

Final Report

**Removal of Arsenic and Mercury Contamination in
Museums using a Natural Environmentally Benign
Chemical**

Grant No. MT-2210-05-NC-06

9/30/2006

**The Arizona State Museum
University of Arizona, Tucson, Arizona**

Principal Investigator: Peggi S. Cross

Project Team:

Dr. Nancy Odegaard, Arizona State Museum, University of Arizona

Dr. Werner Zimmt, Arizona State Museum, University of Arizona

Teresa Moreno, Arizona State Museum, University of Arizona

Dr. Wendell Ela, Environmental Engineering Dept., University of Arizona

Contact Information:

Arizona State Museum

Conservation and Preservation

P.O. Box 210026

Tucson, Arizona 85721-0026
520-621-6314
www.statemuseum.arizona.edu

TABLE OF CONTENTS

EXECUTIVE SUMMARY.....	3
1. INTRODUCTION.....	4
1.1 Pesticide contamination on museum artifacts.....	4
1.2 American Indian use of objects	5
1.3 Existing processes for the removal of arsenic and mercury.....	6
1.4 The structure and properties of α -lipoic acid.....	6
1.5 Photochemical reaction of α -lipoic acid.....	7
1.6 The solvency of α -Lipoic acid and reduced α -lipoic acid	8
1.7 The reaction of α -lipoic acid with arsenic	8
1.8 Sorption/desorption studies of arsenic and mercury using XRF.....	8
1.9 Analysis of materials changes using ATR-FTIR.....	9
2. MATERIALS AND METHODS.....	10
2.1 Preparations of Solutions.....	10
2.1.1 Solubility of organic solvent based solutions.....	10
2.1.2. Effect of acid/base concentration on solubility.....	10
2.2 Photolysis of α -lipoic acid.....	11
2.2.1 Acid/base effects on reduction rate.....	12
2.2.2 Effect of reduction on the pH and solubility.....	13
2.2.3 Effect of different light sources.....	13
2.2.4 Effect of temperature on the photolysis rate.....	15
2.3 Reaction of α -lipoic acid with sodium arsenite or sodium arsenate.....	15
2.4 Selectivity of binding of reduced α -lipoic acid to arsenite versus anions and cations.....	15
.5 Preparation of test materials and sorption studies.....	16
.6 Cleaning of materials.....	16
3. RESULTS AND DISCUSSION.....	17
3.1 Reaction of α -lipoic acid with sodium arsenite and arsenate in solution..	17
3.2 Selectivity of binding of reduced α -Lipoic acid to arsenite versus anions and cations.....	19
3.3 Sorption rates of sodium arsenite and mercuric chloride to materials.....	21
3.4 Results of removing arsenic and mercury from materials.....	22
2.5 ATR-FTIR Results.....	28
4. CONCLUSIONS AND RECOMMENDATIONS.....	29
5. ACKNOWLEDGEMENTS.....	30
5. REFERENCES.....	30

EXECUTIVE SUMMARY

Some natural science specimens and ethnographic artifacts in museums were historically treated with arsenic and mercury salts. This has created an environmental concern for museum workers and the public who may be exposed to these toxins. In addition, museums are frequently being asked to return sacred objects under the Native American Graves Protection and Repatriation Act (NAGPRA). Artifacts that are otherwise well contained and rarely handled are being returned to tribes for culturally appropriate use which may involve direct human contact. The need for a method to decontaminate artifacts and museum surfaces without causing degradation to the surfaces or exposure to personnel poses limitations on the methods that can be employed. The cultural beliefs of the Native American people also limits appropriateness of potential techniques, as some objects may be considered to be living spiritual beings.

α -Lipoic acid is a natural environmentally benign chemical that is integral to all plants and mammals and is patented as an agent for the cure of many diseases. It has also been demonstrated that α -lipoic acid acts in-vivo for the detoxification of both arsenic and mercury in biochemical studies dating back to the late 1950's (Reiss et al (1958); Reiss et al (1958); Grunert (1960); and Wagner (1956)). It is known that α -lipoic acid does not require reduction to bind to mercury (Brown, 1968), however, the literature does not indicate whether reduction is required for binding to arsenic. It has been demonstrated that reduced α -lipoic acid binds strongly to arsenic in water (Spuches et al, 2005). Thus, due to the attributes of α -lipoic acid as a natural healing and detoxification agent and its known ability to react with arsenic and mercury, it is deemed to be the most appropriate cleaning agent for artifacts.

In this study, aqueous α -lipoic acid solutions were developed and reduced using natural sunlight and laboratory ultra violet lamps. The solubility in various organic and inorganic solutions were examined, and variables that may impact the reduction and solubility, such as pH and temperature, were examined. Arsenic and mercury on natural materials such as cotton, wool, paper and feathers were studied using a Niton handheld X-ray Fluorescence Spectrometer (XRF) to monitor contamination levels. A processing sequence that optimized decontamination was developed by running a series of full factorial experiments, which were analyzed using analysis of variance (ANOVA) techniques.

Up to 1000 $\mu\text{g}/\text{cm}^2$ arsenic (of sodium arsenite) was removed to levels near the lower detection limit of the XRF (1 $\mu\text{g}/\text{cm}^2$) without leaving residues. Similar results were achieved in removing mercury (of mercuric chloride) from cotton and paper; however, the solutions and processes developed were not capable of removing mercury from sulfur-bearing materials such as wool and feathers.

1. INTRODUCTION

.1 Pesticide contamination on museum artifacts

The Native American Graves Protection and Repatriation Act (NAGPRA) became law on November 16, 1990 [§25 U.S.C. 3001-3013]. The law governs the ownership rights of Native American human remains, funerary objects, sacred objects and objects of cultural patrimony giving priority ownership to the lineal descendents and mandating the preparation and delivery of inventory lists by museums to the appropriate descendents. NAGPRA regulations mention the topic of pesticides only once (Section 10 under Repatriation 10.1 Part [e]): *The museum official or Federal agency official must inform the recipients of repatriations of any presently known treatment of human remains, funerary objects, sacred objects, or objects of cultural patrimony with pesticides, preservatives, or other substances that represent a potential hazard to the objects or to persons handling objects.* The ability to do this with any accuracy is highly variable, as past record-keeping was not “state-of-the-art,” as it is now. In some cases, whole areas were fumigated and specimens may have even been treated in the field or by private collectors before the museums received the artifacts.

In 2000 the first workshop of the pesticide contamination issue was held at the Arizona State Museum. The realization that the artifacts were contaminated brought deep shock and anger to the tribes who worked so hard in the initiation of the repatriation act (Loma’Omvaya, 2001). On April 4, 2000 representatives from the Arizona State Museum presented testimony to the NAGPRA Review Committee and urged attention to this issue. Subsequently, the NAGPRA Grant Program has funded collections surveys, educational workshops, and consultations directed to pesticide residue identification (Odegaard and Sadongei 2005).

The artifacts in question are sacred beings of the Native Americans and any treatment or handling of the objects must be done in accordance with their cultural beliefs and systems. Some believe that “The artifacts are living spirits who cry to come to the ceremonies back home and dance with “their people” (Hostler et al, 2001). The complexity of the health issues associated with the presence of the toxins are preventing their return to use. A natural method of detoxification is desired that can be integrated into the ceremonial practices of the Native American peoples and which does not drive the toxins further into the artifacts or cause harm to the environment or the person performing the act of detoxification.

.2 American Indian use of objects

The belief systems of the Native Americans and the cultural use of Native American Indian objects is integral to the potential disposition of repatriated objects. The need to define the scope of treatment required to insure that the objects are cleaned sufficiently for a particular use is important. The chemistry developed must be incorporated into a wide variety of ceremonial procedures. Following development it is critical that Native American representatives be consulted prior to any pesticide removal treatments.

The use of objects by American Indians can be physical, symbolic or for life ending use (Sadongei, 2001). The use of sacred or ceremonial objects is based on religious practices and is not commonly known by tribe members not directly affiliated and trained in the practice. Handling or activating of such objects is restricted to specific religious leaders or trained individuals and this is sometimes gender restricted. In some cases, the physical presence of the object symbolically provides a connection to the tribal ancestors and cultural legacies. In some cases, the objects must be allowed to naturally decay or be burned in order to complete the end of life use of the object. This release of the life energy from the object completes the purpose for which the object was created. The Zuni War Gods are an example of this type of object. The Zunis repatriated the War Gods in order to expose them to the elements and allow them to naturally decay (Sadongei, 2001).

The Haudenosaunee Standing committee, or Iroquois Confederacy, which represents several nations of Indians in the New York and Ontario Canada area, repatriated 455 medicine masks in 1998 from the National Museum of the American Indian. A sampling of the masks using spot test papers indicated that 7 percent of the masks tested positively for arsenic. The sacred medicine masks are considered to be the Haudenosaunee's helpers and are referred to as their "grandfathers" (Jemison, 2001). Members of these tribes had hoped to wear the objects in ceremony. The Hopi Katsina kwatsi or "friends" are also objects worn on the head. Hopi elders considered the use of preservatives as poisoning and endangering the friends. The Hopi tribe has actively sought the assistance of researchers at the University of Arizona to test hundreds of objects repatriated from museums throughout the United States for heavy metal pesticides prior to any cultural use.

It was the goal of this research to develop and scientifically test a chemistry that can remove mercury and arsenic toxins from materials representing those used on Native American artifacts. Principles that guided the research were that (1) they must be done in a manner that does not promote the diffusion of

toxins further into the objects (2) Artifacts would not be used in this study and it would **not** be a goal of this work to create the ceremonial procedures for detoxifying the “friends,” “grandfathers” or “Gods” of the Native American people, and (3) that a presentation would be made to tribal representatives to obtain comments.

1.3 Existing processes for the removal of arsenic and mercury

The literature involving the removal of arsenic from materials is limited to studies done on skin (Abdel-Rahman et al, 2005; Wester et al, 1992). Arsenic has an affinity for the sulfhydryl groups in the proteins of skin that causes it to accumulate. The arsenic binding to the skin has been shown to be reversible so that arsenic that has built up in the skin can be slowly released within the body after exposure occurs (Dutiewicz, 1977).

There are numerous studies on the sorption and removal of mercury from wool, as it was studied in the late seventies as a method for removing and recovering mercury from water (Friedman et al, 1972; Masri et al, 1972; Tratnyek et al, 1972; Friedman et al., 1973; Miuamoto et al, 1977; and Laurie et al, 1979).

A limited number of attempts have been made to decontaminate artifacts in museums. A fume cabinet was designed in which museum objects were placed and subjected to compressed air cleaning. This resulted in a maximum removal of 40 percent of the arsenic residues as determined by physically removing samples and analysis using gas chromatography (Glastrup, 2001). The Onondaga Nation of the Haudenosaunee developed a procedure to clean the “grandfathers,” which comprised of repetitions of washing them with soap and water and vacuuming them. This helped to reduce the levels of arsenic on the surface of the objects by an unspecified amount (Jemison, 2001).

There are no published studies of the use of α -lipoic acid to remove arsenic or mercury from natural materials such as cotton, wool, paper and feathers.

1.4 The structure and properties of α -lipoic acid

α -Lipoic acid, also called 1,2-dithiolane-3-pentanoic acid or 6,8-epidithiooctanoic acid and formerly called 6-thioctic acid, or 1,2-dithiolane-3-valeric acid was first discovered in the 1940s by several labs independently (Reed, 1956). Figure 1 illustrates the structure of α -lipoic acid and the reduced form, dihydrolipoic acid .

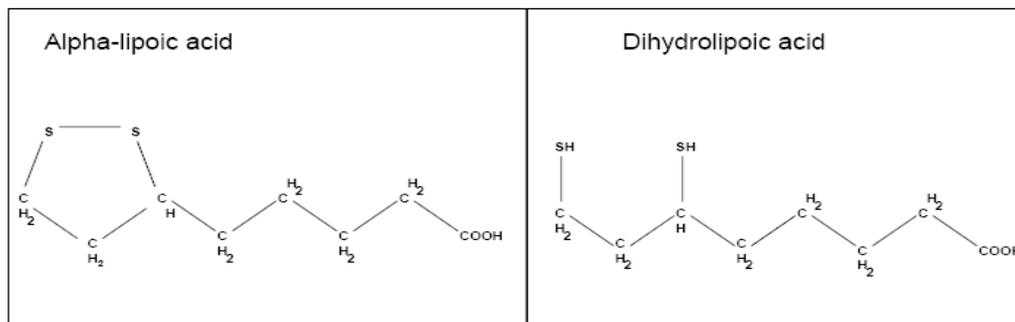


Figure 1. The structures of α -lipoic acid and dihydrolipoic acid redrawn from Packer et al (1995).

.5 Photochemical reduction of α -lipoic acid

It has been demonstrated that mercury can bind to α -lipoic acid without reduction; however, reduction of α -lipoic acid may be necessary for binding to arsenic. This reduction can occur via photochemical reduction, but the chemical nature of the solution, as well as the concentration of the α -lipoic acid can affect the rate and extent of reduction and limit the potency and viability of the solution.

α -Lipoic acid can be reduced using the ultraviolet light in sunlight or laboratory lamps. It is desirable to minimize the photochemical reduction rate for purposes of preparing and using solutions within a day. The reduction of α -lipoic acid to form dihydrolipoic acid (DHLA) occurs by homolytic rupture of the S-S bond followed by protonation. This can be monitored using a UV spectrometer by the disappearance of the 330 nm absorbance peak (Matsugo et al, 1996). As an example, Figure 2 shows the results of photochemical reduction of a 0.01 M solution of α -lipoic acid reduced using the 302 nm UV lamp.

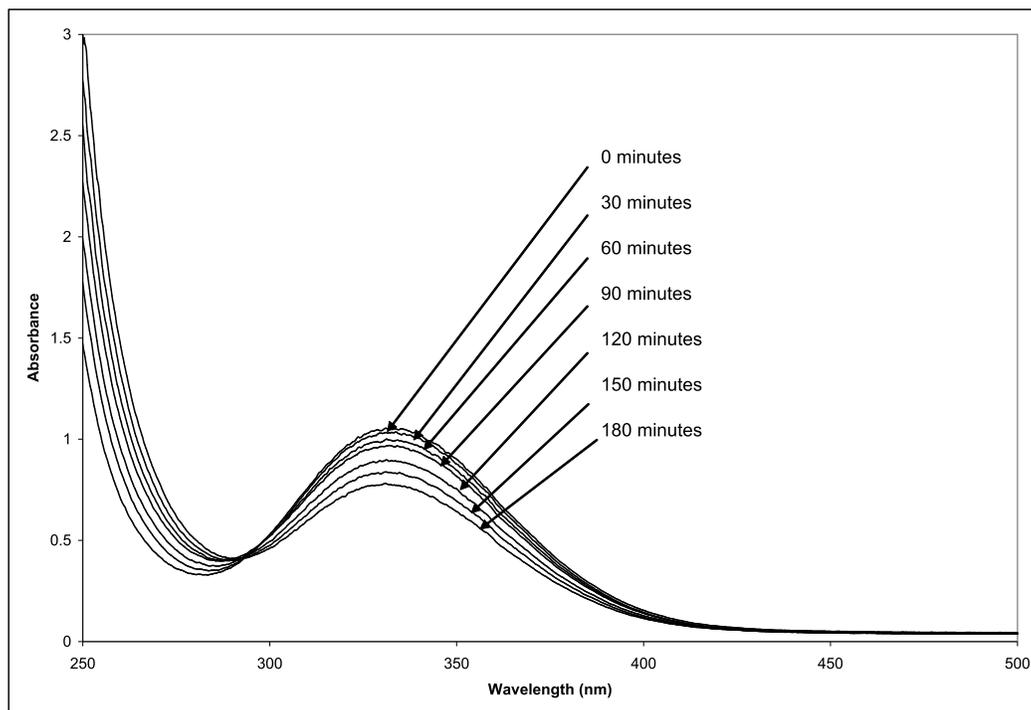


Figure 2. Time-dependent UV Spectral Change of 0.01 M α -lipoic acid.

1.6 The solvency of α -lipoic acid and reduced α -lipoic acid

A concentrated solution, at near neutral pH, that does not contain solvents is desired for purposes of detoxifying fragile artifacts. One of the key barriers to the use of α -lipoic acid in aqueous solutions is its hydrophobic nature, which limits solubility and hence the ability to form concentrated aqueous solutions. The photolysis of α -lipoic acid in organic solvents was extensively cited in the literature (Brown and Edwards, 1969; Whitney and Calvin, 1955; Zimm and Bragg, 1952; Walton et al, 1956; Barltrop et al, 1954), however work done on the photochemical reaction in strictly aqueous solutions was limited (Matsugo et al, 1996).

1.7 The reaction of α -lipoic acid with arsenic

The binding of the arsenic(III) to α -lipoic acid in water occurs at the sulfhydryl end groups of reduced α -lipoic acid and can be studied by monitoring the 270 nm absorbance with UV-VIS Spectroscopy (Spuches et al, 2005). There is no literature on the binding of arsenic(V) to reduced α -lipoic acid and it is unknown whether α -lipoic acid must be reduced to react with either arsenic (III) or (V). Other anions and cations may be present on the artifacts that may interfere with binding to arsenic and this must be accessed as well.

1.8 Desorption studies of arsenic and mercury using XRF

The sorption rates of arsenic and mercury salts must first be studied so that a comparison can be made to the de-sorption rate using the developed solutions. The sulfhydryl content of the material being cleaned may have an impact on the ability of the solutions to remove the metal salts causing differences in the ability of the α -lipoic acid solutions to remove metal salts from sulfur containing and non-sulfur containing materials.

In addition, the process sequence for using the cleaning agent must be developed to optimize the use of the cleaning agent and minimize the amount of rinsing that must occur. Any vigorous processing requirements may damage fragile artifacts being cleaned with this technique.

The instrument used to analyze the arsenic and mercury content on materials after contamination and cleaning was a Niton X-ray Fluorescence Spectrometer. The instrument capability was established by the Arizona State museum in a previous NCPTT grant funded project. The instrument demonstrated the ability to repeatedly detect levels of arsenic and mercury from 1 to 5000 $\mu\text{g}/\text{cm}^2$.

9 Analysis of material changes using ATR-FTIR

The developed process should not alter the materials being cleaned or leave residues on the materials. Attenuated Total Reflectance Fourier Transform Spectroscopy (ATR-FTIR) was used to study the key chemical functional groups of interest for α -lipoic acid and the materials treated. The resulting transmitted interferogram is transformed into a spectrum with peaks characteristic of the chemical functional groups that the material is made of. An analysis of how these peaks change during treatment is useful in understanding if the material was altered or residues remained.

In summary the objectives of this study were:

1. Develop high concentration α -lipoic acid solutions without the use of solvents.
2. Study the reduction rate of α -lipoic acid using natural sun light and laboratory ultraviolet lamps.
3. Study the binding of arsenic to reduced and unreduced α -lipoic acid at the carboxyl end group and the sulfur and sulfhydryl moieties.
4. Study the preference for binding of reduced α -lipoic acid to arsenic over other anions and cations.
5. Develop a processing sequence that minimizes the volume of chemicals used for cleaning and the amount of vigorous treatment to the materials.
6. Study the de-sorption of arsenic and mercury using the α -lipoic acid solutions developed.
7. Determine whether there are changes in the materials or residues from the cleaning process.

2. METHODS AND MATERIALS

.1 Preparations of Solutions

In a typical solubility test, α -lipoic acid (Sigma-Aldrich, >99%), designated as lipoic acid from here on after, was directly dissolved in ethanol (Sigma-Aldrich, >99%), 1-propanal (Fluka, >99.5%), and isopropyl alcohol (Sigma-Aldrich, Reagent Plus >99%), and the solutions were diluted with de-ionized water in order to determine the minimum amount of solvent required to keep the lipoic acid in solution. These solutions were then added to various concentrations of citric acid (Sigma-Aldrich, 99.5%) or bases such as tetra methyl ammonium hydroxide (Aldrich, 0.2 M Reagent Grade), ammonium hydroxide anhydrous (Sigma-Aldrich), potassium hydroxide (Mallinkrodt) and sodium hydroxide (MCB Reagents).

Solubility could be achieved without organic solvents by addition of the lipoic acid to 2 M ammonium hydroxide or sodium hydroxide, followed by ultrasonic dispersion for five minutes and then dilution to the concentration of interest. This procedure was used in all experiments that did not include the use of organic solvents.

.1.1 Solubility of organic solvent-based solutions

The solubility of lipoic acid in ethanol, 1-propanal and 2-propanal (isopropyl alcohol) were studied. A solution with a maximum lipoic concentration of 1.92 mg/ml (9.3×10^{-3} M) using 9 percent alcohol was achieved using this technique.

2.1.2. Effect of acid/base concentration on solubility

Various concentrations of lipoic acid in 1-propanol were added to 0.002 M KOH and NaOH in order to determine whether solubility could be enhanced. The regime for obtaining solutions was not significantly enhanced at this molar base concentration, and when solutions were monitored over time at room temperature some of the solution formed dispersions.

The solubility of lipoic acid was eventually examined in the concentrated base solutions of 2 M ammonium hydroxide and 1 M sodium hydroxide. Concentrated solutions could then be diluted without precipitation of the lipoic

acid, resulting in an alcohol-free solution. Solutions that yielded concentrations of 1, 2, 3 and 4 mg/ml lipoic acid and 0.04, 0.07, 0.1 and 0.2 M ammonium hydroxide (without the use of alcohol) were prepared and compared to like solutions that contained various concentrations of isopropyl alcohol.

2 Photolysis of α -lipoic Acid

Solutions were placed in 12 x 75 mm borosilicate test tubes (VWR 12578-165) and sealed with 13mm Neoprene stoppers (VWR 28296-602) during photochemical reduction. Test tubes were placed flat in a black tray and exposed to direct sunlight at an angle perpendicular to the sun or the UV lamp. The actinic flux exceeded 900 Watts/m² (in all reported cases) as confirmed by an Eppley Normal Incidence Pyrheliometer on the roof of the Atmospheric Sciences Building at the University of Arizona, where the tests were performed. 8-watt 365 nm or 302 nm ultraviolet light source (UVP Model UVLM-28 (265/302) with relative intensity of 500/640 μ W/cm²) were used by placing the black tray of test tubes 16 cm under the light source. Tests done to vary the temperature of the solutions during exposure were accomplished by placing the test tubes on top of an ice water bath or a hot plate.

Photochemical reduction was monitored using a Shimadzu UV-2101 PC, UV-VIS Spectrometer or a Hitachi U-2000 UV-VIS Spectrometer. The reduction of the lipoic acid was monitored by measuring the decrease in absorbance at the maxima (330 nm) for the S-S bond.

Ellman's technique (Ellman, 1959) for the detection of SH by reaction of 5,5'-Dithiobis 2-nitro-benzoic acid or "DTNB" (Sigma) with the solution of interest was used. An SH calibration curve was prepared using 2-mercapto ethanol (J.T. Baker, > 99.5%) in de-ionized water as a standard and measuring the absorbance at 412 nm.

A comparison of the reduction rate of 2 mg/ml lipoic acid solutions under 302nm UV Lamp exposure in pure IPA vs. aqueous based solutions is shown in Figure 3.

The difference in the reduction rate is insignificant; and the offset in the magnitude of the absorbance diminishes with longer exposure times.

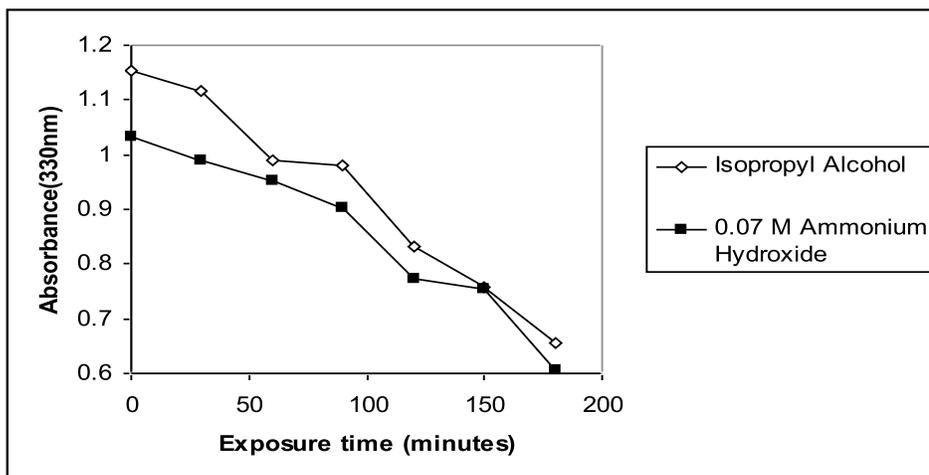


Figure 3. The reduction of lipoic acid using a 302 nm UV lamp for isopropyl alcohol vs. aqueous solutions as indicated by a decrease in the UV absorbance at 330nm.

Using the Ellam technique, it was determined that reduction in isopropyl alcohol and 0.07 M ammonium hydroxide is complete in three hours using the 302nm UV Lamp or 60 minutes in sunlight.

2.2.1 Acid/base effects on reduction rate

A comparison of 2.2 mg/ml lipoic acid reduced in sunlight in ammonium hydroxide vs. sodium hydroxide solutions indicated no change in the rate of reduction or the extent of reduction as shown in Figure 4 below.

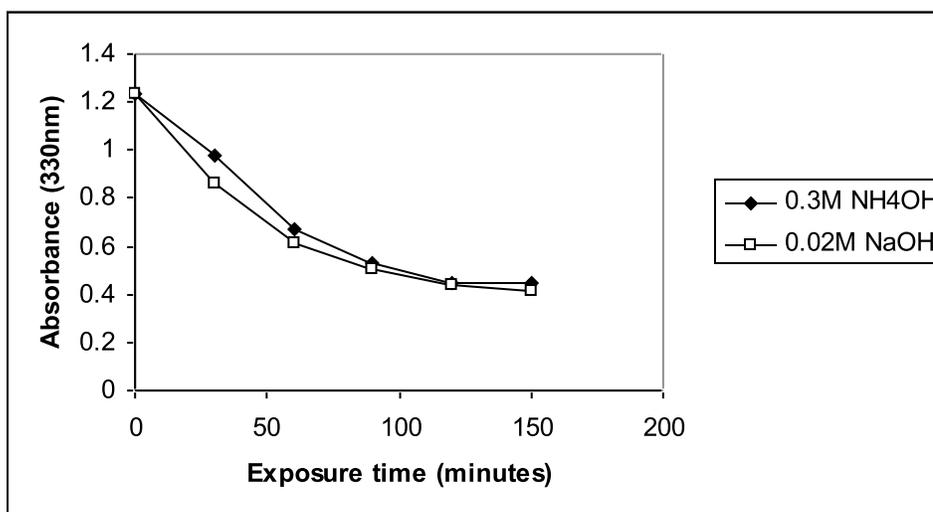


Figure 4. The reduction of 2.2 mg/ml Lipoic acid in basic solutions by actinic exposure as indicated by the decrease in the UV absorbance at 330nm.

The absorbance (330nm) of varying lipoic acid concentration in various concentration ammonium hydroxide solutions were measured after exposure to sunlight for 60 minutes in order to optimize the solution concentration without compromising the reduction rate. 0.2 M ammonium hydroxide did alter the ability to reduce the lipoic acid but lower concentrations did not. Based on these results, it was concluded that optimum reduction occurs for 2, 3 or 4 mg/ml lipoic acid solutions using 0.07 to 0.1 M ammonium hydroxide without the presence of isopropyl alcohol.

.2 The effect of reduction on the pH and solubility

While there originally appeared to be several viable solutions, turbidity was often induced during the photochemical reduction. The influence of pH on the formation of colloids was studied in order to understand the mechanisms leading to turbidity. Figure 5 shows the shift in pH for ammonium hydroxide solutions was more moderate than those from sodium hydroxide. Solutions of lipoic acid in ammonium hydroxide that resulted in pH values of 6 and under were turbid. The 0.07 M NH_4OH , 5 percent IPA case was slightly turbid before photochemical reduction and extremely turbid afterwards, indicating that any increase in solubility due to the presence of the solvent was depleted during photolysis.

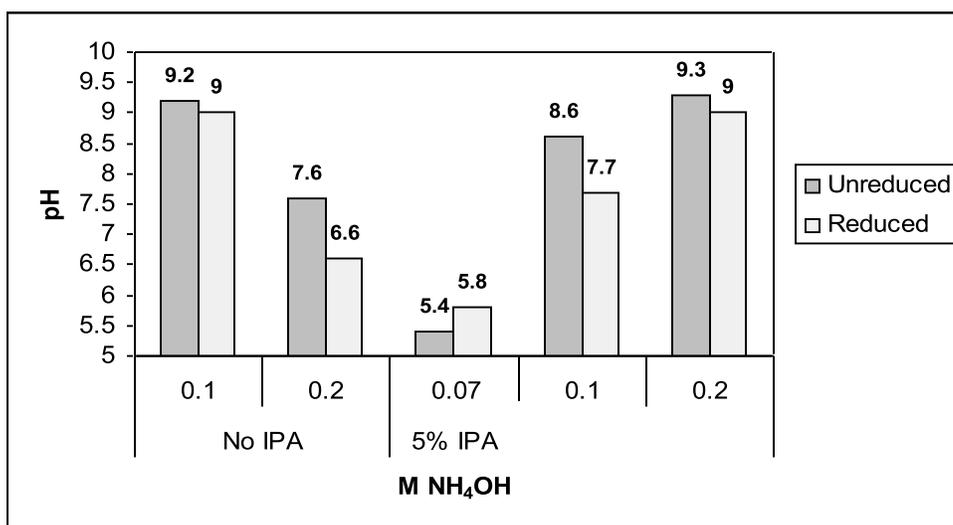


Figure 5. The change in pH after 60 minutes exposure to sunlight for 4 mg/ml lipoic acid solutions with various concentrations of ammonium hydroxide.

.3 Effect of different light sources

Eight watt UV light sources with wavelength intensities predominate in the 302nm and 365 nm ranges were compared. The supplier spectrum of the 302 nm light source indicated more light at the wavelength of interest (330nm); however, it was necessary to look at the integrated system due to decreased transparency of the borosilicate glass test tubes at the lower wavelengths. As shown in Figure 6, the two light sources showed an overlap in the ability to

reduce lipoic acid as indicated by the decrease in the 330nm absorbance. However, using Ellman's reagent, it was shown that the quantity of reactive -SH formed was greater for solutions reduced using the 302 nm UV Lamp (see Figure 7).

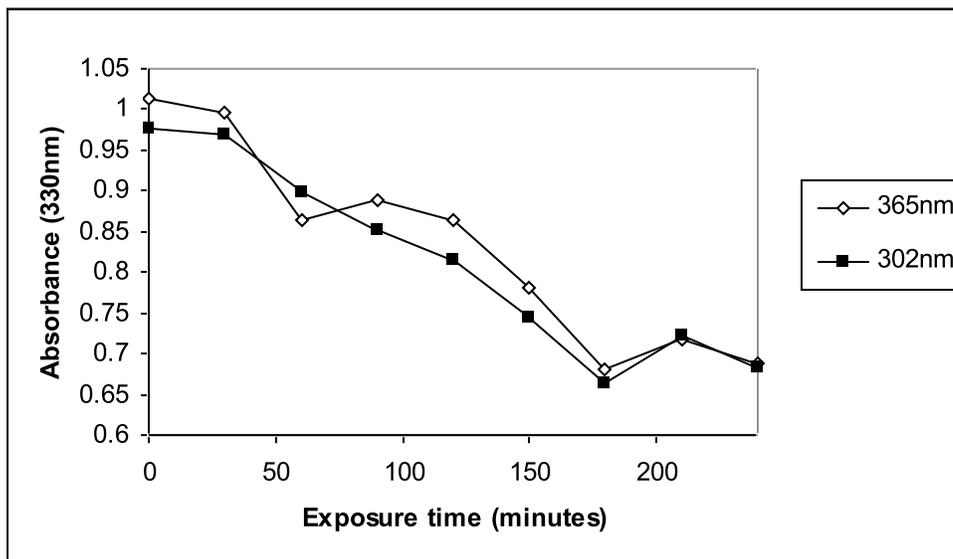


Figure 6. The reduction rate of 2 mg/ml lipoic acid (in 0.07 M ammonium hydroxide) as indicated by the decrease in the 330nm absorbance for two different 8-watt UV light sources.

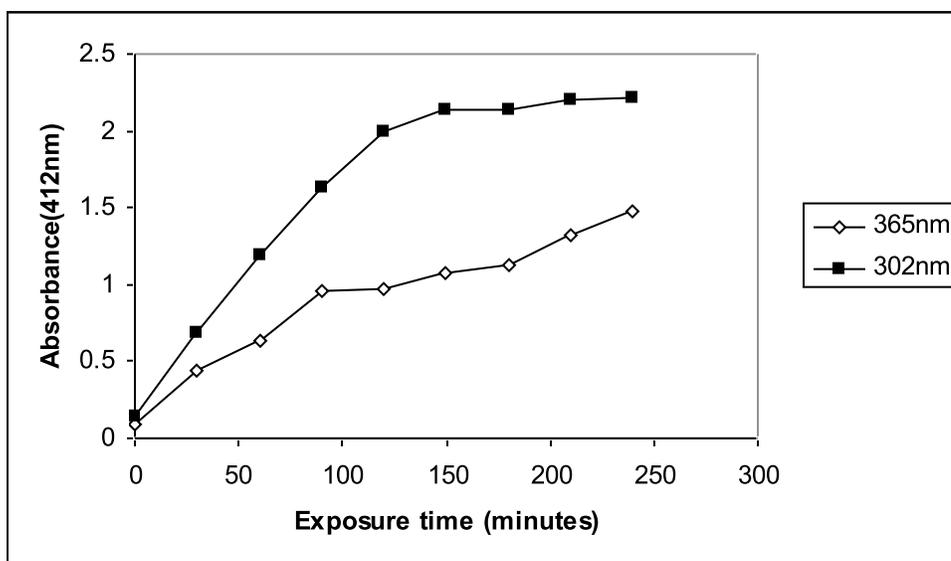


Figure 7. The formation of –SH during reduction of 2 mg/ml lipoic acid (in 0.07 M ammonium hydroxide) as indicated by the decrease in the 330nm absorbance for two different 8-watt UV light sources as indicated by reaction with Ellman's reagent and absorbance at 412nm.

.4 Effect of temperature on the photolysis rate

The temperature during photochemical reduction was examined in the regime for practical use. No significant effects of temperatures from 5 to 30 °C could be detected as shown in Figure 8 for solutions reduced using a 302 nm UV Lamp, and subsequent studies of the –SH formed upon reduction indicated no significant difference due to temperature.

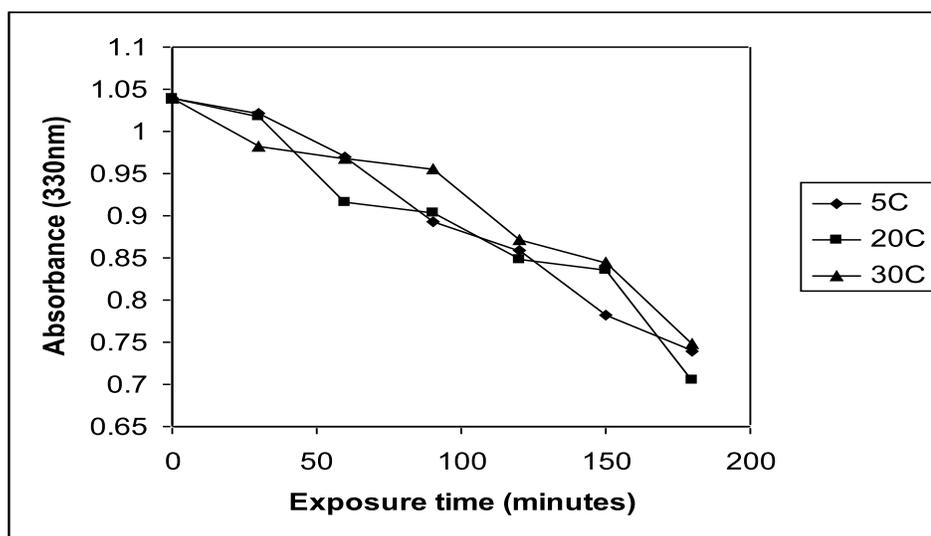


Figure 8. The reduction of lipioic acid vs. temperature for a 2 mg/ml sample in 0.07 M NH₄OH using a 302 nm UV light source as indicated by the decrease in the 330 nm absorbance.

.3 Reaction of α -lipoic acid with sodium arsenite and sodium arsenate in solution

The reaction of sodium meta-arsenite (Sigma-Aldrich, 90 percent) or sodium arsenate dibasic (Sigma-Aldrich) with lipoic acid to form an As-S bond was monitored in solution using UV-VIS spectroscopy absorbance at a wavelength of 270 nm (Spuches et al, 2005).

.4 Selectivity of binding of reduced α -lipoic acid to arsenite versus anions and cations

The ability of arsenic to bind to the sulfur in the lipoic group in preference to various anions was studied by first adding solution of the anions or cations to the lipoic acid cleaning solutions and then adding sodium arsenite to the solutions and measuring the absorbance at 270 nm. The following chemicals were used: nickel sulfate, iron sulfide, silver sulfate (Fisher Scientific); iron sulfate, mercuric chloride (Mallinckrodt); cadmium chloride, magnesium chloride, copper sulfate (J.T. Baker); calcium chloride, barium chloride (Matheson, Coleman and Bell); zinc acetate, sodium fluoride, sodium nitrate, sodium chloride, sodium sulfate (Sigma-Aldrich); and ammonium bicarbonate (Fluka.)

.5 Preparation of test materials and sorption studies

Solutions of sodium arsenite or mercuric chloride (Mallinckrodt) were dispensed onto 550 mm diameter filter paper (Whatman No.1), wool or cotton fabric test pieces (Test Fabrics, Pittston, Pa., Style 532 wool jersey knit or Style 46011 unbleached cotton interlock knit), or feathers (free range Quail) and then allowed to dry prior to measurement of the levels of contamination, using XRF. Feather pieces were approximately 3/4 inch square and were measured for contamination prior to testing to insure that there were no detectable levels of arsenic or mercury. Even dispersions were obtained using a pumped spray bottle.

Sorption of the metal salts onto the materials was studied by cutting the circular test pieces into quarters and placing the materials into magnetically stirred metal salt solutions for various lengths of time at room temperature.

.6 Cleaning of materials

Cleaning of materials was accomplished by dispensing cleaning solutions onto the contaminated materials. Various cleaning sequences included the pre-wetting and rinsing of the materials using de-ionized water or carbonated water (Safeway Select brand.) Pre-wetting and cleaning solutions were dispensed using a measuring pipette with the sample held horizontally and clipped to the edge of a tray. Rinses were done, with the samples held at a 45 degree angle, by dispensing the rinse solution from a wash squeeze bottle (VWR 16651-573).

In the initial stages of cleaning solution development, the entire circular test piece was subjected to cleaning. Multiple post cleaning XRF readings were taken in the center of the test piece and at four points (45, 135, 225 and 315 degrees) midway between the center and edge of the sample. In the final stages of development, the test pieces were cut into quarters and cleaned and measured for residuals, individually.

3. RESULTS

3.1 Reaction of α -lipoic acid with sodium arsenite and sodium arsenate in solution

The ability of lipoic acid to bond to arsenic in its unreduced state was tested, as this information was not available in the literature. No reaction occurred when arsenic (of sodium arsenite) was mixed with unreduced lipoic acid solutions. Reduction of the lipoic acid is necessary for use as a chelating agent for arsenic. Arsenic could be added to the reduced lipoic acid before or after reduction without impacting the extent of bonding to arsenic. As indicated in Figure 9, the lipoic acid or reduced lipoic did not react with arsenic (V).

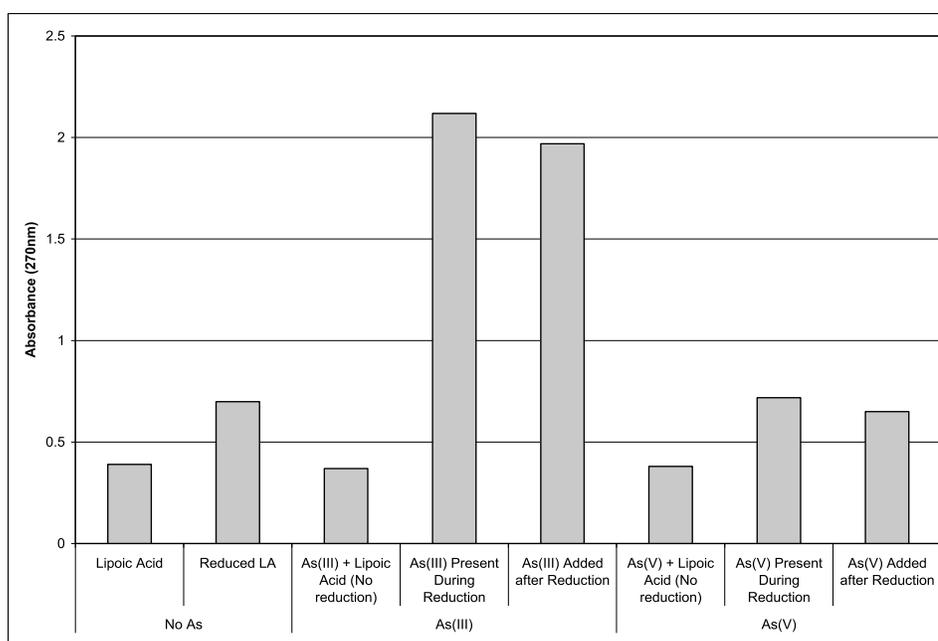


Figure 9. As-S Formation from As(III) and As(V) present during photochemical reduction of lipoic acid or added afterwards (180 min exposure to 302nm UV Source).

The pH of reduced solutions were adjusted using acetic acid, and the reaction rate of the arsenic to the reduced lipoic was tracked using individual samples for each test. The pH 6.2 sample was initially turbid before addition of the arsenic. As indicated in Figure 10, this caused a notable delay in the formation of the As-S bond but all cases approached comparable levels after 10 minutes.

Based on this study, a reaction time of 10 minutes should be allowed and turbid solutions should be avoided.

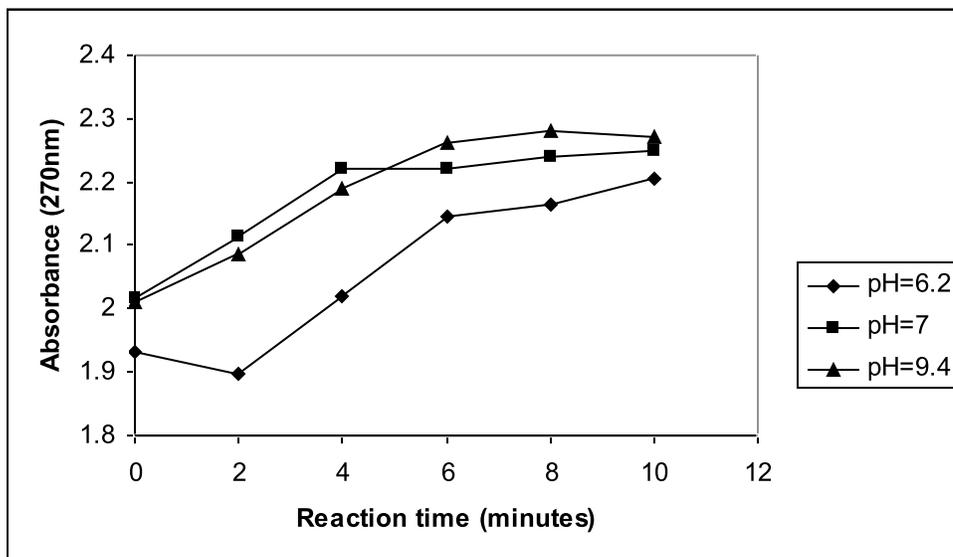


Figure 10. As-S bond formation for a 1:1 mole ratio of arsenic to lipoic acid exposed to sunlight 60 minutes for various pH adjustments made using acetic acid.

The formation of As-S after reaction of arsenic(III) with lipoic acid reduced using the 302 nm and 365 nm ultra violet source was tested using Ellman's technique and monitoring the absorbance at 412 nm. The formation of As-S measured using this technique was highly correlated to development of the 270 nm peak. Figures 11 and 12 show the development of the various absorbance curves over time for solutions reduced using the 302 nm and 365 nm sources.

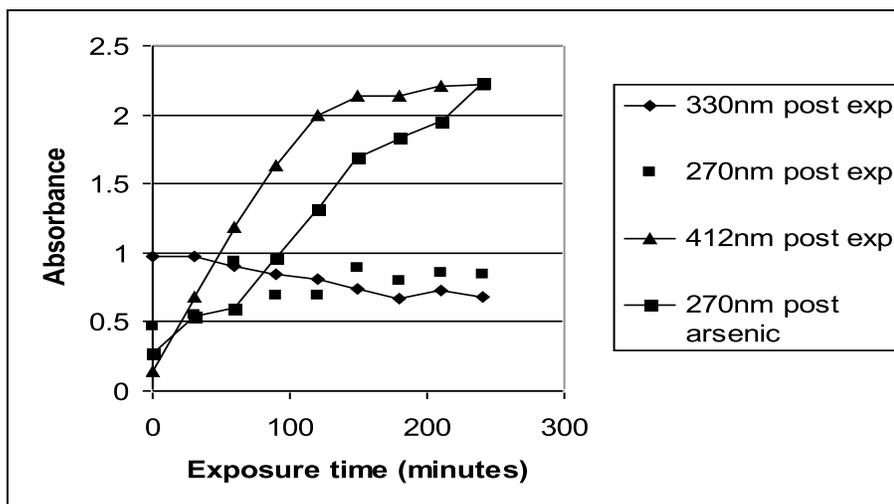


Figure 11. As-S bond formation (270 nm), S-S bond rupture (330 nm) and As-S bond formation using the Ellman technique (412 nm) absorbance. (Starting solution 2 mg/ml lipoic acid and 0.07 M ammonium hydroxide; 1:1 Mole ratio of arsenic(III) to lipoic; 302 nm UV lamp.)

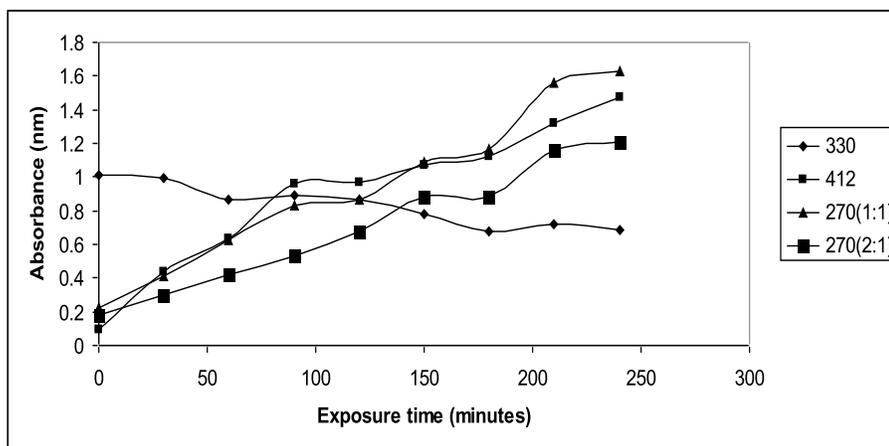


Figure 12. As-S bond formation (270 nm), S-S bond rupture (330 nm) and As-S bond formation using the Ellman technique (412 nm) absorbance. (Starting solution 2 mg/ml lipoic acid and 0.07 M ammonium hydroxide; Mole ratio of arsenic(III) to lipoic 1:1 or 2:1; 365 nm ultra violet exposure lamp.)

3.2 Selectivity of binding of reduced α -lipoic acid to arsenite versus anions and cations

Phosphate, sulfate, nitrate, fluoride and chloride anions were introduced in the form of sodium salts in order to understand if the use of regular tap or river water rather than de-ionized water or the presence of anions on the artifacts would influence the binding of arsenic to reduced lipoic acid. The results shown in Figure 13 indicate that phosphates, sulfates, and chlorides would not interfere with the bonding process for concentrations up to 1:1 mole ratio anion to lipoic acid. There was a lowering of the absorbance for higher concentrations of nitrates. Nitrates have a characteristic absorbance in the 270 nm range and the absorbance should increase due to this, thus nitrates may cause some interference with the bonding of arsenic(III) to reduced lipoic acid.

The study was repeated for the cations: Ni^{2+} , Fe^{2+} , Ag^{2+} , Hg^{+} , Cd^{2+} , Cu^{2+} , Mg^{+} and Zn^{2+} . Figure 14 shows the results. Turbidity was present in all cases except magnesium and zinc. The cadmium sol settled for reduced lipoic to cation ratios of 1:1. This was indicated by a reduction in the absorbance at 270 nm. Mercury formed a white turbid sol and the absorbance decreased at higher ratios, however, there was no visible settling. Thus one could expect these solutions to react with most metal cations to form colloids and possibly flocs. This could be advantageous if a passive coating were formed to protect metal layers on artifacts from further attack during treatment but metal ions in solution will compete with the binding sites for arsenic removal.

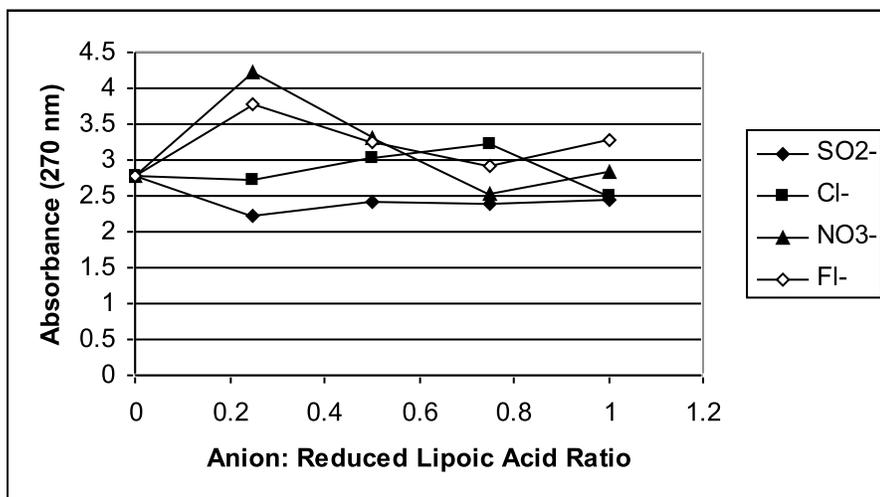


Figure 13. The effect of the presence of anions on the reaction of arsenic (III) with lipoic acid as indicated by 270 nm absorbance. (Starting solution 2 mg/ml lipoic acid and 0.07 M ammonium hydroxide; solution exposed to 302 nm UV light source for 3 hours; 2:3 mole ratio of arsenic(III) to lipoic.)

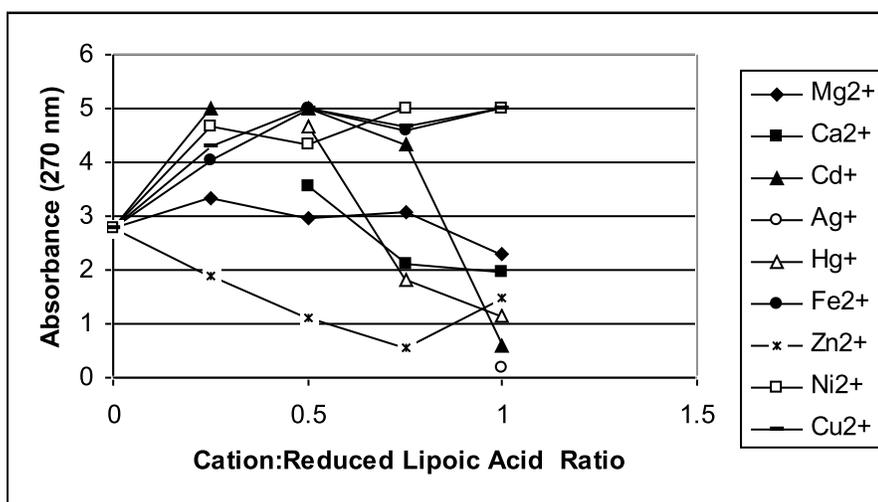


Figure 14. The effect of the presence of cations on the reaction of arsenic (III) with lipoic acid as indicated by 270 nm absorbance. (Starting solution 2 mg/ml lipoic acid and 0.07 M ammonium hydroxide; solution exposed to 302 nm UV light source for 3 hours; 2:3 mole ratio of arsenic(III) to lipoic.)

3.3 Sorption rates of sodium arsenite and mercuric chloride to materials

The room temperature (25°C) sorption of arsenic(III) to feathers and wool over time is shown in Figure 19. A linear increase in the sorption of 1000 ppm arsenic to wool over time was found to follow the equation $y = 1.25X + 73$ where y is the $\mu\text{g}/\text{cm}^2$ arsenic (III) absorbed over “ x ” time in minutes. The sorption to feathers did not increase significantly over time. This is most likely due to the hydrophobic nature of the feather.

There were no distinct desorption trends when an attempt was made to leach samples with $200 \mu\text{g}/\text{cm}^2$ arsenic from the materials by immersions in 25°C de-ionized water over time. The desorption curves for arsenic from wool indicated that as much as 95 percent of the arsenic was removed in the first minute.

Sorption of 1000 ppm mercury on feathers and wool was systematic as shown in Figure 15, however, no desorption of mercury from these materials could be achieved.

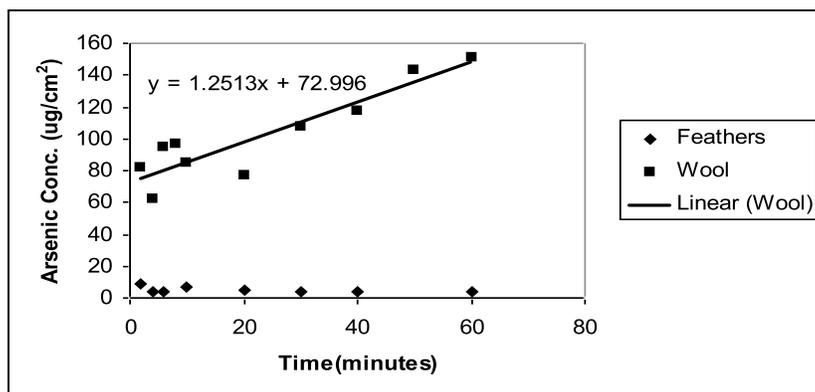


Figure 15. Sorption of 1000 ppm arsenic (III) in water to feathers and wool versus exposure time.

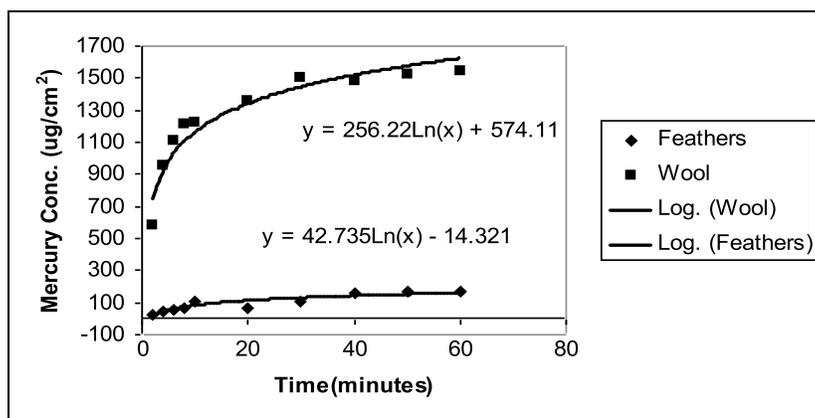


Figure 16. The sorption of 1000 ppm mercury (of mercuric chloride) onto wool and feathers versus time.

3.4 Results of removing arsenic and mercury from materials

Table 1 shows the results of treatment of 50 $\mu\text{g}/\text{cm}^2$ arsenic(III) contaminated filter paper using a de-ionized water rinse (twice) vs. various lipoic acid solutions with and without 5 percent isopropyl alcohol followed by the de-ionized water rinse. The results showed an improvement for the reduced lipoic acid solution and indicated that elimination of the isopropyl alcohol would give

better results. A t-test indicated a significant ($P=0.0001$) improvement in the reduced lipoic acid solutions without isopropyl alcohol.

Table 1. Results of removing $50 \mu\text{g}/\text{cm}^2$ arsenic from filter paper using different techniques and solutions.

Treatment	# of readings	Mean	Standard Deviation	% of baseline Removed
DI water, 2X rinse only	5	14.04	5.4	71%
5% IPA 0.07 M NH_4OH 1,2,3 mg/ml Lipoic <u>60 min solar radiation</u> DI water, 2X rinse	15	11.13	3.32	77%
NO IPA 0.07 M NH_4OH 1,2,3 mg/ml Lipoic <u>60 min solar radiation</u> DI water, 2X rinse	15	5.32	1.39	89%

Figures 17, 18 and 19 show the results obtained when highly contaminated materials were cleaned using different processing sequences. The process sequence was divided into three steps: 1) a presoak for 1 minute using either de-ionized water or carbonated water; 2) a cleaning step using a 2 mg/ml reduced α -lipoic acid in 0.07 M ammonium hydroxide and 3) a rinse step which involved four serpentine rinses from the top to bottom using either de-ionized water or carbonated water. The reduced lipoic acid clean showed improvements in the arsenic removal for all cases except when carbonated water was used as a presoak agent for cotton. The carbonated water was used to facilitate the wetting of the thickly woven cotton samples.

Table 2 shows the results of the ANOVA's run to analyze the experimental results. Reduced lipoic acid showed the most significant effect for the removal of arsenic from filter paper ($p=0.0005$).

The interaction between the clean step and pre-soak reagent was significant in the removal of arsenic from cotton ($p=0.0440$) while the clean step alone was not ($p=0.1135$). De-ionized water was the best pre-soak reagent.

Reduced lipoic acid showed the most significant effect on arsenic removal from wool as well ($p<0.0001$). The pre-soak ($p=0.0030$) and rinse reagents ($p<0.0001$) also showed significant differences. The effect of the interaction

between the reduced lipoic acid clean and the rinse solution was also significant ($p < 0.0001$). A de-ionized pre-soak and rinse were better when reduced lipoic acid was not used.

Table 2. Significance of the Variable Cleaning Sequences on the Residual Arsenic Levels (Variables that cause a significant effect ($p < 0.05$) are highlighted.)

Variable	Filter Paper (p-value)	Cotton (p-value)	Wool (p-value)
Pre-Soak Reagent	0.5850	0.3711	0.0030
Clean Step	0.0005	0.1135	<0.0001
Pre-soak x Clean	0.3980	0.0440	0.1200
Rinse Step Reagent	0.2230	0.7255	<0.0001
Pre-soak x Rinse	0.4352	0.2008	0.2777
Clean x Rinse	0.0890	0.7946	<0.0001
Pre-soak x Clean Step x Rinse	0.4015	0.6221	0.2934

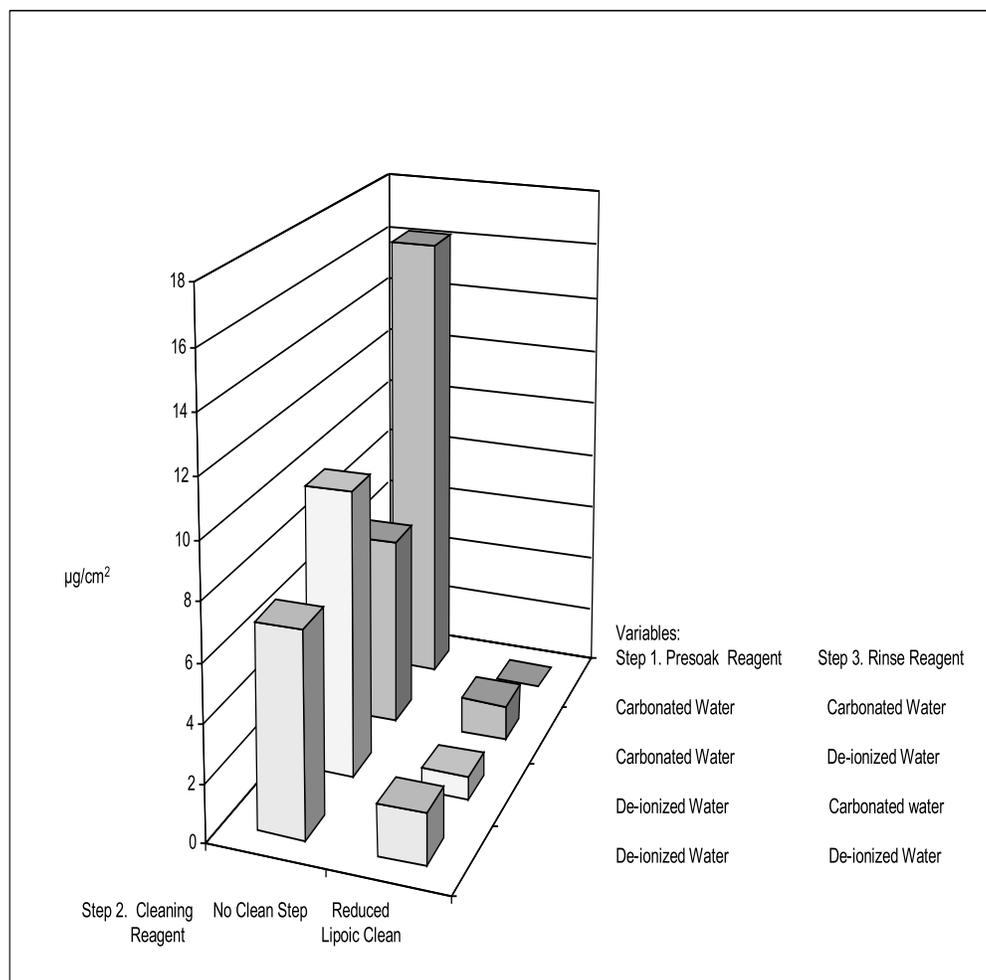


Figure 17. Average residual Arsenic(III) ($\mu\text{g}/\text{cm}^2$) on filter paper after different cleaning sequences. (Original contamination : $307 \mu\text{g}/\text{cm}^2$ Arsenic as NaAsO_2 .)

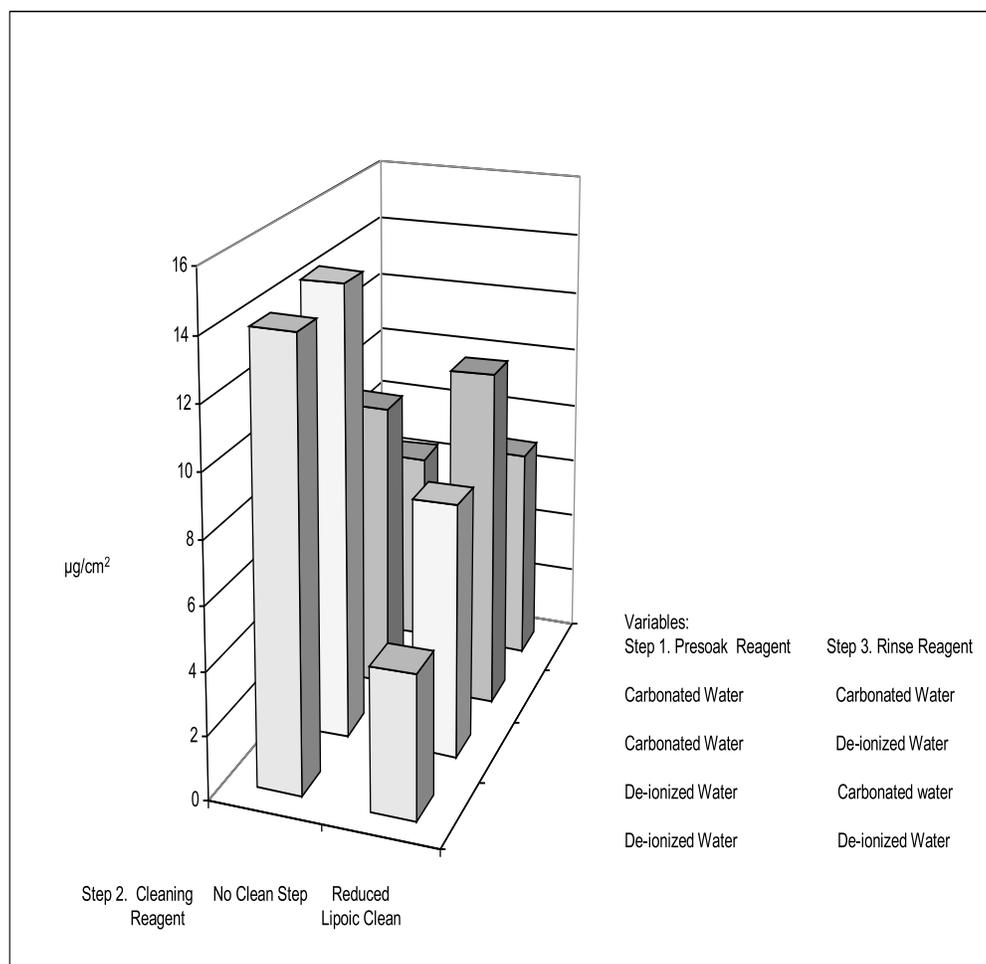


Figure 18. Average residual Arsenic(III) ($\mu\text{g}/\text{cm}^2$) on cotton after different cleaning sequences. (Original contamination: $403 \mu\text{g}/\text{cm}^2$ Arsenic as NaAsO_2 .)

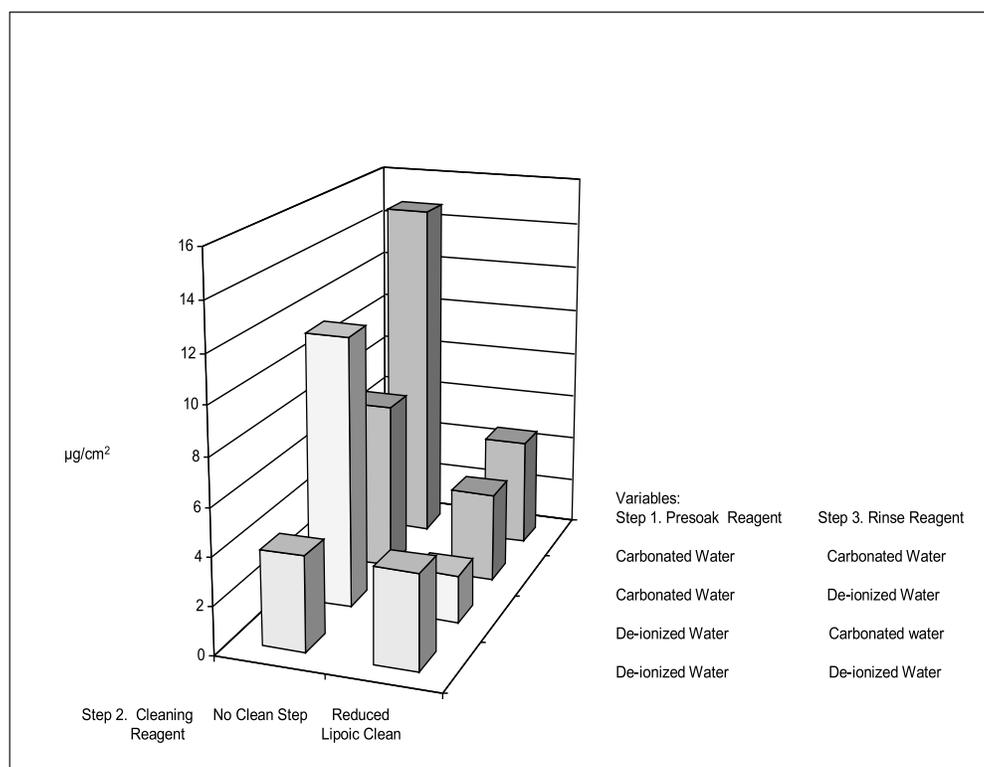


Figure 19. Average residual Arsenic(III) ($\mu\text{g}/\text{cm}^2$) on wool after different cleaning sequences. (Original contamination: $525 \mu\text{g}/\text{cm}^2$ Arsenic as NaAsO_2 .)

A series of experiments were run to determine if a process sequence that included a one minute presoak in de-ionized water and excluded the use of reduced lipoic acid would be effective. The process sequence used for the testing was as follows:

- Presoak- in 2 ml de-ionized water for 1 minute
- Rinse- using a serpentine pattern from top to bottom repeated four times using de-ionized water for filter paper, wool and feathers and carbonated water for cotton.

The ability of this process to remove arsenic (III) from heavily contaminated materials is shown in Table 3, 4 and 5 for samples that were cleaned twice using this process. The process sequences were effective in removing over 90 percent of the arsenic of sodium arsenite from filter paper, cotton, wool or feathers and

arsenic trioxide from filter paper. Over 90 percent removal of mercury was demonstrated from filter paper and cotton as well, however, the solutions were not capable of removing the mercury from wool.

Table 3. Percent Removal of Arsenic from Materials Contaminated with Sodium Arsenite.

	Initial Arsenic Conc. ($\mu\text{g}/\text{cm}^2$)	Percent Removed from 1 Cleaning Sequence	Percent Removed after 2 Cleaning Sequences
Filter Paper	1484	96.5	99.8
Cotton	1224	90.8	98.0
Wool	1354	95.6	99.7
Feathers	565	92.6	92.5

Table 4. Percent Removal of Arsenic from Materials Contaminated with As_2O_3 .

	Initial Arsenic Conc. ($\mu\text{g}/\text{cm}^2$)	Percent Removed from 1 Cleaning Sequence	Percent Removed after 2 Cleaning Sequences
Filter Paper	152	93	96.4

Table 5. Percent Percent Removal of Mercury from Materials Contaminated with Mercuric Chloride.

	Initial Mercury Conc. ($\mu\text{g}/\text{cm}^2$)	Percent Removed from 1 Cleaning sequence	Percent Removed after 2 Cleaning Sequences
Filter Paper	1548	93	99.3
Cotton	1496	65.1	93.2
Wool	2161	8.7	36.7

Table 6 shows the results of another experiments run in order to attempt to remove mercury (average 499 $\mu\text{g}/\text{cm}^2$) from wool using hot acetic acid. The temperature of the removal was shown to be the only significant factor ($p=0.0062$) with the lower temperature (60°C) removing more mercury. However, the maximum removal level was 43 percent.

Table 6. Experiment designed to test the removal of mercury deposited on wool (average level = 499 $\mu\text{g}/\text{cm}^2$) by immersion in acetic acid at different concentrations, temperatures and times.

Temperature	Time(min)	Acetic(%)	Residual Mercury ($\mu\text{g}/\text{cm}^2$)	Percent Removed
60°C	5	5	350.62	42.84
60°C	5	10	327.36	38.42
60°C	10	5	372.46	34.12
60°C	10	10	360.01	22.98
100°C	5	5	451.96	18.97
100°C	5	10	393.98	20.82
100°C	10	5	396.69	18.12
100°C	10	10	366.72	18.71

3.5 ATR-FTIR results

Analysis of cellulose filter paper samples indicated changes in the spectra upon the addition of arsenic (III) that were eliminated by treatments with reduced lipoic acid solutions, but were not eliminated by de-ionized water treatments alone. Similar results were not indicated for the removal of arsenic from other materials nor for the removal of mercury from materials. There was no indication that the lipoic acid solutions left residues on the materials using the techniques developed.

4. CONCLUSIONS AND RECOMMENDATIONS

Concentrated α -lipoic acid solutions could be produced without the addition of an organic solvent. It was determined that α -lipoic acid must be reduced to react with arsenic(III) and reduction could be accomplished in one hour using natural sunlight or 3 hours using an 8 watt UV lamp.

α -Lipoic acid or reduced α -lipoic acid will not react with arsenic(V). As the arsenic used on artifacts was historically arsenic(III) this is not a concern for artifact remediation.

The reduced α -lipoic acid solutions and processing sequences developed were shown to be effective in removing high concentrations of arsenic and mercury from non-sulfur bearing materials such as paper and cotton. The solutions could also effectively remove arsenic from feathers and wool but were ineffective in removing mercury from the sulfur-bearing materials. In general, there is a clear indication that further testing of the solutions on the more complex structure of wood could result in an effective and safe treatment for many of the wooden artifacts that are waiting to go home.

It was demonstrated that anions did not interfere with the binding of reduced lipoic acid to arsenic, however several of the cations tested reacted to form precipitates. This may be advantageous to cleaning if a passive metal organic layer is formed on the surface of metal or metal based painted objects to prevent them from corroding during cleaning. The anions present in regular ground water should not interfere with the cleaning process, however, there was an indication that carbonates which are very common in Southwestern waters could degrade the efficacy.

While de-ionized waters that did not contain reduced lipoic acid were capable of removing a large percentage of the arsenic and mercury, the volume of water required for treatment and the waste generated will be substantially greater. This may pose limitations to field work which could be minimized by the use of reduced lipoic acid. The binding of the metals to the reduced lipoic acid molecule will also provide safer solutions in case of inadvertent contact during cleaning. It was demonstrated that the metal organic complexes formed precipitated out under certain conditions. With further research, a process could be developed to filter those precipitates from the water so that special waste treatment is not required.

The proposal for using alpha-lipoic acid for the removal of arsenic and mercury from artifacts was presented to the Southwest Native Nations Advisory

Board at the Arizona State Museum, Tucson, Arizona in April of 2005 and the proposal was well received. The main concern during discussions on this topic was that there is a need for a better understanding of the diffusion of toxins in soils from objects that are buried after repatriation. The concern is that burials are often taking place near populated areas and waterways. The solutions developed could be used to minimize the contamination on artifacts prior to burial.

5. ACKNOWLEDGEMENTS

The work described in this paper was supported by a grant from the National Park Service and the National Center for Preservation Technology and Training. Its contents are solely the responsibility of the author and do not necessarily represent the official position or policies of the National Parke Service or the National Center for Preservation Technology and Training.

The author would like to thank the project team for their generous support throughout the project as well as the following individuals who have provided inspiration, encouragement and technical advice: Dr. Mark Riley, Dr. Eric Betterton and Dr. David Lynch all of the University of Arizona and Dr. Timberly Roane of the University of Colorado.

6. REFEENCES

Adel-Rahman, M., Skowronski, G.A., Turkell, R.M. In vitro Penetartion of Pig Skin by Heavy Metals in Soil. *Soil and Sediment Contamination* (2005) 14: 123-134.

Avery, M., Pershadsingh, H., Antioxidants. United States Patent 6,664,287, 2003.

Bartrop, J., Hayes, P., Calvin, M. The Chemistry of 1,2-Dithiolane (Trimethylene Disulfide) as a Model for the Primary Quantum Conversion Act of Photosynthesis. *Journal of the American Chemical Society* (1954) 76, 4348-4367.

Biewenga, G.; Guido, R.M.M.; Bast, A. An Overview of Lipoate Chemistry in Lipoic Acid in Health and Disease ed. J. Fuchs, L. Packer and G. Zimmer. Marcel Dekker, Inc. NY (1997) 1-66.

Brown, P., The Investigation of Some Reactions of Alpha-Lipoic Acid. Ph.D. Dissertation, Brown University, 1968.

Brown, P., Edwards J. , Effect of Solvent on the Photolysis of α -Lipoic Acid, The Journal of Organic Chemistry (1969) 34(10) 3131-3135.

Dutiewicz, T. Experimental Studies on Arsenic Absorption Routes in Rats. Environmental Health Perspectives (1977) 19: 173-177.

Ellman G.L. Tissue Sulfhydryl Groups. Archives of Biochemistry (1959) 82: 70-77.

Friedman, M., Masri, M.S. Interactions of Mercury Compounds with Wool and Related Biopolymers. Protein Metal Interactions Plenum Press, New York (1972) 505-550.

Glastrup, J. The Effectiveness of Compressed Air in Removal Of Pesticides from Ethnographics Objects. Collection Forum (2001) 16(1-2) 19-22.

Grunert, R. Effect of DL- α -Lipoic Acid on Heavy Metal Intoxication in Mice and Dogs. Archives of Biochemistry and Biophysics (1960), 86, 190-194.

Hostler, D., Kane, S., Davis, L. The Hopoa Tribel Museum's Experience with Chemical Contamination of Repatriated Materials. Collection Forum (2001) 16(1-2) 54.

Jemison, G.P. Poisoning the Sacred. Collection Forum (2001) 17(1-2) 38-40.

Laurie, S. H., Barraclough, A. Use of Waste Wool for the Removal of Mercury from Industrial Effluents, Particularly those from the Chlor-alkali Industry. Intern. J. Environmental Studies. (1979) 14: 139-149.

Masri, M.S., Friedman, M. Interactions of Keratins with Metal Ions: Uptake Profiles, Mode and Binding, and Effects on Properties of Wool. Protein Metal Interactions Plenum Press, New York (1972) 551-587.

Matsugo, S.; Han, D.; Tritschler, H.J.; Packer, L. Decomposition of α -lipoic Acid Derivatives by Photoirradiation-formation of Dihydrolipoic Acid from α -lipoic Acid. Biochemistry and Molecular Biology International (1996) 38(1) 51-59.

- Miyamoto, T., Ito, M., Sugitani, M., Inagaki, H. Sorption Mechanism of mercuric Ions by Keratine Gels of Thiol-Type. *Sen-I Gakkaishi* (1978) 34(9) T405-T411.
- Packer, L.; Witt, E.H.; Tritschler, H.J. Alpha-Lipoic Acid as a Biological Antioxidant Free Radical *Biology and Medicine* (1995) 19(2) 227-250.
- Reed, L., *Chemistry and Function of Lipoic Acid. Comprehensive Biochem.* (1966), 14, 99-126.
- Reiss, O. Hellerman, L. Pyruvate Utilization in Heart Sarcosomes Inhibition by an Arseno Compound and Activation by Lipoic Acid. *Journal of Biological Chemistry* (1957) 231, 557-569.
- Sadongei, A., *American Indian Concepts of Object Use, Collection Forum* (2001) 17(1-2) 113-116.
- Spuches, A., Kruszyna, H., Rich, A., Wilcox, D., Thermodynamics of As (III)-Thiol Interaction: Arsenite Monomethylarsenite Complexes with Glutathione, Dihydrolipoic Acid and Other Thiol Ligands. *Inorganic Chemistry* (2005) 44(8) 2964-2972.
- Tratnyek, J. P. Little, AD. *Waste Wool as a Scavenger for Mercury Pollution in Waters. Published by the Office of Research and Monitoring of Environmental Protection Agency* (1972)
- Wagner, A.F.; Walton, E.; Boxer, G.E.; Pruss, M.P.; Holly, F.W.; and Folkers, K. Properties and Derivatives of α -lipoic acid. *Journal of the American Chemical Society*, 78, 5079-5081.
- Walton E., Wagner, F., Bachelor, L., Peterson, L., Holly, F., Folkers, K., Synthesis of (+) α -lipoic acid and its optical antipode. *Journal of Chemical Society* (1955) 77, 5144.
- Wester R.C., Maibuch, H.I., Sedik, L., Melendres, J., Wade, M. In Vivo and in Vitro Percutaneous Absorption and Skin Decontamination of Arsenic from Water and Soil. *Fundamentals of Applied Toxicology* (1993) 20: 336-340.

Whitney, R., Calvin, M., Chemical and Photochemical Studies on 6,8-Thiotic Acid and 1,2-Dithiolane (Trimethylene Disulfide) *The Journal of Chemical Physics* (1953) 23(10) 1750-1756.

Zimm, B., Bragg, J. Mechanisms of Biradical-Initiated Polymerization, *Journal of Polymer Science* (1952) 9, 476-478.