

PAPER • OPEN ACCESS

## Interaction between Lead and Bone Protein to Affect Bone Calcium Level Using UV-Vis Spectroscopy

To cite this article: Z Noor *et al* 2018 *IOP Conf. Ser.: Mater. Sci. Eng.* **350** 012009

View the [article online](#) for updates and enhancements.

You may also like

- [3D cell-printing of tendon-bone interface using tissue-derived extracellular matrix bioinks for chronic rotator cuff repair](#)  
Suhun Chae, Yucheng Sun, Yeong-Jin Choi *et al.*
- [The benefit of biomedical explorations in elderly patients with respiratory pathology and dental diseases](#)  
D Munteanu, L I Perea, G Sttescu *et al.*
- [Three-dimensional bioprinted BMSCs-laden highly adhesive artificial periosteum containing gelatin-dopamine and graphene oxide nanosheets promoting bone defect repair](#)  
Xin Sun, Jin Yang, Jie Ma *et al.*



**ECS**  
The  
Electrochemical  
Society  
Advancing solid state &  
electrochemical science & technology

**DISCOVER**  
how sustainability  
intersects with  
electrochemistry & solid  
state science research

# Interaction between Lead and Bone Protein to Affect Bone Calcium Level Using UV-Vis Spectroscopy

Z Noor<sup>1</sup>, A Azharuddin<sup>2</sup>, I Aflanie<sup>3</sup>, N Kania<sup>4</sup>, and E Suhartono<sup>5</sup>

1 Department of Orthopaedic Surgery, Ulin General Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan 70232, Indonesia

2 Department of Orthopaedic Surgery, Faculty of Medicine, Syiah Kuala University, Aceh 24415, Indonesia

3 Department of Forensic, Ulin General Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan 70232, Indonesia

4 Research Center for Toxicology, Cancer, and Degenerative Disease, Department of Pathology, Ulin General Hospital/Faculty of Medicine, Lambung Mangkurat University, Banjarmasin 70232, South Kalimantan, Indonesia

5 Department of Medical Chemistry/Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, South Kalimantan 70712, Indonesia

E-mail: esuhartono@unlam.ac.id

**Abstract.** This present study aim to evaluate the interactions between lead (Pb) and with bone protein by UV-Vis approach. In addition, this prsent study also aim to investigate the effect of Pb on bone calcium (Ca) level. The present study was a true experimental study design to examine the impact of Pb exposure in bone of male rats (*Rattus novvergicus*). The study involved 5 groups, P1 was the control group, while the other (P2-P5) were the case group with exposure of Pb in different concentration within 4 weeks. At the end of the exposure, the interaction between Pb and protein was determined using UV-Vis spectrophotometric method, and the Ca level was determined using permanganometric method. The results shows that that there is an interaction between Pb and bone protein. The result also shows that the value of the binding constant of Protein-Pb is 32.71. It means Pb have an high affinity to bind with bone protein, which promote a further reaction to induced the release of bone Ca from the bone protein. In conclusion, this present study found an obvious relationship between Pb and bone protein which promote a further reaction to increase the releasing of bone calcium.

## 1. Introduction

Lead (Pb) is a heavy metal belonging to period IV of the periodic table. This element is grey in color, malleable, soft, and can be extracted from the mineral galena (PbS). Pb is beneficial to humans for industrial purposes such as battery industry, X-ray protection, paint coloring, PVC industry and others. Widespread use of Pb, causing this element to be a pollutant to the environment and harmful to human health [1]. Pb can enter the body through the digestive tract, breathing, and skin. Pb entering through the gastrointestinal tract will be distributed to bone (60%), liver (25%), kidney (4%), reticuloendothelial system (3%), intestinal wall (3%), and to other tissues. The accumulation of Pb in these organs can result in various organ disorders, such as impaired kidney function, cardiovascular, liver, brain, and hematopoietic system [2].



Disorders of various organs due to Pb are caused by the interaction of Pb with various cations, especially those of the divalent cation, such as calcium (Ca), zinc, and iron [3]. Pathological processes can be found in cell membranes and mitochondria, in the synthesis and function of neurotransmitters, heme synthesis, cellular redox status, and nucleotide metabolism. In addition, Pb also interacts with sulfhydryl groups, carboxyl groups, amine groups of enzyme amino acids, cell membrane constituents, and other macromolecules [4].

Side effects of Pb have been shown in many previous studies. The study by Monir et al. [5] proved that Pb may decrease collagen and increase the risk of fracture in adult female C57 / B6 rats given drinks containing Pb for 4 months. A study in the Hezhang region of China found an association between Pb levels in the blood and a decrease in BMD in women [6]. Furthermore, a study conducted by Todorovic et al. [7] revealed that mice exposed at 100 mg Pb<sup>2+</sup> per kg of body weight for 30 days may have a decrease in bone Ca by 16%. This is due to the greater Pb<sup>2+</sup> affinity than Ca<sup>2+</sup>, causing Pb<sup>2+</sup> to replace Ca<sup>2+</sup> in bone mineral matrix, thus lowering Ca<sup>2+</sup> bone levels [7].

Many methods are used to explain the interaction of metals with proteins, one of which is visible light spectroscopy (UV-Vis). The basic principle of UV-Vis is that the molecule has an electron energy level analogous to the energy level of the electrons in the atom. The energy level of this molecule is called a molecular orbital. Study on the interaction between metal and protein with the UV-Vis method has been described previously [8]. Zhang et al. [8] investigated the interaction between flavonoid-divalent metal Cu<sup>2+</sup> and protein. The result revealed that Cu<sup>2+</sup> interacts with flavonoids to form flavonoids-Cu<sup>2+</sup> and a change in protein conformation due to flavonoids-Cu<sup>2+</sup>. Curvale et al. [9] studies have examined the interaction between Arsenic (As) with amino acids in proteins by UV-Vis. The result revealed that the As could interacted with proteins. The interaction between As (III) and protein is an ionic interaction, and between As (V) with the protein is a hydrophobic interaction [9].

However, as far as we know there is a view literature that explains the effect of Pb on bone protein through the UV-Vis approach. Thus, our present study aim to evaluate the interactions between Pb and with bone protein by UV-Vis approach. Furthermore, this study will explain a deeper understanding how the Pb could changes in the bone Ca level.

## 2. Material and methods

### 2.1. Study design

This study was of pure experimental post test-control group design, conducted for 4 weeks from April to May 2017.

### 2.2. Experimental section

Male albino rats (*Rattus norvegicus*) aged 2-3 months, weighing 80-200 grams, obtained from BPPV, Banjarbaru were used in this study. Before intervention, the rats were acclimatized for 7 days, and kept in separate cages according to their assigned group. During the acclimatization period the animals were given exactly the same animal feed pellets and tap water as were to be used during the intervention. Immediately before the intervention, the rats were fasted for 1-2 hours to ensure that their proventriculus was empty.

Furthermore, the subjects were divided into 5 groups, P0 = control groups; P1 = administration of 0.01 mg / L PbCl<sub>2</sub>; P2 = administration of 0.1 mg / L PbCl<sub>2</sub>; administration of 1 mg / L PbCl<sub>2</sub>; administration of 10 mg / L PbCl<sub>2</sub>; administration of 100 mg / L PbCl<sub>2</sub>. Selection of the animals was by simple random sampling, while the sample size was calculated by means of Federer's formula, yielding a sample size of 5 rats per group. After treatment, the rats of each group was killed with ether and their bones taken for determination of calcium content by the permanganometric method, as performed in a previous study [10].

### 2.3. Bone collection

The collected bones were cleaned and washed, then left to dry in an oven. The dried bones were then pulverized and passed through a 40 mesh sieve. The bone powder was dissolved in HCl (1:4), the resulting solution was concentrated by evaporation and then placed in a water bath for 1 hour. The dry residue was redissolved in 5 ml concentrated HCl and 50 ml distilled water, then again placed in the water bath for several minutes, and filtered through #52 Whatman filter paper.

The filtrate was collected in a 200 ml volumetric flask. The precipitate still remaining on the filter paper was washed in distilled water and the wash water was added to the collected filtrate. Distilled water was added to the mixture of filtrate and wash water to yield a volume of 200 ml. This solution was labeled aliquot A.

### 2.4. Calcium concentration determination

A volume of aliquot A equivalent to 2 g bone ash was pipetted into a 300 ml beaker and diluted to 200 ml with distilled water. The solution was made slightly alkaline by the addition of  $\text{NH}_4\text{OH}$  (1:4) using methyl orange as indicator. HCl (1:4) was then added to slightly acidify the solution, after which 10 ml 0.5 N HCl and 10 ml 2.5 % oxalic acid solutions were added, and the mixture heated to a boil. Under continuous stirring, 15 ml saturated ammonium-oxalate solution was added. The mixture was further heated until a granular precipitate was formed, then the mixture was left to cool. 8 ml of 20% sodium acetate was added and the mixture left for 12 hours. Next the mixture was filtered and washed with hot water until free of chloride (washed with hot water and then with a small amount of HCl (1:4) and finally with hot water until free of chloride). The residue was then put into a beaker by perforating the tip of the filter paper cone with a glass stirrer, then sprinkled with just sufficient hot water until all precipitate had fallen into the beaker. Next, 10 ml  $\text{H}_2\text{SO}_4$  (1:4) was added and the dissolved precipitate heated to near-boiling, left to cool, then titrated with 0.1 N  $\text{KMnO}_4$ . The calcium content was calculated using the formula:

$$\text{Ca per 100 mg sample} = \left( \frac{\text{ml titration} \times \text{total volume of dissolved ash}}{\text{volume of dissolved ash} \times \text{weight of sample}} \right) \times 100\%$$

### 2.5. UV-Vis spectroscopy determination

Aliquot A is taken 100 mL and then put into a cuvette. Absorbance was observed at wavelengths of 220-300 nm using a spectrophotometer.

### 2.6. Metal-Protein bonding constant determination

Metal-protein binding constants were calculated using formula:

$$\frac{1}{A - A_0} = \frac{1}{n} + \frac{1}{n \cdot K} \cdot \frac{1}{Cl}$$

Using a linear curve graph between  $1/(A-A_0)$  with  $1/Cl$ , we get the  $n$  value, ie the number of active sites that bind metals to proteins and Metal-Protein ( $K$ ) binding constants.

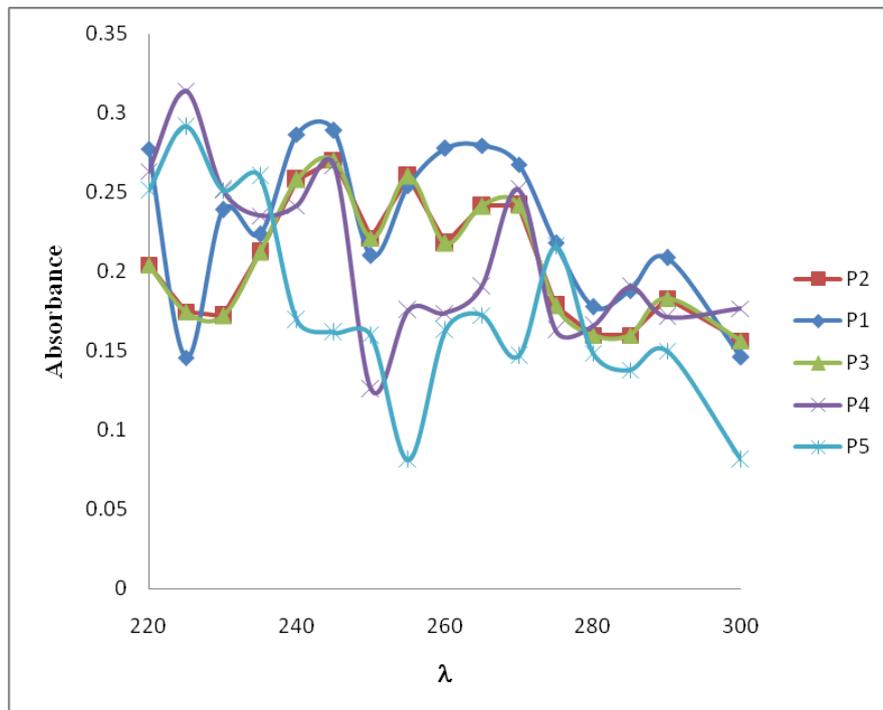
### 2.7. Ethical clearance

The present study was approved by the Research Ethics Commission of the Faculty of Medicine, Lambung Mangkurat University.

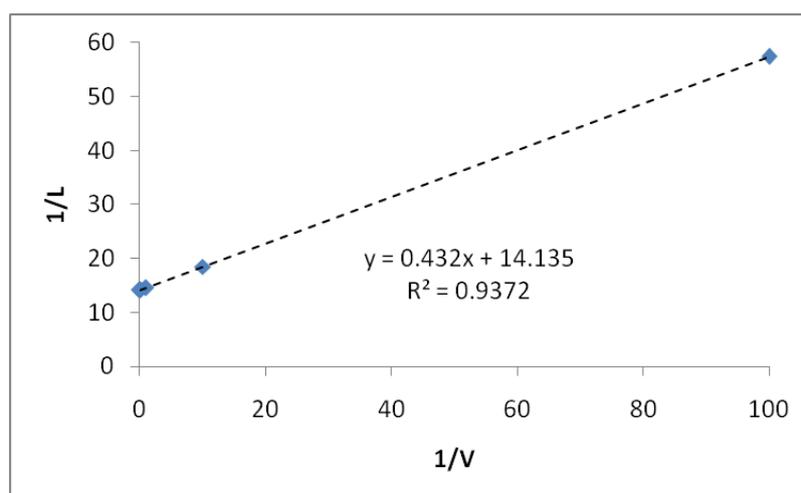
## 3. Results and discussion

Based on the observations, the addition of Pb concentration causes an increase in absorbance at 280 nm wavelength. This shows that there is an interaction between Pb and Tyr, as shown in Figure 1.

Figure 1 shows that an increase in Pb concentration causes the absorbance of the Protein-Pb binding to increase. This is thought to be due to the covalent binding of Pb with the N-terminal amide group, N-terminal of the imidazole group, and the N-terminal of the amine group. This results in changes in the structure of protein molecules [10]. The binding constant of Protein-Pb is determined using the graph as in Figure 2.



**Figure 1.** UV-Vis spectroscopy analysis of bone in different group of treatments. P1 represent control group; P2 represent the 0.01 mg/l  $\text{PbCl}_2$  treatment group; P3 represent the 0.1 mg/l  $\text{PbCl}_2$  treatment group; P4 represent the 1 mg/l  $\text{PbCl}_2$  treatment group; and P5 represent the 10 mg/l  $\text{PbCl}_2$  treatment group.



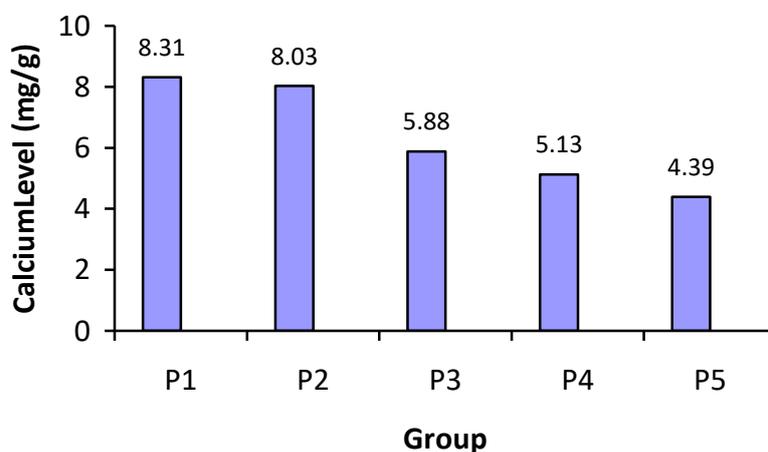
**Figure 2.** The linear curve to determine metal-protein binding constant (K)

According to Figure 2, the value of the binding constant of Protein-Pb is 32.71. The positive value of K means that the reaction between Pb and the protein will shift to the right. This means that the

affinity between protein and Pb is increase, so from this point of view we can said that Pb have a tendency to bind bone protein forming Protein-Pb. The bond between Pb and bone proteins will then lead to the release of bone calcium [11]. This can be seen from the results of this study which is presented in Figure 3.

According to the Figure 3, the level of bone calcium is decrease with the increasing of Pb exposure. It means, the greater the level of Pb exposure, the more calcium will be released from the bone. However, the exact mechanism how the Pb could affected the bone calcium level via the interaction with protein is still unclear. Our previous study indicated that kinetically Pb and calcium has a similar property. Pb and calcium both are divalent cation. However, since Pb has more a much higher affinity than calcium, the presence of Pb in bone could displace calcium from the bone [12].

Furthermore, how the Pb could displace bone calcium, and how is the interaction mechanism between Pb and bone protein still can not be known clearly in this research. It is a limitation of this study that can be used as a reference for further research.



**Figure 3.** Bone calcium level in different group of treatments. Values are mean of three replicates in each group of treatment. P1 represent control group; P2 represent the 0.01 mg/l PbCl<sub>2</sub> treatment group; P3 represent the 0.1 mg/l PbCl<sub>2</sub> treatment group; P4 represent the 1 mg/l PbCl<sub>2</sub> treatment group; and P5 represent the 10 mg/l PbCl<sub>2</sub> treatment group.

#### 4. Conclusion

In conclusion, this present study found an obvious relationship between Pb and bone protein which promote a further reaction to increase the releasing of bone calcium. These findings suggest that many efforts should be made to reduce environmental Pb exposures.

#### 5. References

- [1] Leonas R, Noor Z, Rasyid HN, Madjid TM, and Tanjung FA 2017 Effect of lead nanoparticles inhalation on mesostructure and the osteoprotegerin/receptor activator of nuclear factor-kappaB ligand system in rats. *Cukurova. Med. J.* **41** 48-54
- [2] Zang X, Yang L, Li Y, Li H, Wang W, and Ye B 2012 Impacts of lead/zinc mining and smelting on the environment and human health in China *Environ. Monit. Assess.* **184** 2261-73
- [3] Ani M, Moshtaghi AA, and Aghadavood M 2006 Protective effects of selenium and zinc on the brain acetyl cholinesterase activity in lead intoxicated rat *Res. Pharm. Sci.* **2** 80-4.

- [4] Kasperczyk S, Blaszczyk I, Dobrakowski M, Romuk E, Kapka-Skrzypczak L, Adamek M, and Birkner E 2013 Exposure to lead affects male biothiols metabolism. *Annals. Agric. Environ. Med.* **20** 721–5.
- [5] Monir AU, Gundberg CM, Yagerman SE, van der Meulen MCH, Budell WC, Boskey AL, and Dowd TL 2010 The effect of lead on bone mineral properties from female adult C57/BL6 mice *Bone.* **47** 888-94.
- [6] Chen X, Wang K, Wang Z, Gan C, He P, and Liang Y 2014 Effects of lead and cadmium co-exposure on bone mineral density in a Chinese population *Bone.* **63** 76-80.
- [7] Todorovic T, Vujanovic D, Dozic I, and Petkovic-Curcin A 2008 Calcium and magnesium content in hard tissues of rats under condition of subchronic lead intoxication. *Magnesium. Res.* **21** 43-50.
- [8] Zhang Y, Shi S, Sun X, Xiong X, and Peng M 2011 The effect of Cu<sup>2+</sup> on interaction between flavonoids with different C-ring substituents and bovine serum albumin: Structure–affinity relationship aspect. *J. Inorg. Biochem.* **105** 1529–37.
- [9] Curvale RA, Debattista NB, Pappano NB, Gonzalez DM, and Curvale DA 2011 Spectrophotometric study of the interaction between arsenic and bovine serum albumin *Res. Reprod. Biol.* **6** 131-5
- [10] Suhartono E, Ulfarini YW, Triawanti, Mustaqim WA, and Firdaus RT, and Setiawan MHM 2012 Increase bone calcium dissociation in lead-exposed rats. *Univ. Med.* **31** 151-8.
- [11] Haleagrahara N, Chakravarthi S, Kulur AB, and Radhakrishnan A 2011 Effects of chronic lead acetate exposure on bone marrow lipid peroxidation and antioxidant enzyme activities in rats *Afr. J. Pharm. Pharmacol.* **5** 923-9.
- [12] Lu H, Yuan G, Yin Z, Dai S, Jia R, Xu J, Song X, Li L, and Lu C 2014 Effects of subchronic exposure to lead acetate and cadmium chloride on rat's bone: Ca and Pi contents, bone density, and histopathological evaluation. *Int. J. Clin. Exp. Pathol.* **7** 640-7.

### Acknowledgments

The authors are grateful to the financial support of Faculty of Medicine, Lambung Mangkurat University in funding this project through the Faculty Research grant.