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Spectroscopic study of cyanotoxicity of potassium cyanide on normal human haemoglobin

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Abstract

Potassium cyanide (KCN) one of the salts of cyanide finds its application in several fields of endeavour such as pharmaceuticals, industrial, agricultural, mining, metallurgy, medical, photography and as a biological weapon. Release of cyanide from KCN into the blood stream during its usage has been found to be toxic to human health. Sequel to this, the study aims atinvestigating by spectral analysis, the cyanotoxicity effect of KCN on normal human haemoglobin. The study was divided into 8 groups consisting of control and test groups (0.2, 0.5, 1 and 1.5M KCN + 1000µl Oxyhaemoglobin respectively and 2000, 3000 and 4000µl Oxyhaemoglobin + 1.5M KCN respectively). It was found that KCN caused concentration dependent oxidation on normal human haemoglobin and also a concentration dependent reduction in oxyhaemoglobin concentration which are all precursors to several pathophysiologic conditions in man. Therefore, measures should be put in place to curtail the hazardous effect of cyanide emission on human.

Keywords: Oxyhaemoglobin, Potassium cyanide, Cyanotoxicity, Emission and Oxidation.

Introduction

Potassium cyanide (KCN) is one of the salts of cyanide and highly soluble in water. Upon exposure to air, it easily turns to liquid by absorbing moisture from the air[1]. On dissolution to liquid it hydrolyses to hydrogen cyanide[1]. It is used as chemical additives in electroplating baths to give the article being electroplated a glossy finishing instead of a dull finishing[2]. This colourless crystalline solid is also used as a metal complexing agent in mining, especially in the refinement of metal concentrates[2,3]. In organic synthesis, KCN is used to introduce cyano-groups into organic compounds. This is usually the first step in the synthesis of many compounds used in pharmaceutical, plastic, paper and textile industries[4,5]. It finds many applications in medicine, one of which is its use as a chelating or sequestering agent for the detoxification of poisonous metal agents in the body such that they can be excreted without causing any harm to the body[4]. Other uses of KCN include; as agrochemicals, dryers, specialty chemicals, removal of defective metal coating and in heat treatment[4].

Despite the enumerated importance of KCN, incessant emission of KCN have been reported from a number of industrial sources, such as, in pharmaceuticals, metallurgical, mining, petroleum refineries and other manufacturing industries[6]. Medically, it has been found that intravenous administration of sodium nitroprusside employed in the treatment of hypertension liberates cyanide which could be very lethal when uncontrolled[7]. Other sources of cyanide emission include; emissions from municipal solid waste incinerators, inappropriate disposal of cyanide wastes in landfills and waste ponds, atmospheric release of cyanide from fumigation operations and improper burning of polyamides, acrylonitrile, and polyurethanes[8-11]. Estimation of cyanide emission into the atmosphere from cyanidation processes amounted to 20000 tonnes[2,14,15]. Consequently, this accidental emission of cyanide into the environment results to environmental degradation and contamination of water systems[3,11-14,16,17].

There is paucity of data concerning the control of cyanide emission into the environment resulting from the several uses of KCN[2]. Since no information is available as regards to that, uncontrolled emission of KCN into the environment could pose a serious health risk to man who could be predisposed to the emitted chemical via several means such as; inhalation from air, drinking water contaminated by this chemical, from pharmaceuticals, and by eating agro products contaminated with the chemical[2,12]. Several cases of medical and occupational exposure to cyanide has been reported and this has shown to be fatal to cyanide workers both on a short term and long term[7,19-21]. Since, the red blood cells is the major source of transport of this chemical to the tissues and organs where they cause their toxicological effects when it finds its way into the human body, there is tendency that this chemical may also be toxic to haemoglobin which is the transport protein in red blood cells[22]. Therefore, this study aimed at investigating by spectral analysis the potential oxidative effect of potassium cyanide on normal human haemoglobin.

Materials and methods

Blood sample: Upon approval to conduct research involving human participants by the Ethical board of faculty of biological science, University of Nigeria, blood sample was collected from a human volunteer (a non-smoking male student of the University of Nigeria, Nsukka aged 26 years), who was confirmed to be of genotype Hb AA at Renanscent Hospital, Nsukka, Nigeria. 8ml of whole blood was collected by venipunture into a Venoject tube containing ethylene diaminetetraacetic acid (EDTA) as an anticoagulant and kept at 4˚C until use.

Chemicals and reagents: The chemical and reagents used for this study were of analytical grade and are the following: Potassium cyanide (Panhong Chemical Company, China), Sephadex G-150 (Thomas Scientific, New Jersey, U.S.A), Sodium Chloride (Panhong Chemical Company, China), Methylated Spirit (Coral Health Care, Hyderbad, India), Potassium Phosphate Salts (Panhong Chemical Company, China), Sodium Hydroxide (Panhong Chemical Company, China), Concentrated Hydrochloric acid (Panhong Chemical Company, China), Distilled Water (Energy Centre, University of Nigeria, Nsukka).

Equipment, apparatus and instruments: Ultraviolet-visible (UV visible) Spectrophotometer (Jenway, United Kingdom), Beaker (Pyrex®, Sigma- Aldrich England), Legend X1R Centrifuge (LABSCO, Germany), Spectrumlab 23A Spectrophotometer (Spectrum Laboratories, United States), Erlenmeyer Flask (Pyrex®, Sigma-Aldrich England), Cotton wool (Sri Venkateswara surgical cotton Indian), Curvettes (Pyrex®, Sigma-Aldrich England), EDTA bottles (Guangdong, China), Electronic weighing balance (Adams Equipment Inc. Oxford), Filter paper (Zibo, Shandong, China), Glass Column (Pyrex®, Sigma-Aldrich England), Glass rod (Pyrex®, Sigma-Aldrich England), Glass wool (Gupta Glasswool Industries, Delhi), Gloves (Supermax Latex Products, Malaysia), Hypodermic syringes (Troge Medical GmbH, Germany), Micropipette (Globe Scientific Inc. USA), Pasture pipette (Globe Scientific Inc. USA), pH meter (Hanna Instruments, Italy), Refrigerator (Thermocool Premier, Japan), Test tubes rack (Sanjong Scientific Industries, Delhi), Test tubes (Pyrex®, Sigma-Aldrich England), Tourniquets (Zimmer Biomet, Indiana), Retort Stand (Shree Krishna Scientifics, Ambala, India), Nose mask (Bombay Fabrics, Coimbatore, India)

Preparation of Stock Solution of Potassium cyanide: 0.2, 0.5, 1 and 1.5 molar concentrations of potassium cyanide were prepared according to the method given by Awad et al[23] using the formula are below;

$$\text{Mass} = \text{Molar concentration} \times \text{Molar Mass}$$

Thus, the mass obtained was dissolved in 1 L of distilled water.

Preparation of normal saline: In a clean 250ml beaker, 1g of NaCl was dissolved in 50ml of distilled water[24]. After mixing to homogeneity, the mixture was then made up to 100ml with distilled water taking caution while topping up in order to avoid error due to parallax. The resultant solution was then transferred to a clean reagent bottle and labelled.

Preparation of potassium phosphate buffer: Potassium phosphate buffer (pH 7.6) of molar concentration 10mM was used for this research. The buffer was prepared using Henderson- Hassel Balch equation[25].

Preparation of haemoglobin: Haemoglobin (Hb) was prepared with slight modification of the method given by Robert et al[26]. Briefly, after washing 10ml of the whole blood sample for three times with normal saline, it was spinned at 3000rpm for 30 minutes using a Legend X1R centrifuge (LABSCO, Germany). After centrifugation, the supernatant was aspirated and the pellet was diluted 3 folds with distilled water, rocked gently at room temperature for 10 minutes was and kept in an ice bath for 2 hours (for lysis). The lysate was then centrifuged at 5,000rpm for 45 minutes and was loaded onto a well packed Sephadex G-150 Fast-flow column (Bed dimension 3 × 40cm, 300ml), which was equilibrated with 3 column volume 10mM potassium phosphate buffer of pH 7.6. The elution of the Hb AA was done at 4°C with a linear gradient of 25–100% of the buffer in 2 column volumes. The flow rate of the elution was 2ml/min and the effluent was monitored at 541, 576 and 630nm. Hb AA was collected and was stored at −18°C for future use. For spectrophotometry, stock solution of the haemoglobin and buffer were mixed in the ratio 5:1 with different pipette for sample and reference.

Experimental design: Design of experiment as follows
Group 1: 1000 µl of Oxyhaemoglobin (Normal Control)
Group 2: 1000 µl of 0.2 M KCN + 1000 µl Oxyhaemoglobin
Group 3: 1000 µl of 0.5 M KCN + 1000 µl Oxyhemoglobin
Group 4: 1000 µl of 1.0 M KCN + 1000 µl Oxyhemoglobin
Group 5: 1000 µl of 1.5 M KCN + 1000 µl Oxyhemoglobin
Group 6: 1000 µl of 1.5 M KCN + 2000 µl Oxyhemoglobin
Group 7: 1000 µl of 1.5 M KCN + 3000 µl Oxyhemoglobin
Group 8: 1000 µl of 1.5 M KCN + 4000 µl Oxyhaemoglobin

Measurement of Haemoglobin Concentration: Oxyhaemoglobin concentrations were determined using the Lambert-Beer law[27]. The concentrations of oxyhaemoglobin are expressed as mM and the absorption spectral was scanned between the visible range 400 to 700nm. The concentrations (mM) for oxyhaemoglobin species were determined from the extinction coefficients derived by Meng and Alayash[25]. To calculate the concentrations of oxyhaemoglobin (oxy-Hb), the following equation were considered: $C = A/el$

Where: C = Concentration, A = Absorbance at 541 nm and 576 nm, e = extinction coefficient at 541 nm and 576 nm, l = length of cuvette.

Results and discussion

Characterization of the absorption spectrum of unreacted haemoglobin revealed two distinct peaks (namely β and α band) at 541nm and 576nm respectively (Figure-1). Using this as a baseline, the effects of the different molar concentrations of KCN on the oxidation of oxy-haemoglobin were determined. The result obtained showed that 0.2, 0.5, 1 and 1.5 molar concentrations of KCN led to concentration-dependent reduction in the magnitude of the absorbance maxima of oxy-haemoglobin (Figures 2-5). Whereas, when the molar concentration of KCN were kept constant and the volume of oxy-haemoglobin varied (1000, 2000, 3000 and 4000µl), there were concentration-dependent increase in the magnitude of the absorbance maxima of oxy-haemoglobin (Figure 5-8).

Quantitatively, as shown in Figure-9, the different molar concentrations of potassium cyanide, reduced oxy-haemoglobin concentration. The result revealed that reaction of 1.5M KCN reduced oxy-haemoglobin concentration more than reactions of 0.2, 0.5 and 1M KCN respectively. Reaction of 1M KCN reduced oxy-haemoglobin concentration more than reactions of 0.2 and 0.5M KCN respectively. Reactions of 0.5M KCN reduced oxy-haemoglobin concentration more than reaction of 0.2M KCN. On the other hand, when the molar concentration of KCN were kept constant and the volume of oxy-haemoglobin varied (1000, 2000, 3000 and 4000µl), there were concentration-dependent increase in the concentration of oxy-haemoglobin.

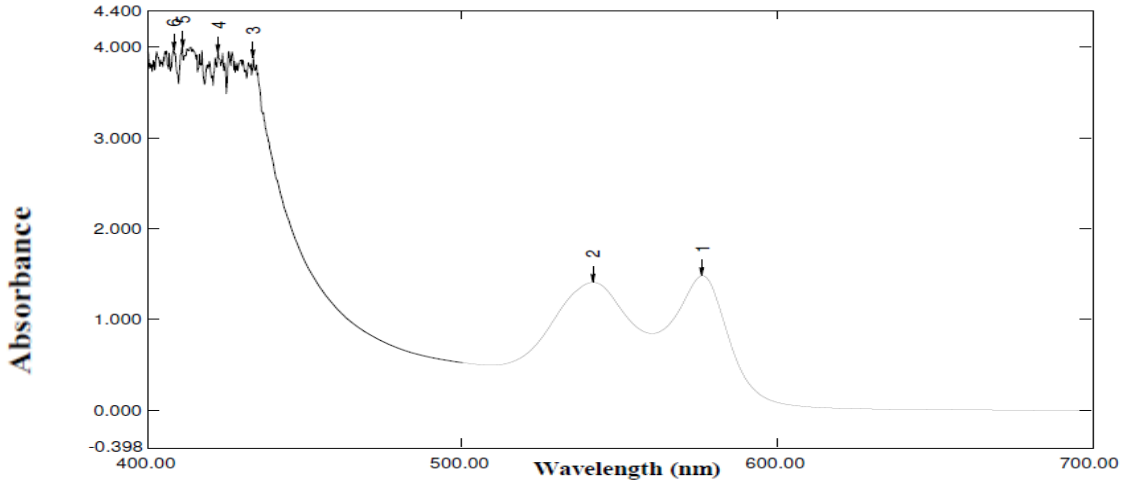

Figure-1: Absorption spectral of 1000 µl of oxyhaemoglobin (89 mM) in 10mM phosphate buffer, pH 7.6 after 24 hours incubation at 25˚C.

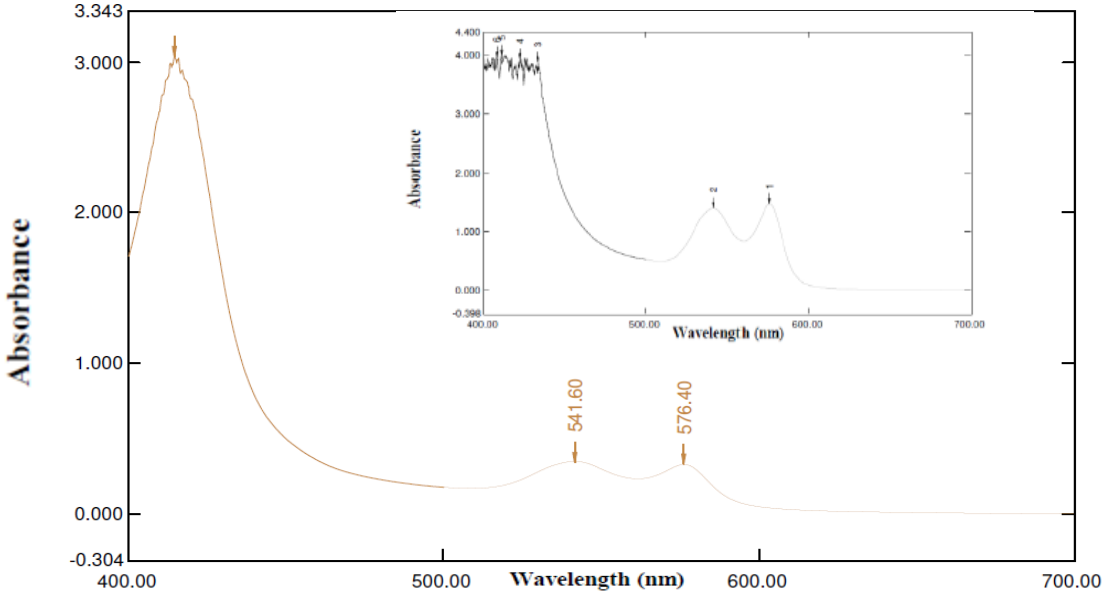

Figure-2: Spectral changes after reacting 1000µl of oxyhaemoglobin (89mM) in 10mM phosphate buffer, pH 7.6 with 1000µl of 0.2M KCN for 24 hour at 25˚C, compared to unreacted oxyhemoglobin.

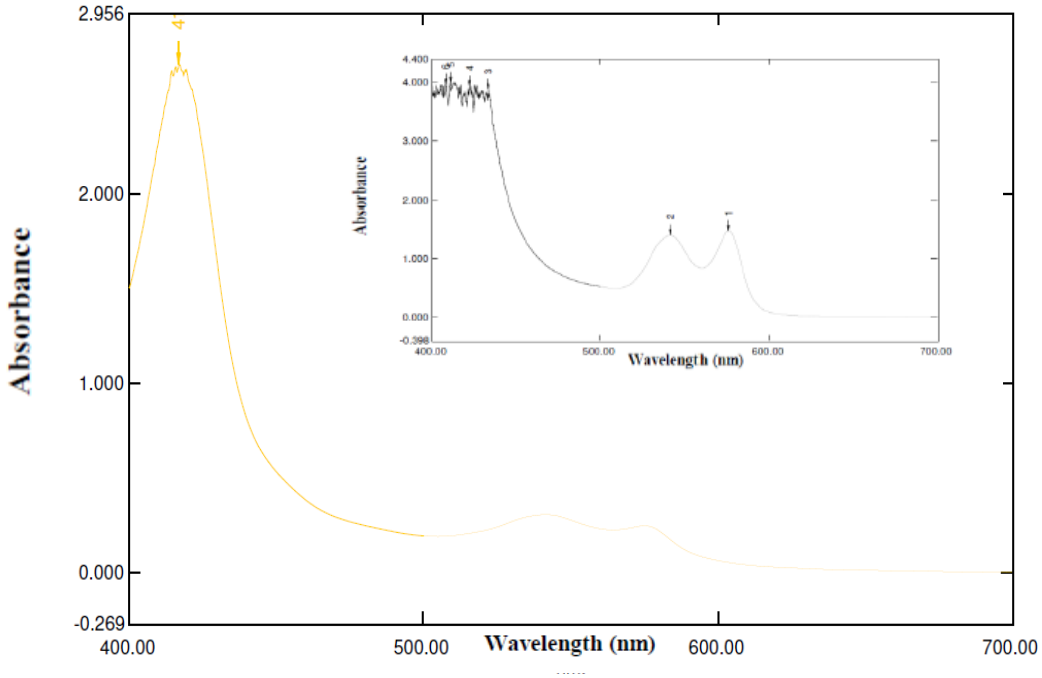

Figure-3: Spectral changes after reacting 1000µl of oxyhaemoglobin (89mM) in 10mM phosphate buffer, pH 7.6 with 1000µl of 0.5M KCN for 24 hour at 25˚C compared to unreacted oxyhaemoglobin.

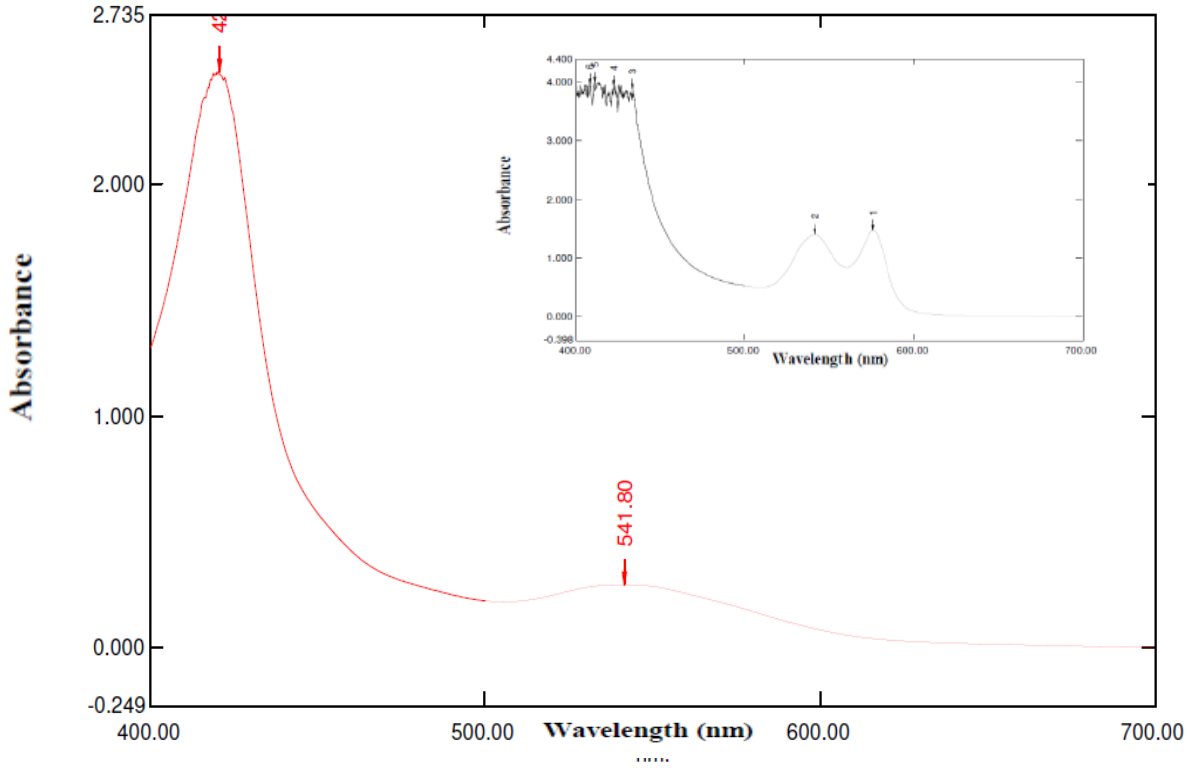

Figure-4: Spectral changes after reacting 1000µl of oxyhemoglobin (89mM) in 10mM phosphate buffer, pH 7.6 with 1000µl of 1M KCN for 24 hour at 25˚C compared to unreacted oxyhaemoglobin.

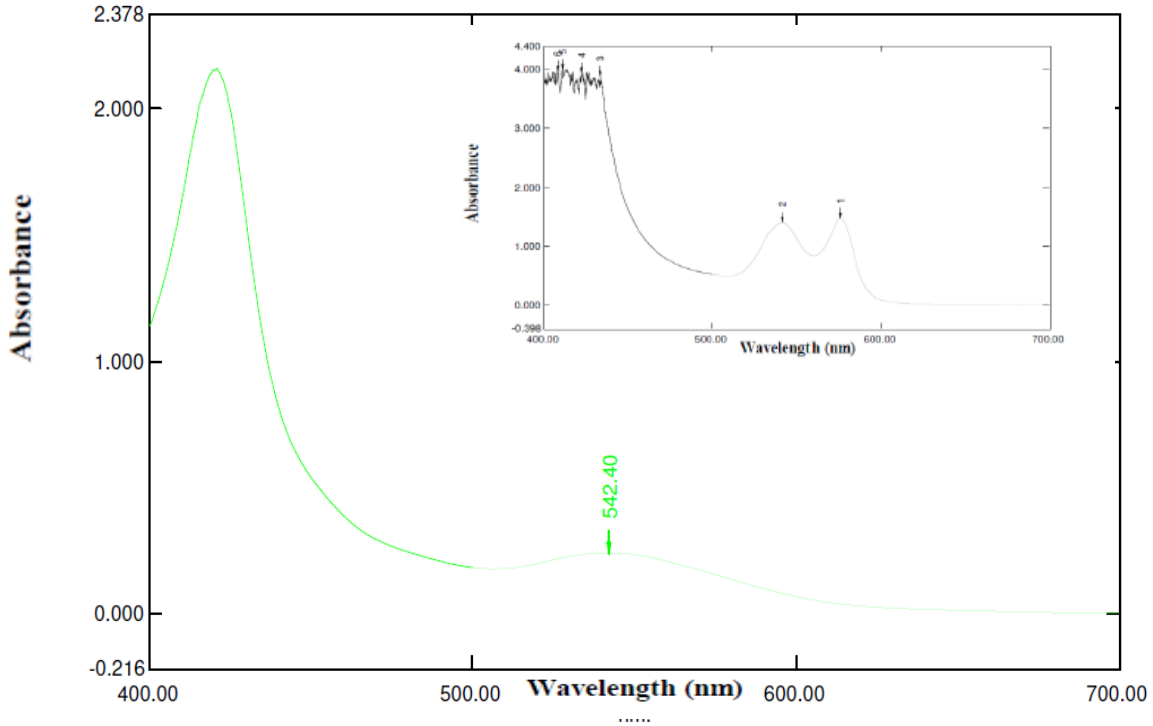

Figure-5: Spectral changes after reacting 1000µl of oxyhaemoglobin (89mM) in 10mM phosphate buffer, pH 7.6 with 1000µl of 1.5M KCN for 24 hour at 25˚C compared to unreacted oxyhaemoglobin.

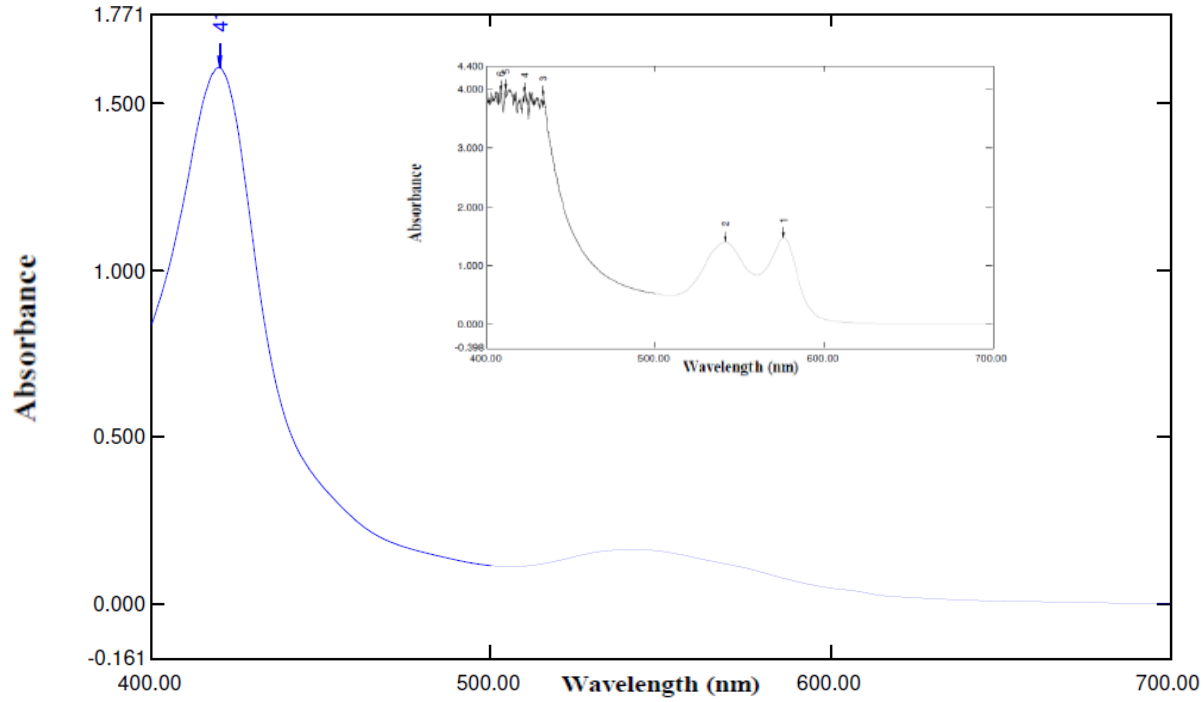

Figure-6: Spectral changes after reacting 2000µl of oxyhaemoglobin (89mM) in 10mM phosphate buffer, pH 7.6 with 1000µl of 1.5M KCN for 24 hour at 25˚C compared to unreacted oxyhaemoglobin.

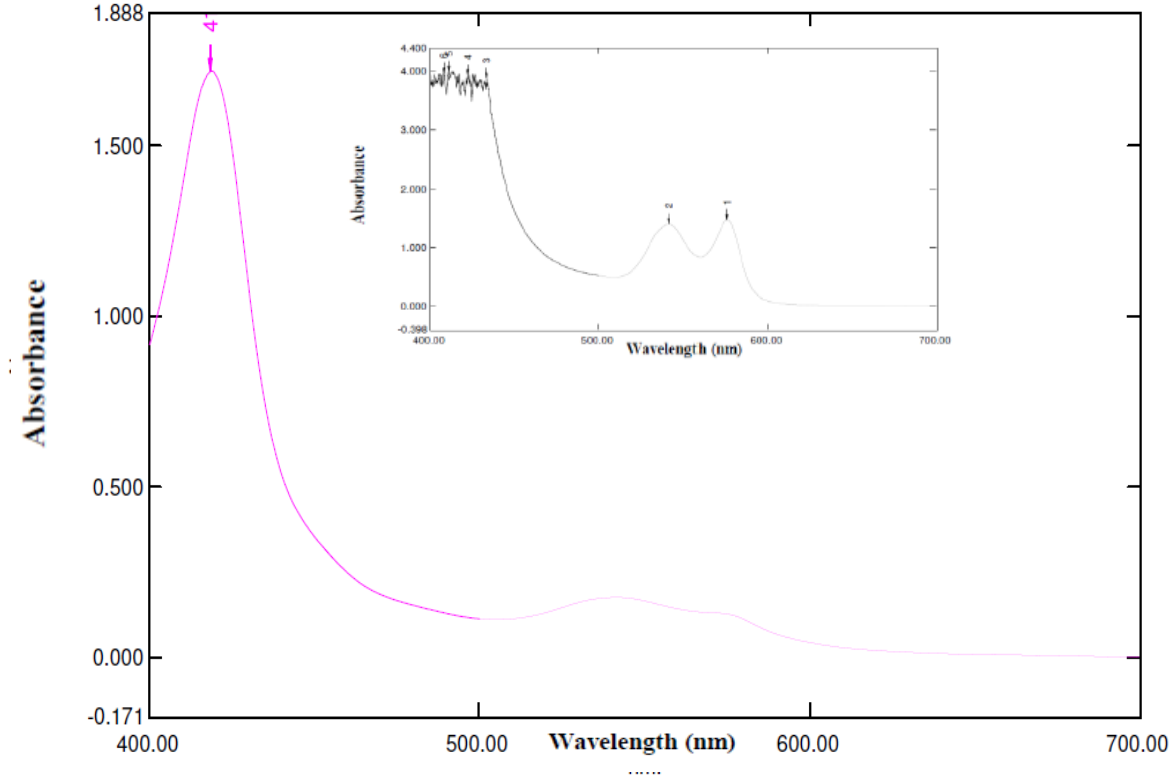

Figure-7: Spectral changes after reacting 3000µl of oxyhaemoglobin (89mM) in 10mM phosphate buffer, pH 7.6 with 1000µl of 1.5M KCN for 24 hour at 25˚C compared to unreacted oxyhaemoglobin.

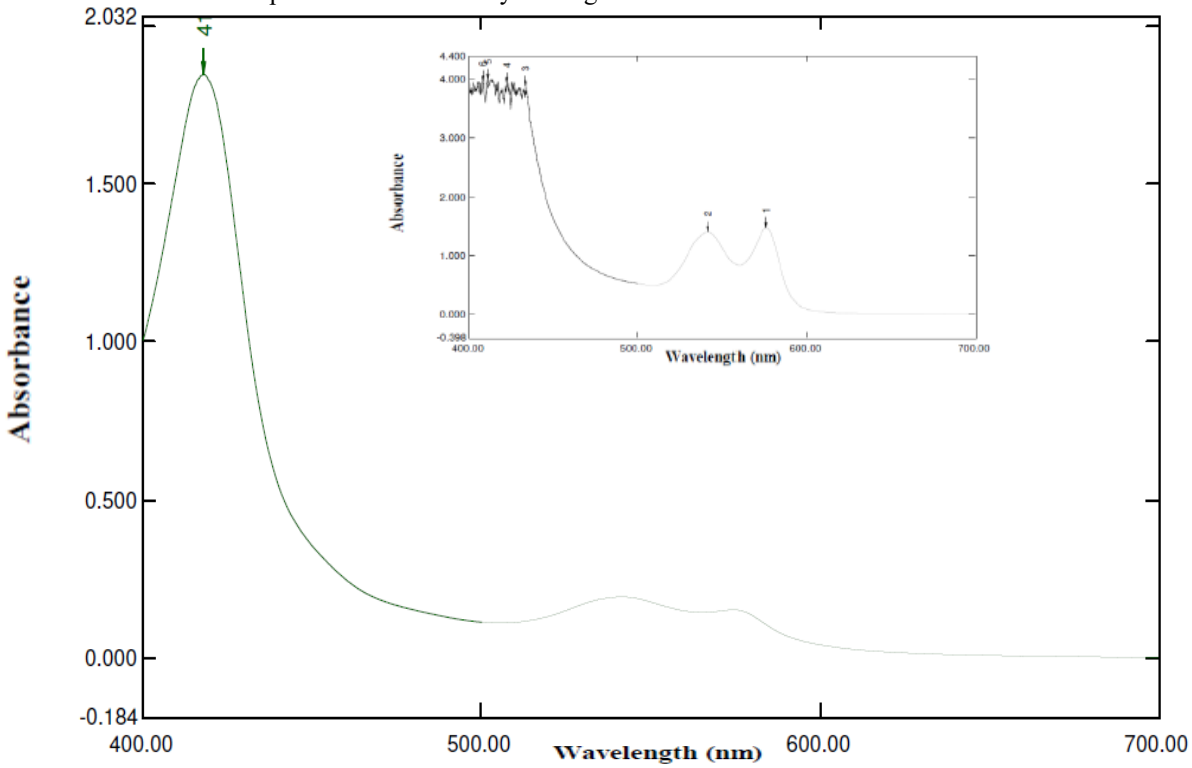

Figure-8: Spectral changes after reacting 4000µl of oxyhaemoglobin (89mM) in 10mM phosphate buffer, pH 7.6 with 1000µl of 1.5M KCN for 24 hours at 25˚C compared to unreacted oxyhaemoglobin.

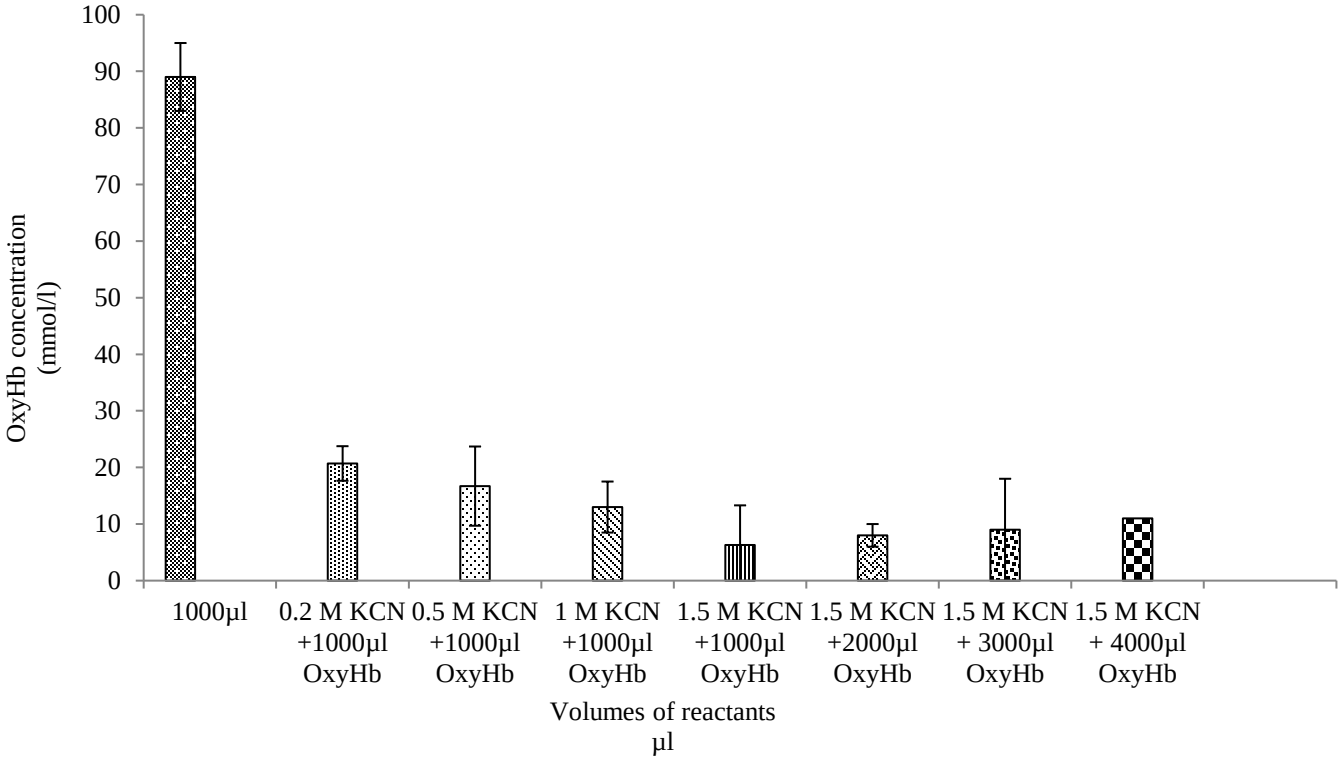

Figure-9: Effect of different molar concentrations of potassium cyanide and constant molar concentration of potassium cyanide with increasing volumes of oxyhaemoglobin on oxyhemoglobin concentration.

Discussion: The different molar concentrations of potassium cyanide caused concentration-dependent oxidation both when oxyhaemoglobin concentrations were kept constant and the potassium cyanide concentrations varied and when the concentration of potassium cyanide was kept constant and the oxyhaemoglobin concentrations varied. The reduction in the magnitude of the absorbance maxima characteristic of oxyhaemoglobin, signifies reduction in oxyhaemoglobin concentration and gradual oxidation of haemoglobin[28,29]. It has been shown that the degree of the reduction in the magnitude of the absorbance maxima characteristic of oxyhaemoglobin is directly proportional to the level of reduction in oxyhemoglobin concentration and oxidation of oxyhaemoglobin[28,29].

This finding suggests that the toxicity of potassium cyanide on human haemoglobin may be concentration-dependent. This implies that in the case of low exposure to potassium cyanide, the oxidative effect on oxyhaemoglobin might be minimal. However, in the case of high exposure to potassium cyanide, the oxidative effect on oxyhaemoglobin tends to be very high and fatal. Furthermore, the concentration-dependent decrease on the oxidation of oxyhaemoglobin when the oxyhaemoglobin volume were gradually increased suggests that oxidation of oxyhaemoglobin by direct or indirect exposure to potassium cyanide tends to be less in the presence of increasing level of haemoglobin in the blood. This simply means that the toxicity of potassium cyanide on oxyhaemoglobin of victims could differ depending on the available level of oxyhaemoglobin in the victim's blood.

The study corroborates the findings of previous researchers who also reported dose-dependent decrease in blood haemoglobin among experimental rats exposed to different concentrations of potassium cyanide. The highest decrease was seen in the high dosed rats[30,31]. Other concentration dependent effects of potassium cyanide reported by some researchers among experimental animals include; dose-dependent neuropathological changes in wistar rats treated with different doses of KCN, hyperactivity, convulsions and laboured breathing in Sprague-Dawley rats exposed to high dose of KCN, and dose-dependent histological damages in the kidney, liver and thyroid in male wistar rats orally treated with different doses of potassium cyanide[32-34].

The implication of this study suggests that the exposure of humans to high levels of cyanide from potassium cyanide through its various uses ranging from the industrial, pharmaceutical, agronomical, medical, metallurgical and mining could lead to severe oxidation of their haemoglobin and drastic reduction in the oxyhaemoglobin concentration and consequently might lead to other pathophysiological conditions that might lead to eventual death of the victims in severe cases[2-4,35-37].

This is consistent with the findings of Banerjee et al.[21] who reported that when thirty-five non-exposed workers who had worked outside the manufacturing building of an Indian electroplating process of a cable industry for more than 5 consecutive years were matched with the exposed workers. There was a significant decrease in blood haemoglobin, serum T4 and T3 concentrations of the exposed workers compared to the non-exposed workers.

The mechanism for the toxicity of potassium cyanide on human haemoglobin has been associated with the oxidation of iron (11) to iron (111)[38]. In this mechanism an electron is abstracted from the heme iron and donated to the bound dioxygen with the subsequent displacement of dioxygen as superoxide, this superoxide subsequently undergo other chemical reactions to produce other free radicals[39]. Consequently, these radicals cause degenerative changes in the erythrocytes, ranging from membrane alteration to Heinze body formation and hemolysis that are frequently observed in diseases of the haemoglobin[38].

Seeing the toxicological effects of potassium cyanide on human haemoglobin, measures should be put in place about its usage and the usage of other cyanide salts so as to forestall the inimical effects it may have on the populace who are occupationally or medically exposed to it.

Conclusion

In this study, it was found that exposure to potassium cyanide could cause oxidation and reduction of oxyhaemoglobin concentration. However, the oxidative effects and reduction in oxyhaemoglobin concentration are concentration dependent.

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References

1. Greim, H. (2003). Hydrogen cyanide, potassium cyanide and sodium cyanide. In: *Occupational toxicants. Critical data evaluation for MAK values and classification of carcinogens*. Weinheim, Germany: *Wiley VCH*, 19, 189-210

2. Korte, F., Spiteller, M., and Coulston, F. (2000). The cyanide leaching gold recovery process is a non sustainable technology with unacceptable impacts on ecosystems and humans: the disaster in Romania. *Ecotoxicol. Environ*., 46, 241–245.

3. Korte, F. and Coulston, F. (1998). Some considerations on the impact on ecological chemical principles in practice with emphasis on gold mining and cyanide. *Ecotoxicol. Environ. Saf*., 41, 119–129.

4. Gail, E., Gos, S., Kulzer, R., Lorösach, J., Rubo, A. and Sauer, M. (2000). Cyano compounds, inorganic. In Ullmann's Encyclopedia of Industrial Chemistry, 2000 electronic release, Weinheim, Germany: VCH-Verlag, pp. 159-189. Accessed 8th March 2020 [http://jwsedck.interscience.wiley.com:8087/]

5. IPCS (2002). Potassium cyanide. Geneva, World Health Organization, International Programme on Chemical Safety.

6. Siller, H. and Winter, J. (1998). Degradation of cyanide in agro industrial or industrial wastewater in an acidification reactor or in a single step methane reactor by bacteria enriched from soil and peels of cassava. *Appl. Microbiol. Biot.,* 50**,** 384–389.

7. Bödigheimer, K., Nowak, F. and Schoenborn, W. (1979). Pharmakokinetik und thyreotoxizität des nitroprussid-Natrium-Metaboliten Thiocyanat. *Deutsche Medizinische Wochenschrift*, 104, 939–943.

8. Carotti, A. A. and Kaiser, E. R. (1972). Concentrations of twenty gaseous chemical species in the flue gas of a municipal incinerator. *J. Air Pollut. Control Assoc.*, 22, 224–253.

9. Fiksel, J., Cooper, C., Eschenroeder, A., Goyer, M. and Perwak, J. (1981). Exposure and risk assessment for cyanide. Washington, DC, US Environmental Protection Agency.

10. ATSDR (1997). Toxicological profile for cyanide. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

11. Eisler, R., Clarke, D. R (Jr.), Wiemeyer, S. N. and Henry, C. J. (1999). Sodium cyanide hazards to fish and other wildlife from gold mining operations. In: Azcue, J. M. (Ed). *Environmental impacts of miningactivities*. Berlin, Springer, pp. 55–67.

12. Mudder, T. I. and Botz, M. (2000). A global perspective of cyanide. A background paper of the UNEP/ICME Industry Codes of Practice Workshop: Cyanide Management Paris, 26–27. Available at http://www.mineralresourcesforum.org/.

13. Ballantyne, B. (1987). Toxicology of cyanides. In: B. Ballantyne, and T. C. Marrs, (Eds.), Clinical and experimental toxicology of cyanides. Wright, Bristol: IOP Publishing, pp. 41–126.

14. Henny, C. J., Hallock, K., and Hill, E. F. (1994). Cyanide and migratory birds at gold mines in Nevada, USA. *Ecotoxicol.*, 3, 45–58.

15. Grosse, D. W. (1986). Treatment technologies for hazardous wastes. Part IV. A review of alternative treatment processes for metal-bearing hazardous waste streams. *J. Air Pollut. Control Assoc.*, 36, 603–614.

16. Clark, D.R. (Jr.) and Hothem, R. L. (1991). Mammal mortality at Arizona, California, and Nevada gold mines using cyanide extraction. *Calif. Fish Game*, 77, 61–69.

17. Ma, J. and Pritsos, C.A. (1997). Tissue specific bioenergetic effects and increased enzymatic activities following acute sublethalperoral exposure to cyanide in the mallard duck. *Toxicol. Appl. Pharmacol.*, 142, 297–302.

18. Haddad, L. M. and Winchester, J. F. (1990). Clinical Management of Poisoning and Drug Overdose. 2nd Ed., W.B. Saunders Company, pp. 11103-1111

19. Okafor, P. N., Okoronkwo, C. O and Maduagwu, E. N. (2002). Occupational and dietary exposures of humans to cyanide poisoning from large-scale cassava processing and ingestion of cassava foods. *Food Chem. Toxicol.*, 49, 1001–1005.

20. Chandra, H., Gupta, B. N. and Mathur, N. (1988). Threshold limit value of cyanide: a reappraisal in Indian context. *Indian J. Environ. Prot.*, 8, 170–174.

21. Banerjee, K. K., Bishayee, B. and Marimuthu, P. (1997). Evaluation of cyanide exposure and its effect on thyroid function of workers in a cable industry. *J. Occup. Environ. Med.*, 39, 255–260.

22. Feher, J.J. (2012). Quantitative Human Physiology; Membrane transport and metabolism. Academic Press, United States. Isac-Garcia, J., Dobado, J. A., Calva-Flores, F. G. and Martinez-Garcia, H. (2016). Experimental organic chemistry laboratory manual (Academic press, New York).

23. Awad, S., Allison, S. P. and Labo, D. N. (2008). The history of 0.9% saline. *Clin. Nutr.*, 27, 179-188.

24. Hills, A. G. (1973). pH and the Henderson-Hasselbalch equation. *The Am. J. Med.*, 55, 131–133.

25. Meng, F. and Alayash, A. I. (2017). Determination of extinction coefficients of human hemoglobin in various redox states. *Anal. Biochem.*, 521, 11–19.

26. Robert, W. R., Mauri, D. and Lisa, P. N. (1994). Discovering the Beer-Lambert Law. *J. Chem. Educ.*, 71, 983.

27. Ibrahim. M. A., EL-Gohary, M. I., Saleh, N. A. and Elashry, M.Y. (2008). Spectroscopic study on oxidation reactions of normal and pathogenic haemoglobin molecules. *Rom. J. Biophys.*, 18, 39-47.

28. Horecker, B. L. (1942). The absorption spectra of haemoglobin and its derivatives in the visible and near infra-red regions. *J. Biol. Chem.*, 148, 173-183.

29. Monsanto Co. (1985). Male fertility study of Sprague-Dawley rats exposed by the inhalation route to acetone cyanohydrins St. Louis, MO, Monsanto Co., Report ML-82-144; US EPA/OPTSPublic Files No. 878216404.

30. Olusi, S. O, Oke, O.L. and Odusote, A. (1979). Effects of cyanogenic agents on reproduction and neonatal development in rats. *Biol. Neonate*, 36, 233–234.

31. Sousa, A. B., Soto-Blanco, B., Guerra, J. L., Kimura, E. T., and Gorniak, S. L. (2002). Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity?. *Toxicology*, 174, 87–95.

32. Soto-Blanco, B., Maiorka, P. C. and Gorniak, S. L. (2002). Neuropathologic study of long term cyanide administration to goats. *Food Chem. Toxicol*, 40, 1693–1698.

33. Gerhart, J.M. (1987). Ninety-day oral toxicity study of potassium silver cyanide [$KAg(CN)_2$] in Sprague-Dawley rats. Prepared for the Dynamac Corporation, Rockville, MD, by IIT Research Institute, Chicago, IL., IITRI Project No. L06183, Study No. 4.

34. Chen, Q., Vasquez, E. J., Moghddas, S., Hoppel, C. L., and Lesnefsky, E. J. (2003). Production of reactive oxygen species by mitochondria. *J. Biol. Chem.*, 278, 36027-36031.

35. Jones, D. C., Gunasekar, P. G., Borowitz, J. L. and Isom, G. E. (2000). Dopamine-induced apoptosis is mediated by oxidative stress and is enhanced by cyanide in differentiated PC12 cells. *J. Neurochem*, 74, 2296-2304.

36. Leavesley, H. B., Li, L., Prabhakaran, K., Borowitz, J. L. and Isom, G. E. (2008). Interaction of cyanide and nitric oxide with Cytochrome c oxidase: Implications for acute cyanide toxicity. *Toxicol. Sci.*, 101, 101-111.

37. Winterbourn, C.C. (1985). Free-radical production and oxidative reactions of haemoglobin. *Environ Health Persp.*, 64**,** 321-330.

38. Faivre, B., Menu, P., Labrude, P. and Vigneron, C. (1998). Hemoglobin autooxidation / oxidation mechanisms and methemoglobin prevention or reduction processes in the bloodstream. *Artif. Cell Blood Sub.*, 26, 17-26