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A Liquid-State Glucose Sensor for Hg and Pb: U V Spectrophotometric Study

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Abstract: ***The investigation of the sugar-metal ion interactions remains one of the main objectives of carbohydrate coordination chemistry because the interactions between metal ions and carbohydrates are involved in many biochemical processes. This study was focused on determining the effect of mercury and lead exposure from various sources. Inorganic mercury and may enter food products during the various manufacturing processes. The main objective of this study is to get a preliminary idea about the effect of poisonous metal ions (lead and mercury) on glycolysis using direct UV detection without using any additional chemical reagent.***

Keywords: sugar-metal ion interactions, carbohydrate coordination chemistry, mercury exposure, lead exposure, glycolysis

1. Introduction

Lead and Mercury are very common pollutants in water which have dangerous potential causing serious disease and health problems to human.[1] Mercury (Hg) is a global pollutant that affects human and ecosystem health. Mercury is a naturally occurring element, but has been directly mobilized by humans for thousands of years into aquatic and terrestrial ecosystems through mining, the use of Hg in precious metal extraction, its presence as a trace contaminant in many materials (e. g., coal, metal ores), and its use in products (e.g., paint, electronic devices) and by industry (chlor-alkali plants; as a catalyst).[2] The atmosphere is the foremost transport pathway of Hg emissions, whereas land and ocean processes play an important role in the redistribution of Hg in terrestrial, freshwater, and marine ecosystems and the production of CH_3Hg that drives the major human exposure route, consumption of fish, particularly marine fish. The temporal and spatial scales of Hg transport in the atmosphere and its transfer to aquatic and terrestrial ecosystems depend primarily on its chemical and physical forms.

It has several sources both natural and manmade. Its presence in water is mainly due to industrialization. Chlor-alkali plants are the real threat to water bodies due to the possible exchange of Hg containing contaminants with fresh water. Mining activities are the yet another major source of inorganic Hg. there are also several other sources in the form of fungicides and preservatives. The source chart is ever expanding in Hg thermometer, batteries, dental amalgam fluorescent light bulb, Hg contaminated foods and other measuring equipments. The environmental burden of Hg is mainly because of its non-biodegradability and bio accumulation over the food web. Well there are whether organic or inorganic Hg has a lot bad impacts on health especially on the central nervous system.

Types of Mercury		
Elemental	Mercury vapor (Hg°) Stable monoatomic Gas	Dental amalgams
Inorganic	Divalent mercury ($Hg2^{+}$)	Toxic species in human tissue after conversion
Organic	Methyl mercury (CH_3Hg^{+}) Ethyl mercury ($CH_3CH_3Hg^{+}$)	Fish, sea mammals, Thimerosal vaccines

Lead is toxic and prolongs exposure to lead ions will caused serious brain and nervous system damage. According to WHO, the concentration was limited to 0.01 mg/L for lead and 0.001 mg/L for mercury in drinking water regulation.[3] Most of the current analytical method for lead and mercury are inductively coupled plasma-optical emission spectroscopy (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS), atomic absorption spectrometer (AAS), and graphite furnace atomic absorption spectrometer (GFAAS). However, those methods required highly trained operator and expensive equipment. Besides that, those methods are much more complicated in terms of calibration and sample preparation. Spectroscopy method without chemical reagent proves useful and it is a non-destructive method. In year 2012, Ahmad Fairuz introduce a near infrared spectroscopy analysis on aqueous sucrose, glucose, and fructose solution without using any additional chemical reagent.[4]

Mercury exposure can be determined through the analysis of a variety of tissues to include blood, urine, finger or toe nails, breast milk and hair. Many studies have measured total Hg in blood without distinguishing the forms of mercury found in the blood. This study was focused on determining the effect of mercury exposure from consumption of processed foods. Inorganic mercury may enter food products during the various manufacturing processes. For example, mercury cell chlor-alkali chemical products are used extensively in food processing and always contain inorganic mercury residues. Vegetable oil products manufactured using the common alkali refining process may present a moderate risk of mercury contamination.[5] The mercury cell chlorine used to bleach flour is expected to contain a small amount of mercury residue. The corn starch used to manufacture the corn sweeteners in the HFCS product line is treated purposely with inorganic HgCl2 as part of the manufacturing process to inhibit endogenous starch-degrading enzymes.[6] It is thus reasonable to suggest that consumers are routinely exposed to non-elemental inorganic mercury (I-Hg) when they consume heavily processed foods, including corn sweeteners. Our justification for adding the support group intervention to help students and community members eliminate corn sweeteners from their diet is based on the concept that consumption of corn

sweeteners is both a known and potential source of inorganic mercury exposure and a potential factor in the development of insulin resistance.

As a consequence of the anthropogenic activities of our industrial society, a heavy load of mercury in the biosphere led to the widespread contamination of water and soils with attendant environmental and health concerns. Mercuric compounds can accumulate through chemical and biological pathways in microorganisms, plants, animals, and finally human beings with exposure to the source and environmental release. It has been found that mercury damages organisms at low micromolar levels and at intervals as short as minutes. Inorganic mercury species undergo methylation to form methylmercury (MeHg (I)), which is the most toxic and most commonly occurring organomercuric compound, by microbiological transformation. It is generally accepted that mercury binds to the bases of DNA and the binding strength increases with increasing content of the AâT base pair. The interactions of mercury, mainly as MeHg (I), with DNA bases and other model systems have been extensively studied for its toxicity and environmental effects based on the structural evidence obtained primarily from 1H/13C and 199 Hg NMR, X-ray, and infrared spectroscopy.

This study was focused on the effect of lead and mercury on glucose, using UV VIS spectroscopy. The objective of this study is to get a preliminary idea about the effect of poisonous metal ions (lead and mercury) on glycolysis using direct UV detection without using any additional chemical reagent. To study the effect of metal ions on glucose molecule UV/ VIS absorption studies are carried out by varying metal ion concentration. Since, UV/VIS spectroscopy is a reliable method to explore the structural changes under gone by a molecule and to understand about the complex formation According to Beer-Lambert Laws, when light pass through a medium, it will experience scattering, reflection or absorption. The output light intensity will decreases depending on the concentration of the sample and path length of the light passes through. The decreases of light intensity can be calculated via equation A = log (I0/I), where A is the absorption, I0 is the input light intensity, and I is the output light intensity. Besides that, every medium, molecules, or atoms has its own unique absorption capability on different wavelength. The relationship between path length, concentration and absorption can also written in the form of A = Ɛbc, where b is the path length and c is the concentration of the sample.

2. Materials and Methods

All reagents were of analytical grade. The important bioenzyme, Glucose (Glu, 1.0816 g) was used for the interaction studies. Lead nitrate (3.312 g in water) Mercuric chloride (4 g in water) purchased from Aldrich were selected as metal ions without further purification. Glucose solution is prepared in neutral pH. Stock solutions of analyte molecules (Pb and Hg) is prepared at a concentration of 1×10^{-1} M and desired concentrations will be achieved by successive dilution

2.1 Lead Nitrate

Figure 2.1: lead nitrate

Lead is toxic and prolongs exposure to lead ions will caused serious brain and nervous system damage. According to WHO, the concentration was limited to 0.01 mg/L for lead in drinking water regulation

2.2 Mercuric chloride

Figure 2.2: Mercuric chloride

Mercury (Hg) is a global pollutant that affects human and ecosystem health. Mercury is a naturally occurring element, but has been directly mobilized by humans for thousands of years into aquatic and terrestrial ecosystems through mining, the use of Hg in precious metal extraction, its presence as a trace contaminant in many materials (e. g., coal, metal ores), and its use in products (e. g., paint, electronic devices) and by industry (chlor-alkali plants; as a catalyst)

2.3 Apparatus and characterization

UV/Vis absorption studies were carried out using a UV-1800 Shi-madzu spectrophotometer equipped with a 1-cm quartz cell at a wavelength range of 200-900 nm. The bioenzyme Glucose (1×10^{-1} M) in an aqueous solution was used for steady-state absorption measurements. Lead nitrate and Mercuric chloride stock solutions were prepared in water at a concentration of 1×10^{-1} M and the desired concentrations were prepared by successive dilution. For every sample measured, the cuvette will washed with distilled water to prevent any left over from previous sample. Both the spectrometer and deuterium lamp are warm up for at least 30 minutes before starting the measurement. Steady state fluorescence measurements were carried out using LS 55 spectrofluorometer at an excitation wavelength 290/295 nm with emission spectral data in the range 300-550 nm (figure 2.11). Fluorescence excitation spectrum was recorded in the wavelength range 200-350 nm at an emission wavelength 340 nm. For solution state fluorescence measurements OVA (0.1 mg/ml) in an aqueous solution of citrate buffer (pH 4.5) was used.

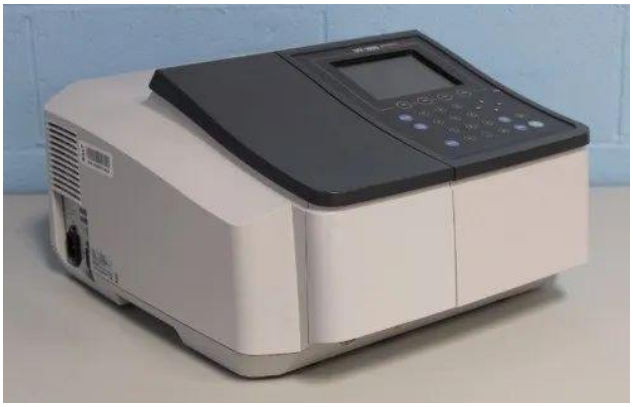

Figure 2.3: UV-1800 Shimadzu spectrophotometer

Figure 2.4: Perkin Elmer LS-55 Spectrofluorometer

For immobilised state fluorescence studies membranes were cut and placed (diagonal dimension) in a 1 cm quartz cuvette (sigma) filled with millipore water to which varying concentrations of quenchers (H_2O_2, and CYT C) were injected with the help of a micro syringe to get a net concentration ranging from subpico and micro molar to level. The experimental setup is shown in figure 2.12. The synchronous fluorescence spectra were also measured at an excitation wavelength of 290 nm keeping a constant wavelength difference of $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm for tyrosine and tryptophan residues respectively.

3. Results and discussion

UV-vis spectroscopy is a reliable method to explore the structural changes under gone by an enzyme molecule and to understand about the complex formation.[7, 8, 9] The UV-vis absorption spectra show the effect of lead nitrate and mercuric chloride on the Glucose absorption spectrum. Glucose possesses an absorption maximum at 270. The concentration of Pb and Hg was varied by successive dilution. In all the studied conditions we observed a decrement at 270 nm on interaction with lead nitrate and mercuric chloride. Figure 1 represents the UV/ VIS absorbance spectra of Glucose (1.8016 g) as a function of various concentration of lead nitrate and mercuric chloride

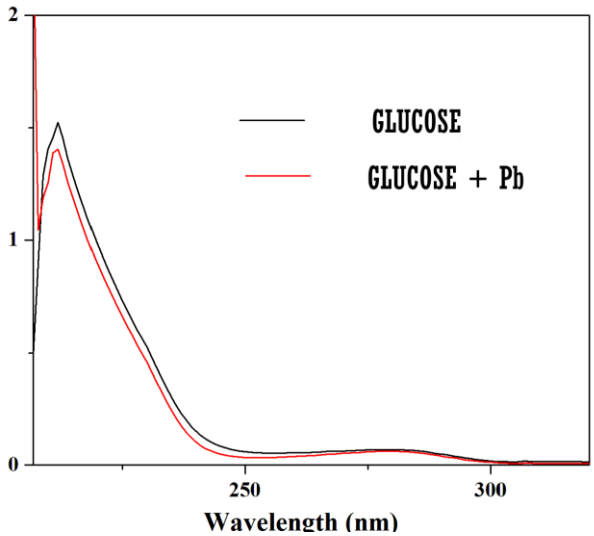

Figure 1: UV-Absorption spectrum of glucose-lead system at pH 7

It is clear from the UV/ VIS absorbance spectra that the peak at 270 nm was decreased and the absorption spectral maximum shows a slight blue shift (from 270 to 265 nm). Which means the microenvironment of the bioenzyme glucose was changed on interaction lead nitrate and mercuric chloride, and assumed to be resulted in the formation of a metal ion bioenzyme complex.[10, 11] Glucose exhibits a

concentration-dependentchange in absorption intensity on varying quencher concentration, due to the change in its micro environment. This again means that the hydrophobicity around glucose molecule was decreased. The obvious decrement of absorbance intensity also indicated the formation of a new complex between ovalbumin and hydrogen peroxide.

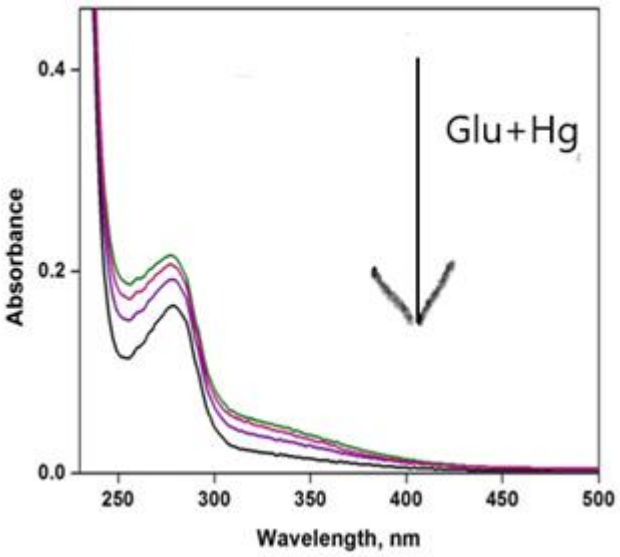

Figure 2: UV-VIS absorption spectrum ofglucose solution-mercuric chloride system at pH 7.

To further confirm the quenching mechanism the UV absorption spectra of glucose, and the mercury (II) –glucose and lead – glucose system were recorded. Typical absorbance spectral changes in the OVA spectrum in the presence of Mercury (II) as well as with lead are presented in Figure 2. The UV–Vis absorption spectrum of glucose exhibits two main absorption bands one strong peak is located at approximately 210 nm, which is mainly caused by a C=O peptide-bond-based n– π*transition related to the α-helix content of bioenzyme, and the other weak peak is at 270 nm.[12] Addition of Hg (II) led to the decrease in absorbance of glucose at 270 and 210 nm. Only a process occurring in ground state can perturb the absorption spectrum. This result suggests the formation of a complex at the ground state (static quenching) between Hg (II) and glucose. Similar results were observed for lead – glucose system. Fluorescence quenching studes carried out at different pH conditions also supported the above data (figure 3).

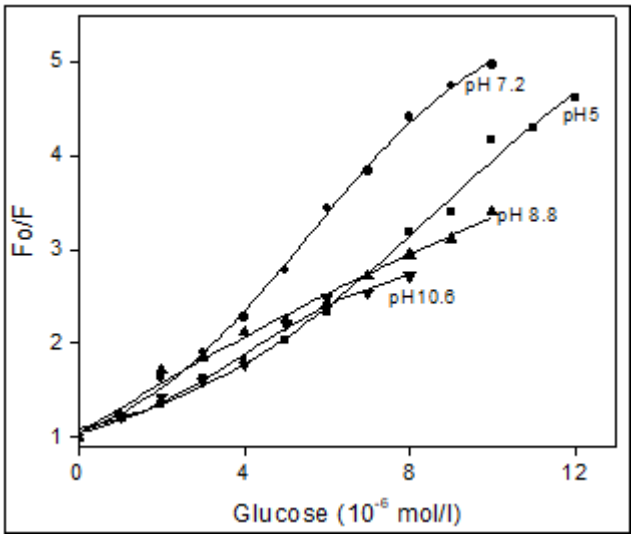

Figure 3: Fluorescence quenching interaction of lead with glucose at four different pHs.

4. Conclusion

The above mentioned data undoubtedly indicate the impact of mercury and lead (toxic metal mions) on glycolysis. This type of action allows for a significant influence of mercury compounds on pathogenesis. Moreover, single studies indicating the association between mercury and lead with glucose results in obesity. However, there is a lack of data indicating the interrelation between mercury exposure and the mechanisms of obesity pathogenesis. Despite this fact, the demonstrative base of the lead and mercury's role in the glycolysis pathway seems to be valuable. Additional investigations of the possible effect of organism's mercuric and lead content modulation on pathogenesis should be undertaken.

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