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ORIGINAL ARTICLE

Spectrophotometric evaluation of effects of heavy metals on human erythrocytes.

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ABSTRACT… Objective: To study the effect of heavy metals on the human erythrocytes. **Study Design:** Experimental Study. **Setting:** Department of Chemistry Hazara University, Mansehra. **Period:** January 2017 to June 2017. **Material & Methods:** Erythrocytes were isolated and prepared by the standard method from blood samples. The standard absorption spectra were recorded in the range of 200-800nm using double-beam UV-visible spectrophotometer. Heavy metals were prepared in laboratory as per given protocol. Erythrocyte samples were treated with prepared metals. Absorbance of these samples was recorded. The spectrum of these treated samples was compared with the standard spectra of erythrocytes to look for any variations that might have occurred. **Results:** It was observed that human erythrocytes exhibited altered behavior with each metal which was evident by shifting in peaks in UV/Visible spectra. The absorption maxima changed from higher to lower wavelengths with every metal tested. It was also observed that this change was more pronounced in case of Lead, Cadmium and Mercury while similar changes were also observed with Arsenic and Selenium. **Conclusion:** This study showed that erythrocytes exhibited different behavior with different metals as evident by the shift of peaks in UV/Visible spectra. The absorption maxima were shifted towards lower wavelengths suggesting that these metals do exert an effect on human erythrocytes.

Key words: Erythrocytes, Heavy, Metal, Spectrophotometry.

INTRODUCTION

Metal ions perform numerous critical functions in human beings. Therefore, deficiency of these metal ions can lead to illnesses. Anemia caused by iron deficiency, growth retardation due to inadequate dietary zinc intake and heart diseases in infants secondary to copper deficiency are some of the examples of these deficient states.[1] These metal ions also cause toxicity in humans and the classical example is heavy metal poisoning such as with lead (Pb) and mercury (Hg). Even excess of essential metal ions can be noxious; iron (Fe) is a commonplace domestic toxin in the United States due to accidental ingestion of the dietary iron supplements usually in children.[2]

Prolonged exposure to industrial toxins particularly heavy metals (cadmium, lead, arsenic, selenium and boron etc.) affect various body systems including gastrointestinal tract, renal, hematopoietic, nervous, cardiovascular (CVS) and immune systems of human body. Toxic effects of heavy metals are attributed to their widespread usage and availability. Humans have been using heavy metals for thousands of years and various studies have reviewed their effect on human health. In developing countries like Pakistan heavy metal soil concentrations, taken from the downstream side of river near capital city, were as follows: chromium (Cr, 149%), nickel (Ni, 131%), cadmium (Cd, 176%), zinc (Zn, 139%), lead (Pb, 224%) and copper (Cu, 182%) as compared to samples from the upstream sites suggesting that there is an increased exposure to toxic metals.[3] Likewise, levels of heavy metals were found to be high in waste water, which is used for irrigation in different areas of Pakistan, as well as in soil, water, and crops like wheat.[4] These metals are either ingested in food and water or inhaled as smaller particles and then after absorption get

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stored in bone marrow and soft tissues leading to an increase in the blood levels of these metals.[5]

There are various industrial heavy metals which are commonly used in households like mercury in disinfectants, lead which is used in lead based batteries and paints and different antifungals. Likewise, work-related exposure to heavy metals like lead, mercury, chromium, gold and silver can occur in workers and residents of such dwellings via inhalation of suspended particles in air.[6] As a result, these heavy metals attach with hemoglobin (Hb) leading to alteration in its structure and thus plummeting the oxygen (O_2) carrying capability of Hb and causing stress on CVS, brain and other body parts.[5]

Human erythrocytes are preferred model for investigating oxidant stress in humans because of following reasons: I) erythrocytes are constantly in direct contact with molecular O_2, II) erythrocytes possess metal ions which catalyze various oxidative chemical reactions, III) erythrocyte membranes possess ample polyunsaturated fatty acids (PUFA) and they are vulnerable to lipid peroxidation, IV) erythrocytes cannot repair and regenerate damaged membranes, V) they circulate in the plasma and the plasma lack antioxidant enzymes for example superoxide dismutase, catalase and glutathione peroxidase.[7] Hence, erythrocytes can be recognized as a valuable and suitable model system in which to assess the effects of different heavy metals.[8]

It is a well-established fact that heavy metals are major industrial toxicants and they are also found in food, water and air. They exert toxic effects on humans even in low concentrations. Therefore, their effect must be carefully analyzed so as to minimalize the human exposure to these heavy metals and subsequently to lessen the risk of various illnesses. Spectrophotometry is a well-established method for determination of effects of industrial heavy metals.[9] Therefore, we have conducted this study to quantify the effects of these metals on human erythrocytes using spectrophotometric method.

MATERIAL & METHODS

This study was conducted from January 2017 to June 2017 at the Chemistry Department of Hazara University, Mansehra. Blood samples were collected aseptically from clinical laboratory and method of Dodge et al was used to isolate and prepare erythrocytes. A 10ml sample of erythrocytes was taken in flask and deionized water was used to dilute it up to 20ml. Double-beam UV-visible spectrophotometer (PerkinElmer, USA) was used to measure standard absorption spectra in the range of 200-800nm.

Preparation of Heavy Metal Standard Stock Solution

Five ppm stock solution of every metal was made in the laboratory as follows:

Stock solution 1: Lead (II) Sulphate
Lead **II** sulphate (1.51g) was dissolved in 100ml of deionized water to get a 5ppm stock solution.

Stock solution 2: Arsenic (III) Chloride
Arsenic **III** chloride (0.90g) was dissolved in 100ml of deionized water to get a 5ppm stock solution.

Stock solution 3: Mercury (II) chloride
Mercury **II** Chloride (0.135g) was mixed in100ml deionized water to get a 5ppm stock solution.

Stock solution 4: Cadmium (II) Sulphate
Cadmium **II** sulphate (0.92g) was dissolved in 100ml of deionized water.

Stock solution 5: Selenium (IV) Chloride
Selenium **IV** Chloride (0.110g) was dissolved in 100ml of deionized water to get a 5ppm stock solution.

Erythrocyte samples were treated with metal salts and the changes were measured by recording UV/Visible spectra of treated samples. Erythrocyte sample of 0.5ml was treated with 01ml of 5ppm stock solutions of lead sulphate, arsenic chloride, mercury chloride, and selenium chloride while 02ml sample of erythrocytes was treated with 01 ml stock solution (5ppm) of cadmium sulfate. UV/Visible absorption spectra were measure between 200-800nm on Double beam UV-Visible

spectrophotometer. Spectra of treated samples were compared with that of standard erythrocyte spectra to observe the variations that might have happened.

RESULTS

Effect of heavy metal on erythrocytes

Our study analyzed effects of various heavy metals i.e. lead, arsenic, mercury, boron, cadmium and selenium on human erythrocytes.

Normal erythrocytes exhibited absorption maxima at 537nm as shown in Figure-1.

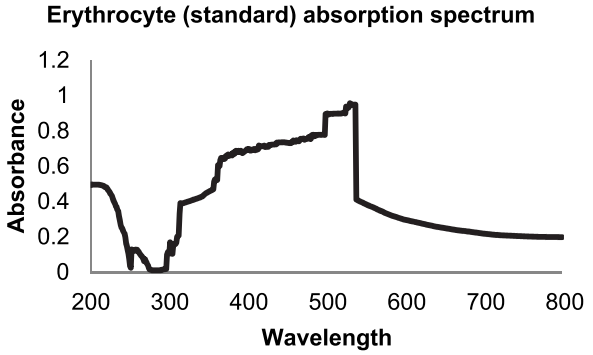

Figure-1. Normal erythrocytic absorption spectra.

The erythrocyte samples treated with lead sulphate displayed absorption maxima at 445nm as displayed in Figure-2. Milky-reddish appearance, after treatment with lead salt, showed interaction of lead with lone pair of carboxylic O_2 within molecular structure of erythrocytes.

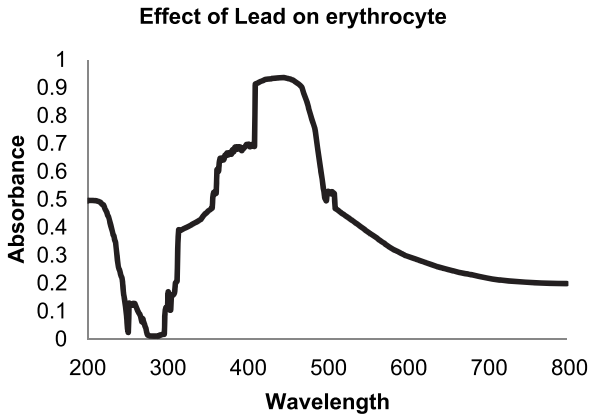

Figure-2. Absorption spectra of lead treated samples.

Arsenic chloride treated samples exhibited absorption maxima at 500nm as shown in Figure-3. Similarly, samples treated with mercury chloride exhibited absorption maxima at 489nm (Figure-4). Mercury complex absorbed light in the region of violet-green. Formation of colored complex showed the transition of metal. This was possibly caused by the coordination of metal, in the complex, with oxygen of the carboxylic group of hemoglobin.

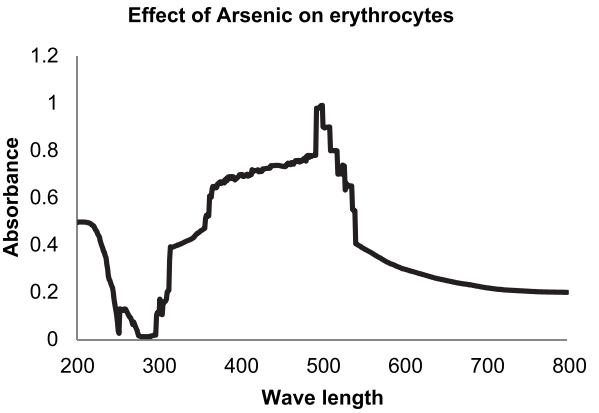

Figure-3. Absorption spectra of arsenic treated samples.

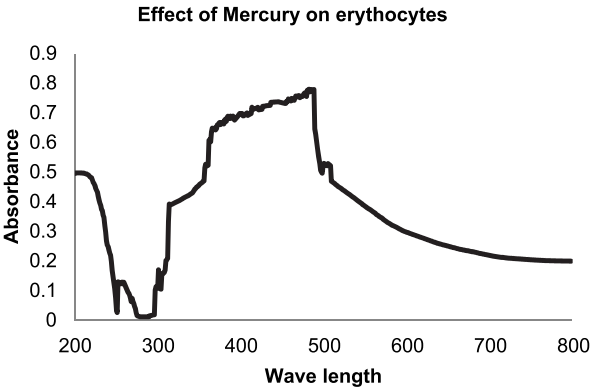

Figure-4. Absorption spectra of mercury treated samples.

Selenium chloride displayed absorption maxima at 475nm as shown in Figure-5. Appearance of characteristic absorption bands along with brownish color of the resulting complex represented transition and creation of co-ordination complex by metal-ligand interaction in the reaction mix.

Cadmium sulfate displayed absorption maxima at 434nm as shown in Figure-6. Erythrocyte and cadmium sulfate mixture exhibited a yellow green color and this specified the creation of a complex because of transition and co-ordination

of cadmium ions and the ligand.of cadmium ions and the ligand.

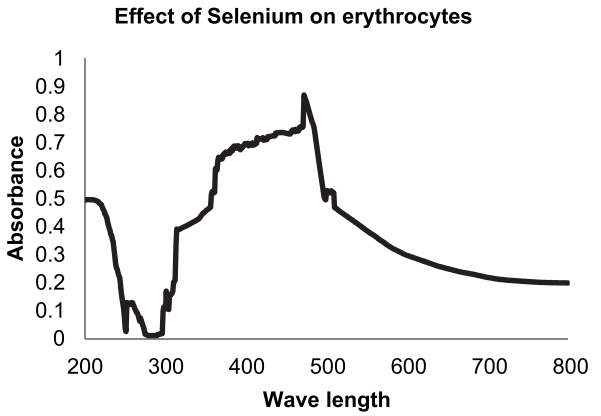

Figure-5. Absorption spectra of selenium treated samples.

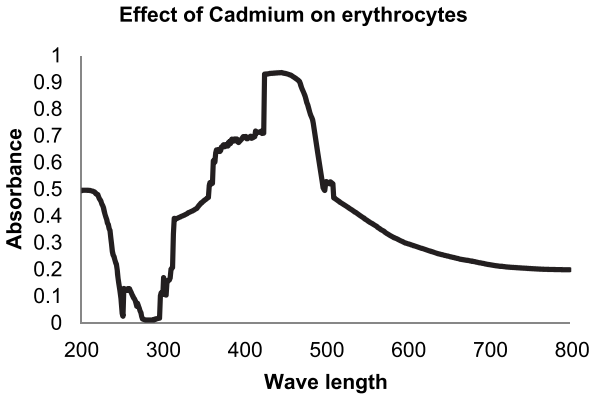

Figure-6. Absorption spectra of cadmium treated samples.

DISCUSSION

Lead

Lead and its associated compounds inhibit the production of hemoglobin; cause dysfunction of joints, kidneys, reproductive and cardiovascular systems and lead to acute and chronic damage to the peripheral and central nervous systems.[10] Substantial reduction in erythrocyte count, hematocrit and Hb levels have been observed in both rats and humans associated with higher blood levels of lead.[10,11]

There was a shift in absorption maxima of lead treated erythrocyte samples at 367nm and 525nm. This alteration in absorption maxima and reddish milky coloration represented lead complexity with active sites on erythrocytes. It was apparent from this decrease in absorption maxima and shift in peaks towards lower wavelength that there was an effect on erythrocytes.[8,12]

Arsenic

Researchers are searching for protective antioxidants so as to fight against arsenic induced toxicity. Dietary antioxidants have received enormous attention recently in order to be used in illnesses associated with oxidative stress. In 2003, WHO recommended the consumption of vitamins A, C, E and trace elements particularly zinc and selenium to mitigate the effects of arsenic poisoning.[13,14]

There was a shift in erythrocytic absorption maxima from 537nm to 500nm after being treated with arsenic solution. It was evident from this shift in absorption maxima that there was a bond formation between arsenic and the oxygen of erythrocytes. This likely complex formation also led to decrease in the number of red blood cells due to their linkage with metals.[15] Similarly, the brick red color of the solution further confirmed the creation of an arsenic complex with erythrocyte.[15,16]

Mercury and Selenium

Mercury is believed to be one of the most noxious contaminants which human beings are confronting these days. It is found naturally in soil (inorganic mercury), air (organic mercury) and water (organic and inorganic forms). High level exposure of mercury and selenium can damage the heart, brain, lungs, kidney and immune system.[17]

It is an established fact that the mercury and selenium have had higher capabilities of interacting with the donor pair of ligands containing oxygen, nitrogen or sulfur groups and in turn forming complexes. Literature reveals that these two metals prefer sulfur over others in making such complexes.[18] In our case, SH groups present in red blood cells provided an excellent opportunity for complex formation. This was confirmed by shift in spectra where new bands appeared at 500 to 550nm which showed

pi-pi interaction of SH groups of erythrocytes with mercury and selenium ions and hence confirmed the interaction of mercury and selenium with the O_2 of erythrocytes.

Cadmium

Ingestion of cadmium in high quantities irritates gastric mucosa. Consumption of acidic food and beverages which are inappropriately stored in cadmium glazed containers is the commonest mode of acute cadmium poisoning.[19,20]

UV/Visible spectra of cadmium treated erythrocytes showed that the interaction of cadmium with human erythrocytes caused disruption of bands in both UV and visible regions. This could be due to the fact that the cadmium coordinated with the active areas of the red blood cells (RBCs) which eventually moved the bands from higher to lower regions. Appearance of a milky white color of the solution also represented some kind of complex formation.

Cadmium disturbed the absorption maxima and other peaks considerably. Literature also supported this fact as decrease in count of erythrocytes was due to the interaction of the active groups of erythrocytes with cadmium and lead to a decrease in absorption maxima.[8,21]

Effect of various toxic metals like lead, mercury, arsenic, cadmium and selenium on human erythrocytes was analyzed in this study. It was observed that erythrocytes exhibited altered behavior with each metal studied which was evident by shifting of peaks in UV/Visible spectra. The absorption maxima shifted from higher to lower wavelengths with every metal tested. The shift was more significant in cases of lead, mercury and cadmium while a similar behavior was identified with selenium and arsenic.

These changes were attributed to the fact that metals interact with erythrocytes. The lipid sites of erythrocytes appeared to be quite sensitive for interacting with a number of metals present in the atmosphere thus altering original RBC structure and their action. Any such interaction of RBCs with external species damage erythrocytes and reduce their number.

CONCLUSION

This study showed that erythrocytes exhibited different behavior with different metals as evident by the shift of peaks in UV/Visible spectra. The absorption maxima were shifted towards lower wavelengths suggesting that these metals do exert an effect on human erythrocytes.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Khawar Anwar	Study design and data collection.	
2	M. Usman Anjum	Gathered and organized data, Written manuscript.	
3	M. Adnan Sadiq	Statistical analysis and supervised the study.	
4	Erum Bashir	Conceived the idea and performed study.	
5	Khawaja Shakeel Ghani	Proof-read, critical revision and final approval of the manuscript.	