



## Effect of toxic gases on human erythrocytes: Spectrophotometric analysis.

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**ABSTRACT... Objective:** This study was carried out to analyze the influence of various toxic gases on 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. Gases were prepared in laboratory as per given protocol. Erythrocyte samples were treated with prepared gases. Absorbance of the samples was recorded. The spectrum of these treated samples was compared with the standard spectra of erythrocytes to check for any changes that might have occurred. **Results:** It was observed that erythrocytes exhibited altered behavior with each gas which was confirmed by shift in peaks in UV/Visible spectra. Absorption maxima were shifted from higher to lower wavelengths and this shift was more pronounced in case of Chlorine, Carbon Monoxide and Carbon Dioxide as compared to that observed in case of Hydrogen Sulfide, Sulfur dioxide, and Nitrogen Dioxide. **Conclusion:** This study showed that erythrocytes exhibit different behavior with different gases as evident by the shift of peaks in UV/Visible spectra. The absorption maxima were shifted towards lower wavelengths suggesting that these gases do exert an effect on human erythrocytes. The lipid site of erythrocytes, being sensitive for absorbing various atmospheric gases, caused deformation of original erythrocyte structure and altered their activity. This type of interaction of red blood cells with such external species, particularly the donor-acceptor case, caused damage to erythrocytes and in turn, decreased their number.

**Key words:** Erythrocytes, Gases, Spectrophotometer, Spectrum, Toxic.

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## INTRODUCTION

Air pollution is a serious problem which threatens human health globally for a long time. There are different contributing sources of air pollution which can be a small unit of cigarettes to a huge amount of toxic gases produced from fossil fuel combustion in motor engines of vehicles and industrial expansions.<sup>1,2</sup> Serious diseases like respiratory infections, cardiovascular diseases and cancers are the major manifestations of continuous exposure to these toxic gases. Globally, Millions of deaths are associated with environmental pollutants each year.<sup>3-5</sup> Sterility in male is also linked with air pollution in the latest research.<sup>6</sup>

Some of the main aerial pollutants include sulfur

dioxide (SO<sub>2</sub>), nitrogen dioxide (NO<sub>2</sub>), carbon monoxide (CO) and ozone (O<sub>3</sub>). SO<sub>2</sub> is described to cause a rise in the daily number of deaths due to cardiovascular and respiratory illnesses. It absorbs readily (99%) through the respiratory tract and displays a wide spread tissue-distribution.<sup>7,8</sup> Amount of SO<sub>2</sub> in blood and other tissues increases proportionately with the concentration of SO<sub>2</sub> in inhaled air. Radioactive sulfur (S35) could be identified in the protein segments of tissues for at least one week.<sup>9</sup> Chow and Hong reported an increase in lipid peroxidation, in rats, of the heart and lung tissues as a result of SO<sub>2</sub> inhalation at the rate of 10 ppm for one hour daily for thirty days.<sup>4</sup> Substantial rise in sulfhemoglobin ratios and erythrocyte activities of superoxide dismutase and glutathione peroxidase, due to exposure to

heavy air pollution, were also reported.<sup>4,10</sup> Various studies reported SO<sub>2</sub> inhalation induced increase in sulfhemoglobin and osmotic fragility ratios, leucocyte and erythrocyte count, hematocrit and hemoglobin levels.<sup>11</sup> Hence, inhalation of several air pollutants (NO<sub>2</sub>, O<sub>3</sub>, cigarette smoke) was described to lead to an increase in osmotic fragility ratios and MDA levels.<sup>12,13</sup> Long term exposure to these toxic gases in humans, even at low concentrations, is very hazardous. Therefore, this study was conducted to analyze the effects of various toxic gases on erythrocytes.

## MATERIAL & METHODS

This study was performed at Department of Chemistry, Hazara University, Mansehra, from January 2017 to June 2017. Blood samples were collected from clinical laboratory under aseptic conditions and erythrocytes were separated and prepared by the method of Dodge et al. The standard absorption spectrums were measured, (range: 200-800nm), using double-beam UV-visible spectrophotometer (PerkinElmer, USA).

Gases were prepared in laboratory as follows:

### Carbon dioxide gas

CO<sub>2</sub> was prepared by reacting calcium carbonate (CaCO<sub>3</sub>) with Hydrochloric acid (HCl) as follows:  

$$\text{CaCO}_3 + \text{HCl} \rightarrow \text{CaCl}_2 + \text{H}_2\text{O} + \text{CO}_2$$

### Carbon monoxide gas

CO gas was prepared by reacting Zinc (Zn) metal with Calcium Carbonate (CaCO<sub>3</sub>) as follows:  

$$\text{Zn} + \text{CaCO}_3 \rightarrow \text{ZnO} + \text{CaO} + \text{CO}$$

### Sulphur dioxide gas

SO<sub>2</sub> gas was prepared by reacting Copper (Cu) metal with Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) with following chemical reaction; 
$$\text{Cu} + 2\text{H}_2\text{SO}_4 \rightarrow \text{CuSO}_4 + \text{SO}_2 + 2\text{H}_2\text{O}$$

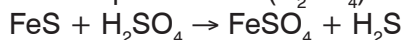
### Chlorine gas

Cl<sub>2</sub> gas was prepared by reacting manganese dioxide (MnO<sub>2</sub>) with hydrochloric acid (HCl) as follows: 
$$\text{MnO}_2 + 4\text{HCl} \rightarrow \text{MnCl}_2 + 2\text{H}_2\text{O} + \text{Cl}_2$$

### Hydrogen sulphide gas

H<sub>2</sub>S was prepared by reacting iron pyrite (FeS)

with Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) as follows:



### Nitrogen dioxide gas

NO<sub>2</sub> was prepared by reacting nitric acid (HNO<sub>3</sub>) with tin (Sn) and NO<sub>2</sub> gas was produced as byproduct. 
$$4\text{HNO}_3 + \text{Sn} \rightarrow \text{H}_2\text{O} + \text{H}_2\text{SnO}_3 + 4\text{NO}_2$$

Erythrocyte samples were then treated with these prepared gases. Absorbance of the gas treated samples was recorded on double beam UV-Visible Spectrophotometer in the range of 200-800nm. Recorded spectra of these samples were compared with standard erythrocyte spectra to analyze for any changes that might have occurred.

## RESULTS

This study examined the impact of different toxic gases such as CO<sub>2</sub>, CO, SO<sub>2</sub>, Cl<sub>2</sub>, H<sub>2</sub>S, and NO<sub>2</sub>, which were normally released during industrial processes from different sources, on the human erythrocytes. Our study showed that the standard normal erythrocyte spectrum showed maximum absorption at 537nm as shown in Figure-1.

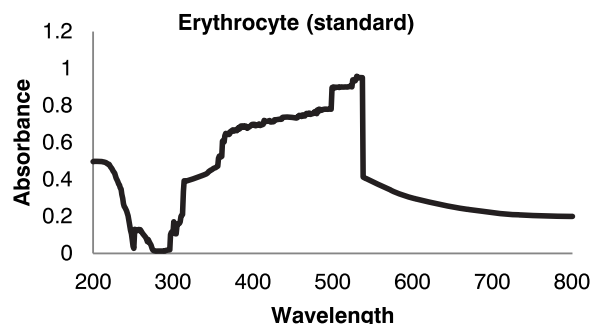
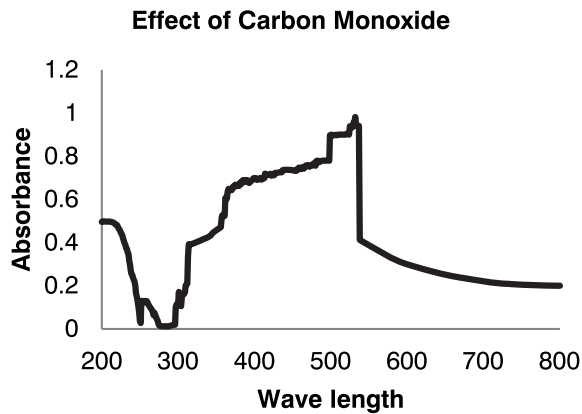
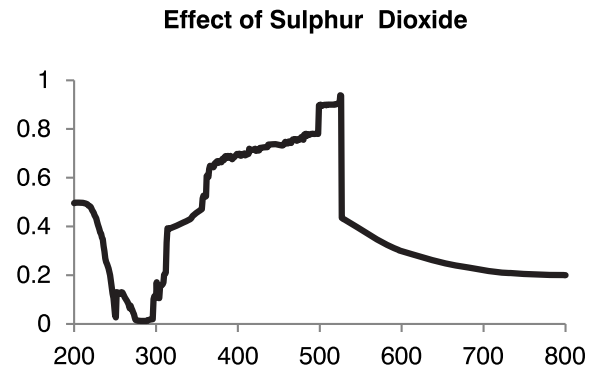


Figure-1. Standard normal erythrocyte absorption spectrophotometric spectra.

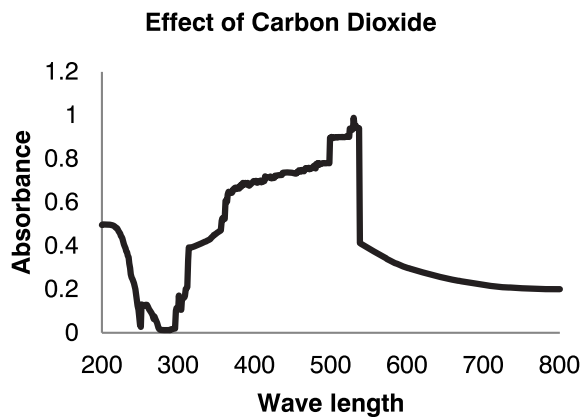
Erythrocytes were treated with toxic gases which were prepared in the laboratory. After treating the erythrocyte samples with these toxic gases, there was a change in the observed spectra. Carbon dioxide and carbon monoxide displayed similar effect on treated erythrocytes as the absorption maxima was obtained at 533nm (for CO<sub>2</sub>) and 532nm (for CO). The spectrum exhibited a minor shift downwards as shown in Figure-2 and 3 respectively.



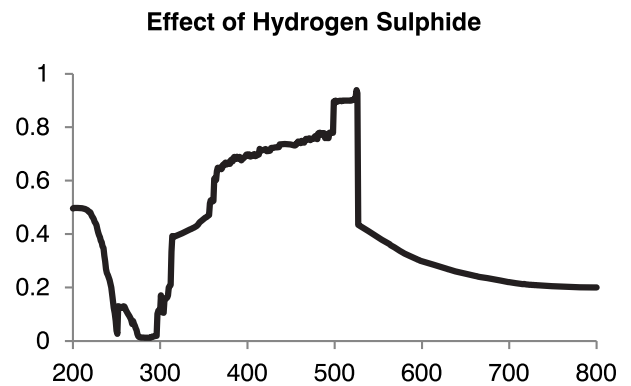
**Figure-2. Spectrophotometric absorption spectrum for CO treated erythrocytes.**



**Figure-4. Spectrophotometric absorption spectrum for SO<sub>2</sub> treated erythrocytes.**



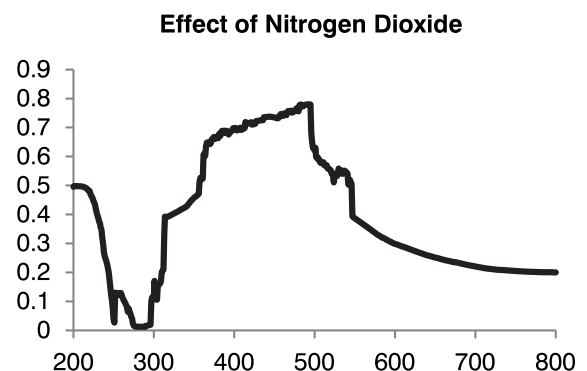
**Figure-3. Spectrophotometric absorption spectrum for CO<sub>2</sub> treated erythrocytes.**



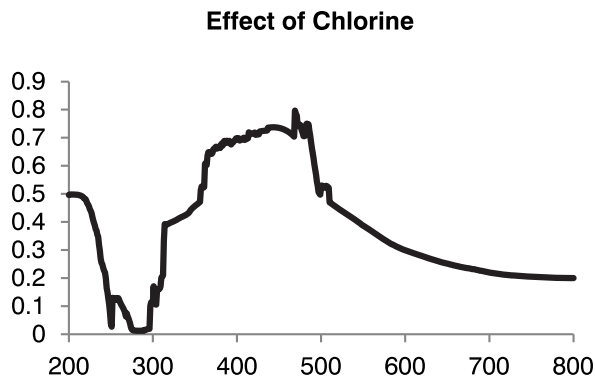
**Figure-5. Spectrophotometric absorption spectrum for Cl<sub>2</sub> treated erythrocytes.**

Sulfur dioxide treated erythrocytes displayed absorption spectra at 526nm and 512nm, as displayed in Figure-4. There were new peaks in SO<sub>2</sub> exposed erythrocyte spectra showing that some new compounds were formed with changing intensities.

Chlorine, hydrogen sulfide, and nitrogen dioxide gas treated samples of erythrocytes showed absorption maxima at 498nm, 526 and 495 as shown in Figures-5, 6 and 7 respectively. These changes could be attributed to interaction of these gases with iron which was present in hemoglobin. Specifically after treatment with NO<sub>2</sub>, it appeared that vital bands of erythrocyte had disappeared and some new compounds were formed by the process of interactions and complications.



**Figure-6. Spectrophotometric absorption spectrum for H<sub>2</sub>S treated erythrocytes.**



**Figure-7. Spectrophotometric absorption spectrum for NO<sub>2</sub> treated erythrocytes.**

## DISCUSSION

Interaction of erythrocytes with various gases like Carbon monoxide, Carbon dioxide, Chlorine, Hydrogen Sulphide, Sulphur dioxide, and Nitrogen Dioxide was analyzed.

### Carbon Monoxide and Carbon Dioxide

CO and CO<sub>2</sub> showed absorption maxima at 530 and 532nm respectively. Interactions of CO and CO<sub>2</sub> with the carboxylic group of hemoglobin resulted in greenish-red appearance.

These gases enter the body via inhalation, contact through skin and eye. Symptoms include headaches, seizures, dizziness, and hallucination.<sup>14</sup> Typically, both these gases behave in a similar pattern. Though, the extensive concentration of CO lead to paucity of blood oxygen because of high affinity of hemoglobin for CO.<sup>15</sup>

### Sulfur Dioxide

It is a byproduct of burning of coal and other fossil fuels. Moreover, other natural sources of atmospheric SO<sub>2</sub> include volcanoes, marine and terrestrial biogenic discharges.<sup>9</sup>

Resulting UV/Visible spectra after SO<sub>2</sub> treatment of erythrocytes showed that there was interaction of SO<sub>2</sub> with erythrocytes which lead to changes in bands in spectra. Interaction of lone pair of electrons on sulfur and oxygen of red blood

cells resulted in alteration in band from 350nm to 450nm. Appearance of two other bands at 510nm and 536nm indicated that there was some kind of bonding among SO<sub>2</sub> and erythrocyte active locations which caused the quantity of red blood cells to reduce considerably.<sup>16,17</sup>

### Chlorine

Mostly, exposure to chlorine occurs via inhalation and low-level exposure to chlorine in air will lead to skin, eye or airway irritation, cough and sore throat while exposures at higher levels result in the dyspnea, wheezing, chest tightness and bronchospasm. Severe exposures to chlorine could lead to non-cardiogenic pulmonary edema and it could be delayed for many hours.<sup>18</sup>

Absorption maxima in case of chlorine treated erythrocytes shifted from 537nm to 470nm. This shift could be attributed to the interaction of chlorine with the hydrogen of erythrocytes. It has been proposed that lowering of absorption maxima due to chlorine was because of absorption of chlorine in the lipid layer of erythrocytes which eventually lower the number of erythrocytes.<sup>19</sup> It was also reported that the long-time chlorine exposure of erythrocytes may lead to severe clotting abnormality which in the end causes death.<sup>20</sup>

### Hydrogen Sulfide

Hydrogen sulfide leads to inhibition of the cytochrome oxidase enzyme system which causes dearth of oxygen for use in cells and tissues. As a result, an acid-base imbalance ensues because of anaerobic metabolism which causes buildup of lactic acid. Cardiac and nervous tissues are chiefly susceptible to the disturbance of oxidative metabolism and death may occur due to respiratory arrest.

H<sub>2</sub>S gas treated samples of erythrocytes showed absorption maxima at 526nm. The UV/Visible spectra after treating erythrocytes with H<sub>2</sub>S was comparable to that of spectra of SO<sub>2</sub> treated sample. This confirmed that the sulfur played a key part in shifting the absorption spectra in both cases. This shifting of absorption spectra could be attributed to the interaction of sole pair of

electrons on sulfur and oxygen of red blood cells

### Nitrogen Dioxide

Nitrogen Oxides (NO<sub>2</sub>) are a group of gases which cause numerous severe health issues. They react with ammonia and other compounds to form nitric acid and other interrelated compounds. These minute particles damage lung tissue and effects breathing and can even cause premature death. These trivial particles could infiltrate deeply into sensitive areas of respiratory system and might lead to or exacerbate lung diseases, for example emphysema and bronchitis, as well as worsen pre-existing heart disease.<sup>21,22</sup>

NO<sub>2</sub> treated erythrocytes showed the shifting of absorption maxima. Presence of electrons in nitrogen and oxygen, which were available for bonding with the active sites of red blood cells, might be responsible for this shift. Longtime exposure of erythrocytes to NO<sub>2</sub> also effects the pH of red blood cells as both active groups (N and O) serve as a bridge among erythrocytes and NO<sub>2</sub>. As a result, the number of erythrocytes decreased<sup>23</sup> which may in turn cause serious diseases.

This study revealed that erythrocytes displayed altered behavior with each gas analyzed. There was a shift in absorption maxima from higher to lower wavelength with each gas used and this shift was more pronounced in cases of Chlorine, Carbon Monoxide and Carbon Dioxide.

### CONCLUSION

This study showed that erythrocytes exhibit different behavior with different gases as evident by the shift of peaks in UV spectra. Absorption spectra shifted from higher to lower wavelengths and this shift was more considerable and noticeable in case of Chlorine and Carbon Dioxide, Hydrogen Sulphide and Nitrogen Dioxide suggesting that these gases do have an effect on human erythrocytes. The lipid site of erythrocytes, being sensitive for absorbing various atmospheric gases, caused deformation of original erythrocyte structure and altered their activity. This type of interaction of red blood cells with such external species, particularly the donor-

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


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### AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Errum Bashir	Conceived the idea, Study design and performed study.	
2	Khawar Anwar	Proof-read, Critical revision and final approval of the manuscript.	
3	M. Usman Anjum	Gathered and organized data, Written manuscript.	
4	Wajid Rehman	Supervised the study.	