduction reaction (redox). A possible reason for the high efficiency could be due to the hydroxylamine also having a high binding affinity towards the manganese oxide surface before electron transfer takes place thus improving the overall reduction efficiency of the dissolution process.

7.5 Comparison of the calcium and manganese evolution rate for a clean versus coated fossil bone

Fossil bone at pH 3.75 and 0.14 mol L^{-1} was compared with an uncoated fossil bone under the same conditions to compare the trends in the rates of calcium and manganese evolution.

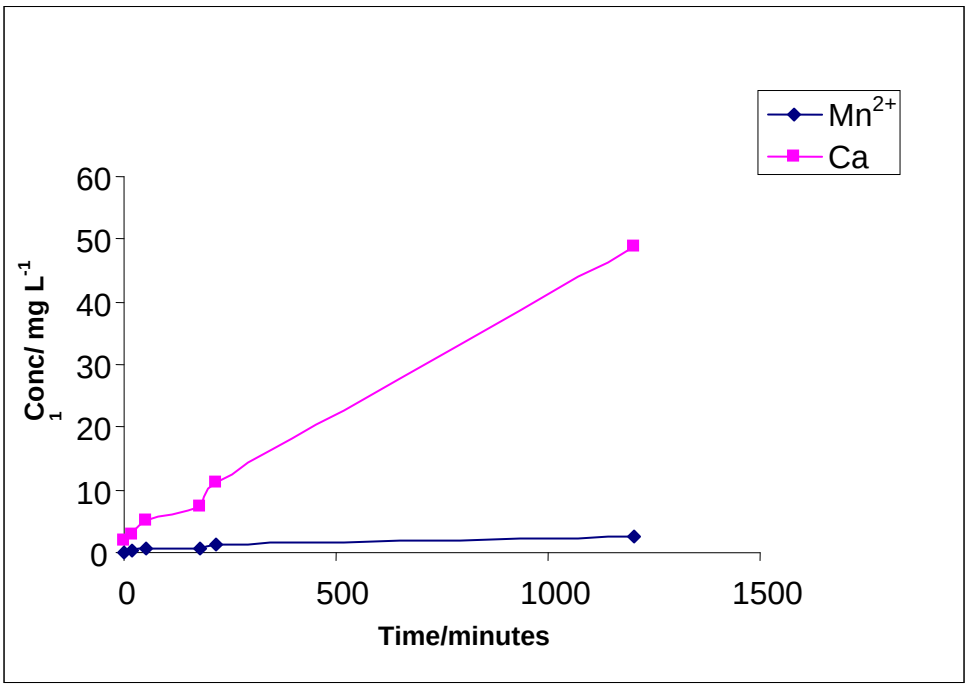


Figure 7.34 Manganese and calcium evolution trends as a function of time for an uncoated fossil bone treated with 0.14 mol L^{-1} hydroxylamine at pH 3.75.

The rate of calcium and manganese evolution differs for an uncoated fossil bone as shown in fig. 7.34. This trend is to be expected as there is less manganese than calcium on the surface of uncoated fossil bones. The hydroxylamine is also a complexing agent for some elements and is thus thought to complex with the weakly bound calcium, and other microscopic soil debris located on the surface of the fossil bones. This removal of

calcium has been shown through gravimetric quantification and macroscopic observation to have a minimal effect on the overall surface features and taphonomy of the bones. Gravimetric quantification also validates that the amount of calcium evolved over the time period required to clean the bones is negligible.

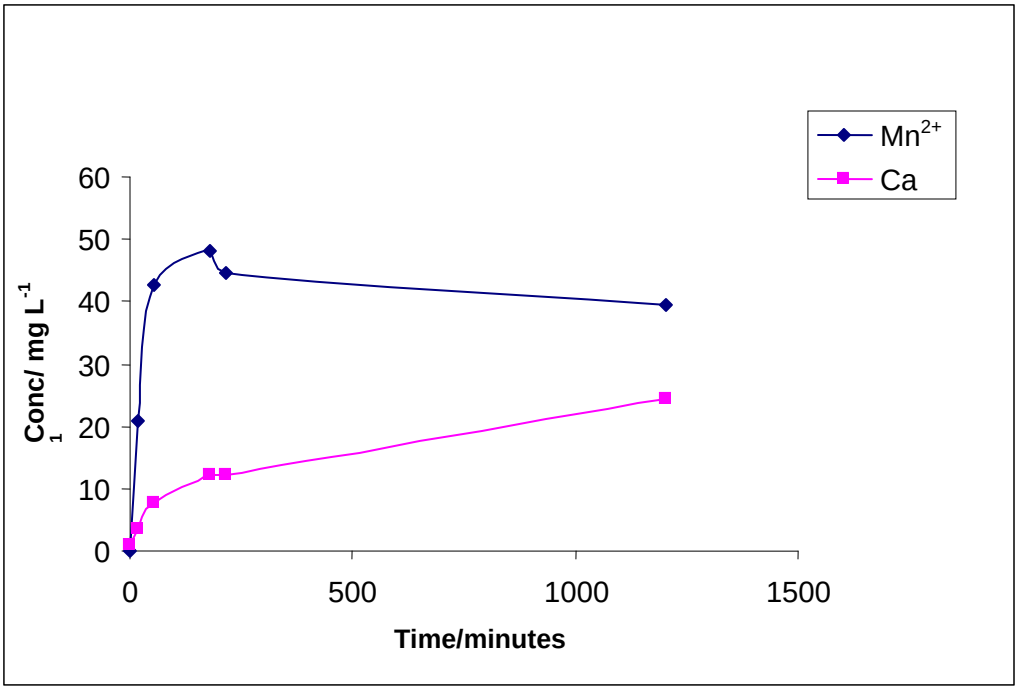


Figure 7.35 Manganese and calcium evolution rate trends for a manganese oxide coated fossil bone treated with 0.14 mol L^{-1} hydroxylamine at pH 3.75.

The rate of manganese and calcium for a coated fossil bone treated with 0.14 mol L^{-1} hydroxylamine is illustrated in fig. 7.35. The manganese evolution rate is much higher than that of calcium. The initial rate (up until an approximate time of 200 minutes) shows a large increase in the concentration of manganese. Thereafter the evolution rate levels off. This large increase in the initial rate of manganese as opposed to the smaller

increase in calcium can be attributed to the fossil bone coating having a high concentration of manganese. This birnessite coating also contains a small percentage of calcium that can account for the slight increase in calcium evolution for the same coated fossil bone (Anthony, 1997; Johnson and Post, 2006).

7.5.1 Comparison of calcium evolution rate in duplicate for non coated fossil bone vs. non coated modern cow bone treated in 0.14 mol L^{-1}hydroxylamine solution

As previously mentioned in chapter one, fossil bones undergo diagenesis resulting in replacement of various elements in the matrix of the bones. Calcium is one of the better elemental indicators of bone dissolution providing the bone has no mineral coatings as these coatings and soil trapped in the porous bone can give elevated results.

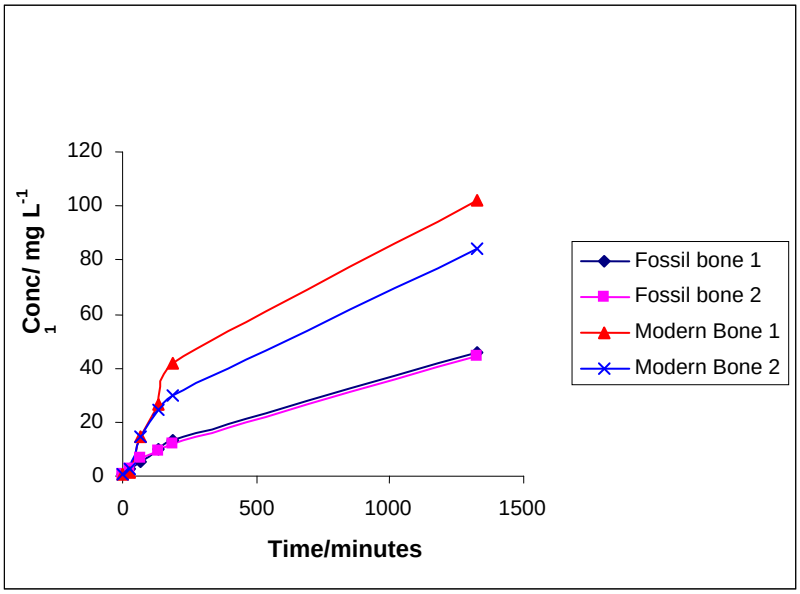


Figure 7.36 Relationship and trend between non coated fossil bones (FB) and uncoated modern bones (CB) as a function of calcium evolution related to time performed in duplicate.

7.5.2 Gravimetric analysis for the comparison of modern vs. fossil bone in hydroxylamine

To validate that the effect of hydroxylamine on fossil bone specimens is negligible, comparative analysis was performed using an uncoated clean fossil bone fragment and a uncoated modern cow bone fragment.

Table 7.3 Gravimetric analysis for a comparison between uncoated fossil bones vs. modern bones treated with 0.14 mol L^{-1} hydroxylamine solution at an initial pH of 3.75 performed in duplicate.

	Initial mass/g	Final mass/g	Final pH
Cow bone 1	0.3066	0.2934	4.72
Cow bone 2	0.1427	0.1308	4.61
Fossil bone 1	0.4886	0.4823	4.22
Fossil bone 2	0.7453	0.7381	4.39

In order to validate the use of hydroxylamine for the non-destructive removal of manganese oxide on a microscopic scale, a comparative experiment was performed whereby the rate of calcium evolution is compared for a modern cow bone and a clean uncoated fossil bone. The rate of calcium evolution is considerably greater for the modern cow bone specimen compared to the fossil bone. This difference can be attributed to the different chemical compositions of the two different specimens. The cow bone primarily consists of calcium carbonate, phosphates and hydroxy appetite that is more susceptible to acidic conditions and ligand dissolution. Conversely the fossil bone

specimen has undergone mineral replacement and diagenesis, thus forming a fluorohydroxyl appetite and silicious material has replaced the less resistant bone matrix making it more resistant to weathering and chemical dissolution. This difference between modern bone and fossil bone specimens shows that fossil bones cannot be treated with the same methodology as modern bone material.

7.6 Treatment of fossil bone fragment soaked in a catechol solution

Analysis was performed in duplicate as shown below. After treatment in catechol followed by treatment in a 0.14 mol L^{-1} hydroxylamine solution at an initial pH value of 3.75, the original dark coating appeared to become even darker with a glossy texture.

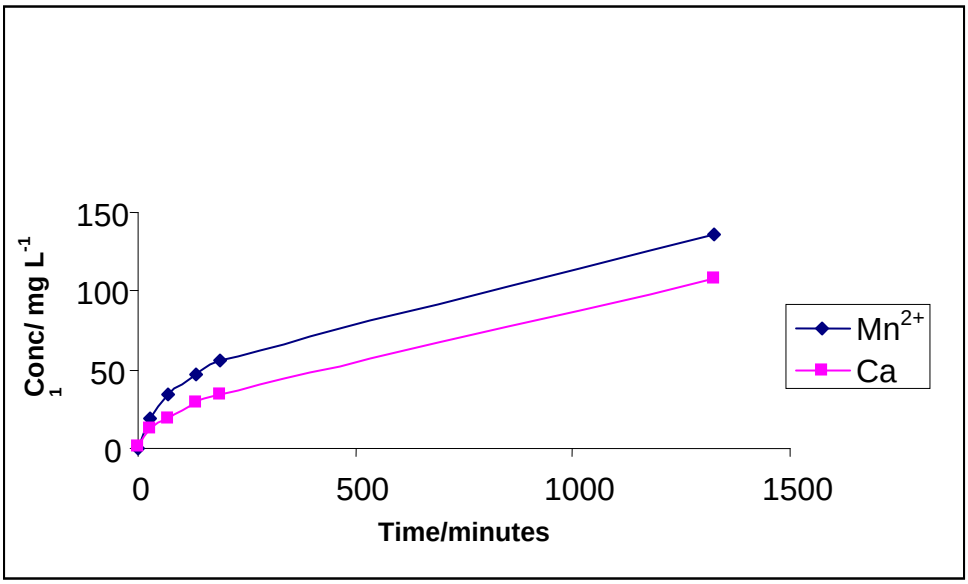


Figure 7.37 Relationship between manganese and calcium evolution trends as a function of time for manganese oxide coated fossil bone (Sample 1) pre-treated with 0.10 mol L^{-1} catechol before being treated in 0.14 mol L^{-1} hydroxylamine at initial pH of 3.75.

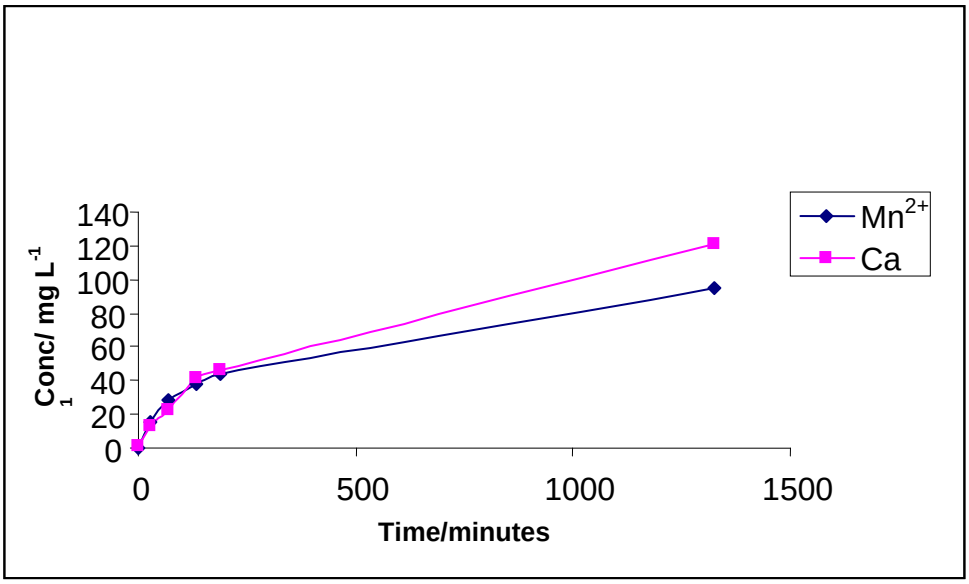


Figure 7.38 Relationship between manganese and calcium evolution rate trends for manganese oxide coated fossil bone (Sample 2) pre-treated with 0.10 mol L^{-1} catechol before treatment in 0.14 mol L^{-1} hydroxylamine.

Catechol is an organic ligand that was used in an attempt to remove the manganese oxide coatings. Although fairly successful, the disadvantage to using this method is the product from the reduction reaction of manganese dioxide and subsequent oxidation of catechol is a polymeric substance (Matocha, *et al*., 2001). This polymer was found to also bind strongly to the porous bone surface, thus obscuring it. In an attempt to remove this new polymeric layer, two bone fragments previously treated with catechol were placed into the optimized hydroxylamine solution. The rate of calcium and manganese evolution was measured in order to determine the efficiency of the catechol treatment. The two samples showed poor reproducibility.

7.7 Method overview

Hydroxylamine removes manganese oxide fossil bone coatings efficiently and the rate of the reduction reaction for the optimized parameters is fast. This method should however only be used when no other chemical fossil specimen preparation methods are available as this is the strongest acid and reducing agent of all the chemical methods developed, optimized and validated. Although validation has shown that this method is non-invasive it should only be used on specimen samples that are not sufficiently cleaned by the hydrogen peroxide and nitrite fossil preparation method.