A Study on Determining the Effect of Acid on Bones

Nikhitha Jose, Kezia Ann Varghese, Sruthy Venugopal, Danie Kingsley*
School of Biosciences and Technology, Vellore Institute of Technology, Katpadi, Vellore, India.
*Corresponding author details: Dr. DanieKingsley. J, Assistant Professor Senior, SBST, VIT, Vellore-632014. Email ID: daniekingsley@gmail.com.Phone number: 9025956491

ABSTRACT
Forensic experts have developed their own method for determining how corrosive chemicals like sulphuric acid, nitric acid harm bone. Dissolving bodies is a contemporary method of disposing human remains and has been practiced throughout the years. In popular media, criminals attempt to dispose of their victims by using various chemicals to dissolve the corpses.This research investigates the effects of sulphuric acid and nitric acid on bones by using an animal analogue (chicken, *Gallus domesticus*). Over time, the appearance, consistency of all specimens was documented and presence of protein was identified from the acid treated bone sample. This study shows a comparison between the effects of both the acids in degradation of bones. Acids catalyze hydrolysis reactions when they come in contact with bone, more the ions in solution, faster will be the reaction. In case of sulphuric acid the degradation of bones takes time as it is a slow process. The protein can be extracted from the residue of bone present in the sample. Whereas in case of nitric acid the bone sample gets completely dissolved without leaving any trace. Therefore no protein or DNA can be extracted from the sample, which makes it difficult in the identification of the victim.
Keywords*:* Forensic, Sulphuric acid, Nitric acid, Bone, Animal analogue

INTRODUCTION

Corrosive substances can harm bone health by causing dissolution, breakdown, and loss of integrity. As with the corrosion and destruction of bone in acid, these effects can accumulate over time. The effect of corrosive substances purposefully applied to remains in order to disguise their potential for identification or the immersion of whole bodies to dissolve them as completely as possible, as has been attempted in some criminal cases, is one topic of forensic research. Corrosive chemicals have the ability to produce localized bone injury (Pokines, 2016).

Different ways may be used to obstruct the identification of the corpse or to entirely destroy it in frequent forensic settings. Such activities eliminate proof that a crime has been committed, as well as evidence tying the victim to the criminal. Burial, dismemberment, and burning are common methods for disposing of a homicide victim's body. Another option worth considering, particularly in the context of organized crime, is attempting to decompose a corpse using excessively acidic or alkaline liquids, resulting in the progressive breakdown of the tissues. There have been reports that such techniques have occurred, and it is clear that such cases would provide a significant challenge for investigators and forensic pathologists. (Amadasi, 2017).

Dissolving bodies in acid is a current method of human remains disposal that has been used for centuries. The "Acid Bath Murderer," John Haigh, an English serial killer who was convicted and hanged for murdering six people in the 1940s, is the most famous case in history. He dissolved his victims' bodies in pure sulfuric acid, believing that if their bodies could not be discovered, a murder conviction would be impossible. (Vermeij, 2015).

Many different techniques and multiple types of tissue are employed to get a positive identification of human remains. Dismemberment, removal of fingers to thwart identification through fingerprints, destruction or removal of teeth, disfigurement of the face, burning of the body, and even dissolution in various household chemicals are all common attempts to conceal a victim's identity and prevent positive identification. Because positive identification methods require both hard and soft tissues, scientists must learn how corrosive compounds affect all of the many types of tissue that make up the human body (Hartnett, 2011).

To our knowledge, this research focused mostly on these chemicals ability to harm human tissues and the time required for such effects to manifest. Furthermore, determining what might be the time for larger samples or even a whole body from the analysis of the times of consumption of a small sample, such as a tooth, is challenging. If time and the amount of acid available are not constraints, a human body can be entirely dissolved in acid without leaving any macroscopic remnants (Vermeij, 2015). The present study used an animal counterpart to investigate the effects of sulphuric acid and nitric acid on bones (chicken, *Gallus domesticus*) (Amadasi A. C., 2015).

MATERIALS AND METHODS

1. **Preparation of sample**

   Chicken bone samples preferably leg bones were collected from a poultry farm from Vellore, Tamil Nādu. These bones were washed in hot water thrice to remove unwanted tissues. After washing the samples were air-dried and stored in closed containers for further use. **(D.Kovacs, 2007)**

2. **Treatment of sample with acids**

   For this, acids like sulphuric acid and nitric acid were used. 250ml of sulphuric acid and nitric acid were taken in separate glass breakers and the air -dried bone samples were immersed in these solutions using forceps. These bones were kept immersed for a week to observe the morphological changes and to check the intensity of degradation. **(D.Kovacs, 2007)**

3. **Collection of residues**

   After treating the samples with acids, they were collected in centrifuge tubes. Three different samples were collected in different tubes. The first tubes contained sulphuric acid along with some minute bone particles. The second and third tubes contained sulphuric acid and nitric acid without bone particles (not pure acids) respectively. **(CaterinaLicini, 2020).**

4. **Precipitation of residues**

   Ethanol precipitation was performed to extract proteins from the obtained residues. 1ml of protein was incubated overnight at -20 0C in 5ml of 100 % ethanol. The solution was

centrifuged for 20 minutes at 3000rpm, the supernatant was discarded, and the pellet was rinsed with 5ml of ice-cold acetone and incubated at 200C for 20 minutes. The pellet was air-dried following centrifugation at 3000rpm for 20 minutes, the supernatant was discarded, and the pellet was resuspended in the chosen buffer. **(CaterinaLicini, 2020)**

5. **Preparation of phosphate buffer**

Solution A was prepared by dissolving 16.095g ofNa2HPO4 in 300mL of distilled water. Solution B was made by dissolving 5.56 g ofNaH2PO4 in 200 mL of distilled water. The phosphate buffer was then made by mixing 61mL of Solution A and 31mL of Solution B and diluting to 200mL with distilled water. **(M.Bradford, 1976)**

6. **Estimation of protein**

400µl of precipitated samples were pipetted into separate test tubes, and 600µl of newly produced pH7.0 phosphate buffer was added to make it up to 1 ml. Then 5ml of Bradford reagent was added and incubated at room temperature for 5 minutes. In a similar way, a blank was created using a buffer. To quantify the quantity of protein in the samples, a graph of absorbance 595nm was constructed. **(M.Bradford, 1976)**

RESULT AND DISCUSSION

The bones were collected from a poultry farm and were washed and cleaned thoroughly later and were subjected for acid treatment (H2SO4, HCL). We observed certain morphological changes. On treatment with sulphuric acid, the shape of the bone was deformed, size of the bone was reduced from 8 to 4 cm in the first sample of bone, and the color of the bone was changed from light brown to blackish brown. Also, the intensity of burns was observed to be increasing at a faster rate at the knee cap and cartilage regions of the bone.

We observed these changes of bone during various time intervals as follows: -

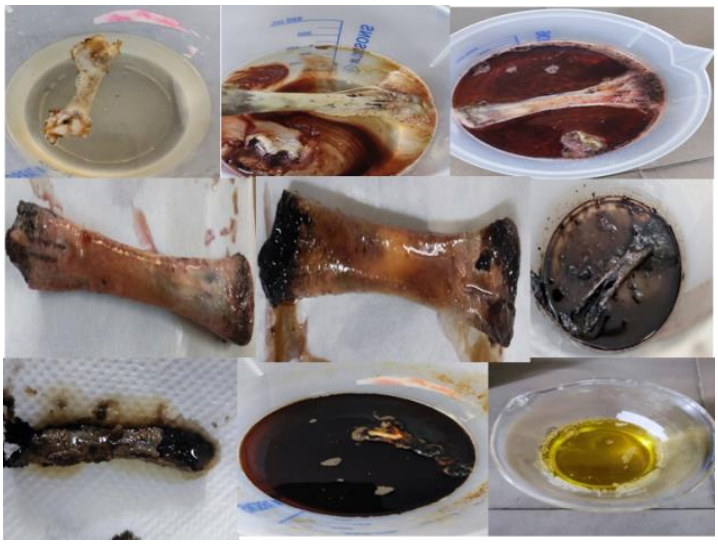

Figure 1. ***Morphological changes observed in different time intervals***

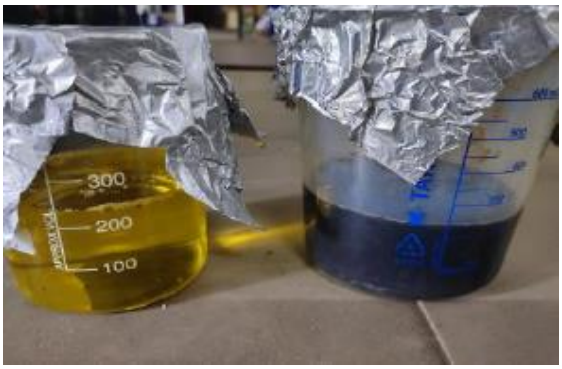

Figure 2. On degradation, the color of sulphuric acid changed from transparent to blackish brown and the color of nitric acid changed from transparent to shiny yellow

Then the resultant material we got was divided into three parts, sulphuric acid with bone, sulphuric acid and nitric acid. And were transferred to centrifuge tubes and centrifuged firstly for 20 minutes at 3000rpm (Fig:3:a) supernatant were discarded and pellet was then washed with ice cold acetone and after 20 minutes again centrifuged for 20 minutes at 3000rpm (Fig:3:b) . And only in sulphuric acid treated bone and sulphuric acid the pellets were formed and pellets were absent in nitric acid no further changes were observed on treating with nitric acid. And the pellet formed was as follows: -

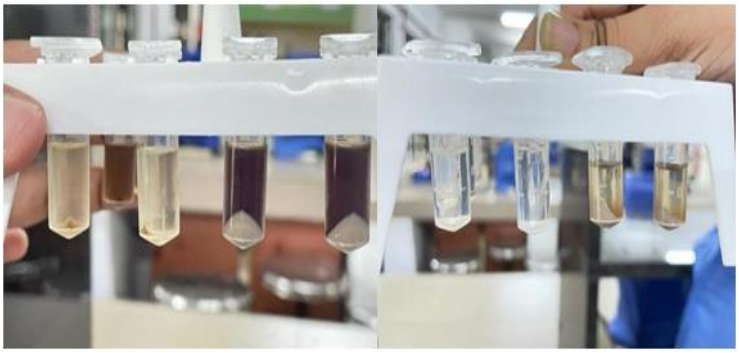

Figure 3. a) Pellets observed at first and b) final centrifugation techniques

And hence the pellets formed at the final step of centrifugation are further proceeded for the Bradfords test for the protein estimation. We added the different samples into different test tubes also added phosphate buffer as well as the Bradford's reagent and observed that the tubes containing the bone with sulphuric acid was positive the colour change was visible to colorless to dark blue colour and the other tubes were resulting reagent's colour , hence we can conclude that the tube that has bone with sulphuric acid contain protein. And which can be used for proteomics study which helps to identify the tissue source of material, also helps in analyzing identifying genetic information, and species determination which helps in forensic investigation to identify the victim who has been found dead at the crime scene. So our study resulted in protein extraction from the tissue bone sample and which can be used in forensic studies for identifying the victim.

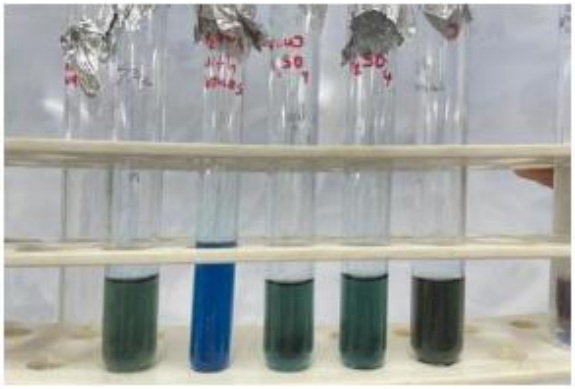

Figure 4. Only H2SO4 with bone showed positive response on treatment with Bradfords test hence protein content is present.

The research work done for this study includes, treating of chicken bones with sulphuric acid and nitric acid separately to examine the morphological changes, and to observe the rate of degradation of the bones at different time intervals. This work was also useful in determining the colour changes observed in the acid solutions during the treatment process. Followed by acid treatment, the residue obtained were precipitated using ethanol precipitation method to observe the pellet formation, which was further used for the estimation of presence of protein. The estimation of protein was done using the Bradford method. The intensity of blue color obtained with an absorbance 595nm, showed the amount of protein present in the sample.

The goal of the study was to use a chicken bone as an analogue for acid treatment and analyze how acids affect bones (humans). Following that, a comparison of sulphuric and nitric acids was conducted to establish which acid is more harmful to human health. The study's end goal was to detect the presence of protein in the samples, which may then be used to determine the sample's tissue or bodily fluid source, a key piece of forensic context. Amino acid polymorphisms are a type of genetic information found in proteins. Protein is far more stable than DNA, in addition to being several orders of magnitude more prevalent in a sample. This means that even when DNA is no longer accessible to investigators in biologically or environmentally damaged material, protein may still be there.

Several studies have been done for determining the effect of corrosive substances on human bone, nail, and other body parts using various acids and alkaline solutions, experts reached different conclusions (Hartnett, 2011). This study focuses mainly on the degradation properties of sulphuric acid and nitric acid. When acids react with the bone particles, it undergo a hydrolysis reaction, more the ions in the solution faster will be the degradation reaction (Amadasi, 2017). It was observed that the degradation starts from the soft tissues attached to the bone which extend to other regions over time.

So many studies are there which show protein extraction using different methodologies. we had gone through a variety of papers and studied the differences also. In one such paper, we saw that they had used HCL for proteome analysis (Xiaogang Jiang, 2007), whereas we got the resultant protein by using H2SO4. They also got various soluble

proteins. they used specialized techniques for protein analysis like 2-D LC-MS, and MS. Whereas we used the Bradfords test for protein estimation.In another paper (Sarah M. Lyon1, 2016), they have used mouse bone for protein extraction for finding protein extraction from the human cranial bone. Whereas we used chicken bone for our experiment. Here also in their paper they have used advanced techniques like Mass Spectrometry to sequence peptides and isolate proteins. Also, they have stored their data to the ProteomeXchange identifier. Whereas we haven't done any of the advanced techniques but we got the same resultant protein from the Bradford test.

In this paper, (Timothy P. Cleland1 and Deepak Vashishth1, 2015)have used a variety of new techniques for protein extraction they haven't used any demineralization but they used hydroxyapatite chromatography to extract the bone proteins. They have also resulted in a higher yield of proteins than other small-scale techniques. Later they used HPLC-MS / MS techniques for vascular proteins and extracellular matrix proteins. Whereas we used simple techniques to yield protein than performing high-cost techniques. Also, we used H2SO4 and HNO3 for our experiment.

The results can be used in determining the time taken for body parts to completely dissolves and how to determine the identity of the person, using the protein extracted from the remaining sample, obtained after the degradation of the body parts. These preliminary findings and observations from this study may be practically used in forensic investigations of bodies found in acidic substances, for which there is no scientific evidence so far.

Table 1. Absorbance of test sample at 595nm

TEST SAMPLES	ABSORBANCE AT 595nm
H_2SO_4 with bone sample	1.657
H_2SO_4alone without bone (not pure)	0.126
Nitric acid alone without bone (not pure)	0.0376

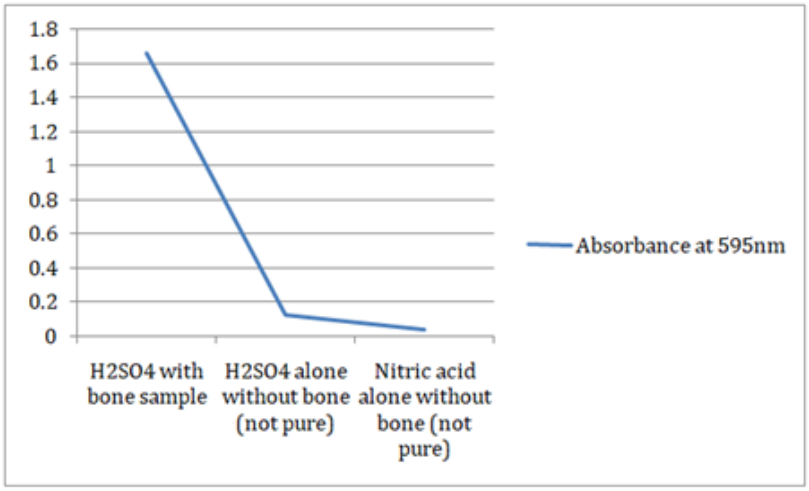

Figure 5. Absorbance graph of test samples at 595 nm

CONCLUSION

According to the findings, acids can have a negative impact on bones, leading to increased rates of deterioration and burn intensity. The bones submerged in the nitric solution were entirely disintegrated, proving that nitric acid is the most harmful acid to human health. The Bradford assay revealed that samples containing sulphuric acid and minute bone particles had increased protein content, while sulphuric acid alone (processed with bone but not pure) had somewhat lower protein content. We may deduce from this that we can extract proteins from acid-treated bones, which can then be employed in forensic proteomics to solve cases involving acid-related crimes or murders.

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