
Effect of Oscillating Magnetic Field on Bovine Haemoglobin Structure and its Stability

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ABSTRACT

The increasing prevalence of electronic devices has raised concerns about the proliferation of magnetic fields, prompting extensive research into their potential impact on living organisms. This study investigates how sub-acute oscillating magnetic fields (OSMF) affect the structure and stability of bovine Haemoglobin (Hb) in the presence of a surfactant and varying pH levels. Haemoglobin, a crucial protein for oxygen transport, undergoes conformational changes in response to external stimuli, including magnetic fields. To explore this, Bovine Haemoglobin solutions were prepared at different pH levels, incorporating the surfactant Sodium Dodecyl Sulfate (SDS). An oscillating magnetic field was generated using a solenoid for exposure. The study measured variations in conductivity and pH before and after OSMF exposure, and SDS denaturation curves were obtained through UV spectrometry. The results revealed shifts in Haemoglobin's pH and conductivity after exposure to OSMF, indicating conformational changes. The presence of SDS affected the solution's colour, suggesting interactions between the surfactant and Haemoglobin. Haemoglobin conductivity was higher before OSMF exposure than after, suggesting potential ionization alterations. Changes in Haemoglobin conductivity, a paramagnetic substance, imply the exposure of amino acid residues under OSMF influence. The study highlights OSMF's potential to induce conformational changes, particularly noting a dimeric conformer at pH 5.9. These findings enhance our understanding of biological responses to magnetic fields, underscoring the significance of pH in mediating these effects. In conclusion, this study elucidates the impact of OSMF on Haemoglobin structure, emphasizing the necessity to consider pH conditions. The observed conformational changes hold implications for understanding biological responses to magnetic fields, with potential applications in medical therapies. Further research is essential to explore long-term effects and translate these findings to human Haemoglobin.

Keywords: Oscillating Magnetic Field, Haemoglobin, Surfactant, pH, Conformational Changes, Conductivity

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I. INTRODUCTION

The proliferation of electronic devices in homes and industries has led to an increased generation of magnetic fields, necessitating extensive research to comprehend their potential impact on living organisms (Atef et al., 1985). Recognizing the capacity of biological systems to detect and respond to various stimuli, including mechanical, electric, or magnetic forces, has underscored the significance of understanding the effects of electromagnetic fields (Schuita, 1986). Over the last few decades, it has been well-established that weak electromagnetic fields across a broad spectrum of frequencies and intensities can exert diverse biological effects, both beneficial and detrimental (Markov, 1984). This holds particular relevance in modern technical applications and medical therapies, where low and extremely low-frequency (ELF) fields are extensively employed to treat a wide range of diseases. Among the biological systems influenced by these fields, Haemoglobin (Hb) plays a crucial role (Markov, 1984). Found in the red blood cells of all vertebrates (excluding fish), Haemoglobin is an iron-containing metalloproteinase responsible for transporting oxygen (Edwin, 1972). It facilitates aerobic respiration, energizes organismal functions in the metabolic process, and aids in the transportation of respiratory carbon dioxide. Haemoglobin significantly augments the total blood oxygen capacity, demonstrating a seventy-fold increase compared to dissolved oxygen in blood (Markov et al., 1975). Structurally, Haemoglobin exhibits a quaternary configuration characteristic of multi-subunit globular proteins, with its four subunits arranged in a tetrahedral fashion (Fabiana et al., 2019).

Previous investigations into the direct effects of magnetic fields, particularly at high strengths (3500 and 4000 G), have revealed distinct conformations of Haemoglobin (Toledo et al., 2008). These alterations were accompanied by changes in intermolecular interactions, as indicated by variations in the slope of $\eta_{sp}/C = F(C)$ lines and Huggins' constant K' . Notably, there were no observable changes in the intrinsic viscosity $[\eta]$ of Haemoglobin, indicating a lack of modifications in the dimensions and shape of the Haemoglobin molecule.

The Blood Oxygen Level Dependent susceptibility effect (BOLD) is a technique which uses the magnetic properties of blood but note that they are not ferromagnetic properties. If blood was ferromagnetic, then people would bleed to death or explode in MRI scanners which produce much stronger magnetic forces. Oxygenated Haemoglobin is diamagnetic, and it is slightly repelled by a magnet (Toledo et al., 2008), whereas deoxygenated Haemoglobin is paramagnetic, it is very slightly attracted to a magnet. MRI uses a very strong magnetic field so this difference in the magnetic properties of oxygenated and deoxygenated Haemoglobin in blood can be detected.

The main purpose of this study is to examine the effect of the combination of a surfactants and sub-acute oscillating magnetic fields on the Haemoglobin as a function of pH. This effect can be confirmed if there are conformational changes in the structure of the Haemoglobin.

2. MATERIAL AND METHOD

2.1 Preparation of Haemoglobin solution

In this study, bovine Haemoglobin procured from the Sigma Company was utilized. A precise amount of dry Bovine Haemoglobin (4.03125g) was carefully introduced into a 100ml standard flask containing a small volume of distilled water. The flask was gently swirled to facilitate the gentle dissolution of Bovine Haemoglobin without generating foam (Kobe et al., 2002). Subsequently, distilled water was added to the flask up to the 100ml mark, resulting in the creation of five distinct Haemoglobin solutions with pH values of 7.34, 7.41, 7.55, 7.58, and 7.63.

For the surfactant component, Sodium Dodecyl Sulfate (SDS) was employed. An exact quantity of SDS (2.8838g) was carefully weighed and added to a 100ml standard flask with a small volume of water. The flask content was gently swirled to ensure the dissolution of SDS without foaming. The buffer solution was concocted by blending NaH_2PO_4 solution, serving as the stock solution, with NaOH alkali solution containing a measured quantity of NaCl salt (Madsen, 2007). The NaH_2PO_4 solution was prepared by dissolving 15.60g of NaH_2PO_4 in 250ml of distilled water. Additionally, a 0.4M alkali solution of NaOH was formulated by dissolving 4g of NaOH pellets in 250 ml of distilled water. From these preparations, five distinct pH buffer solutions were created and are tabulated as follows:

Table 1. Chemical Used in Volume (ml) and Weight (g)

pH	Volume of Alkali Solution (NaOH)	Weight of Salt (g)	NaH_2PO_4 Solution (ml)	Volume of Buffer Solution (ml)
6.5	6.30	2.04	25	1000
6.8	11.8	1.65	25	1000
7.4	19.7	1.42	25	1000
7.8	23.3	1.24	25	1000

This comprehensive methodology ensured the preparation of Haemoglobin solutions, surfactant, and buffer solutions with precise pH values, laying the foundation for subsequent experimental procedures.

2.1.2 Construction of the OSMF

The study employed various materials for the construction of the Oscillating Magnetic Field (OSMF), including a solenoid, a wooden cage located in the animal house of Adekunle Ajasin University, cereal pellets, and a 12-volt step-down transformer. The solenoid, an electrical device used to generate a magnetic field, was crafted using a cylindrical wooden frame measuring 0.5m in length. This frame hosted 2950 turns of a 0.05mm diameter copper coil. The alternating current power source (AC) was linked to the solenoid via a 12-volt step-down transformer, facilitating the production of an AC current output of 5.50A. Refer to Figure 1 for a schematic diagram illustrating the block diagram of the AC power source, the step-down transformer, and the solenoid. This setup was crucial for generating the required oscillating magnetic field in the experimental setup.

2.2 Method

Ten 10ml conical flasks underwent washing and drying in the oven for 10 minutes. Subsequently, 2ml of Haemoglobin was added to the first five flasks. These flasks containing the solution were gently shaken and allowed to stand for two minutes. Following this, a buffer solution with pH values of (5.9, 6.5, 6.8, 7.4, and 7.8) was individually introduced into each of the five flasks, filling them up to the 10ml mark. Using a syringe, haemoglobin, Sodium Dodecyl Sulfate (SDS), and the buffer solution were drawn into a 1000ml flask. The solution underwent agitation for 2 hours at 25°C. After agitation, the solution was analysed using an ultraviolet spectrometer (UV). Sodium Dodecyl Sulfate (SDS) denaturation curves were established by measuring the maximum optical density of the 10ml Haemoglobin-containing solution at 405nm (Alimi et al., 2009). A search-tech model UV\V is spectrophotometer and a 1-cm cuvette, thermostated to maintain a temperature of 25°C, were employed for these measurements. All measurements were conducted post-incubation of Haemoglobin. The two sets of prepared flasks were then placed at the centre of a circular solenoid for a duration of five minutes.

3. Results and Discussion

The first set of results presented is that of the variation of the Haemoglobin pH and conductivity with concentration of the Haemoglobin before and after exposure to OSMF of 41ml gauss for 5minutes without buffer solution. This variation is shown in table 1 and expressed graphically in Fig.1.

Table 2. The variation of the Haemoglobin pH and conductivity with concentration of the Haemoglobin before and after exposure to OSMF of 41ml gauss

	Measured pH of Haemoglobin		Measured Conductivity of Haemoglobin	
Concentration of Haemoglobin	pH before exposure to OSMF	pH after exposure to OSMF	Conductivity before exposure to OSMF	Conductivity after exposure to OSMF
10	7.34	7.49	163	96
20	7.41	7.53	177	105
30	7.55	7.62	200	136
40	7.58	7.67	233	156
50	7.63	7.69	256	180

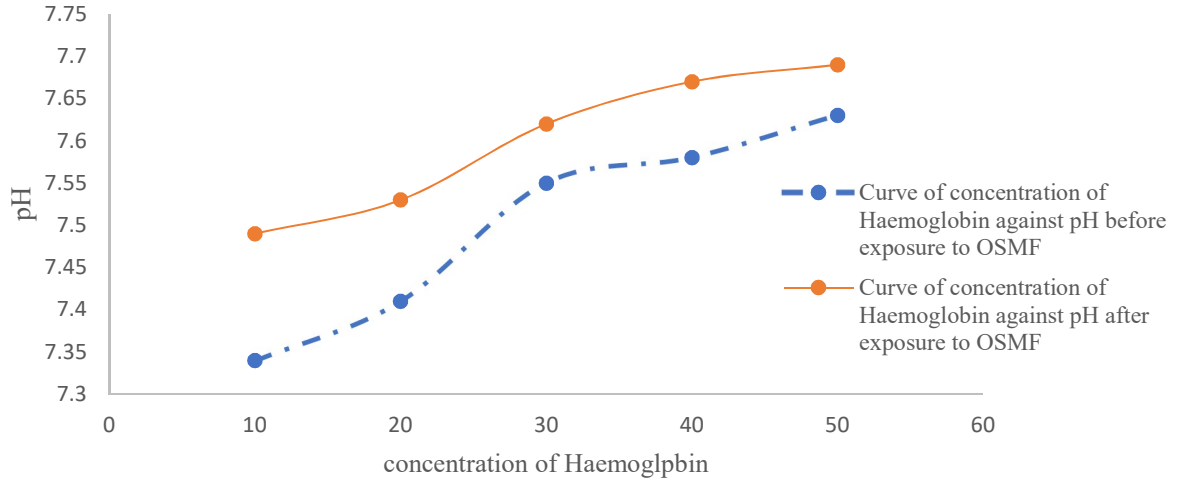


Fig 1. Curve of variation of pH against concentration of Haemoglobin

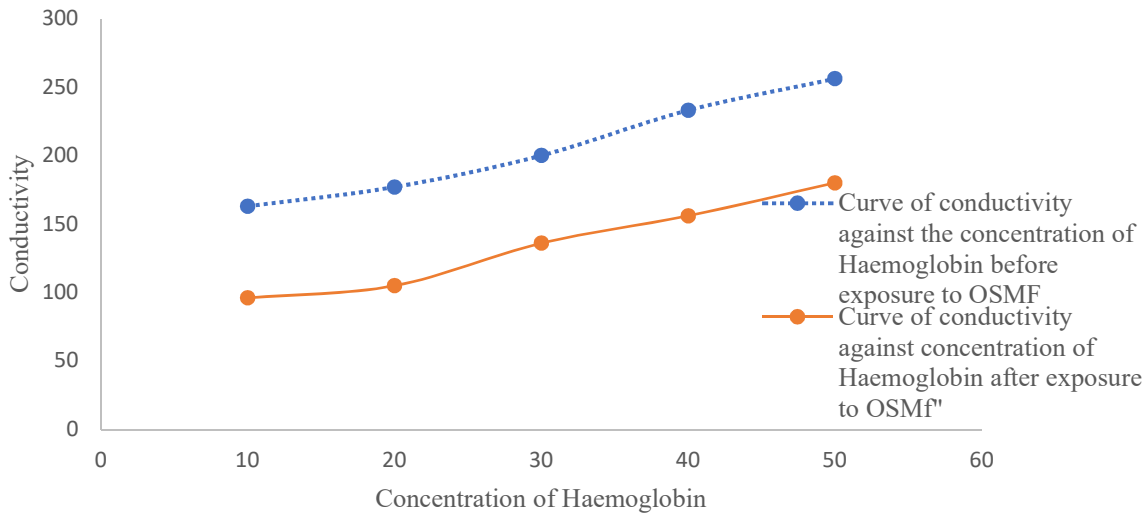


Fig 2. Curve of variation of conductivity against concentration of Haemoglobin

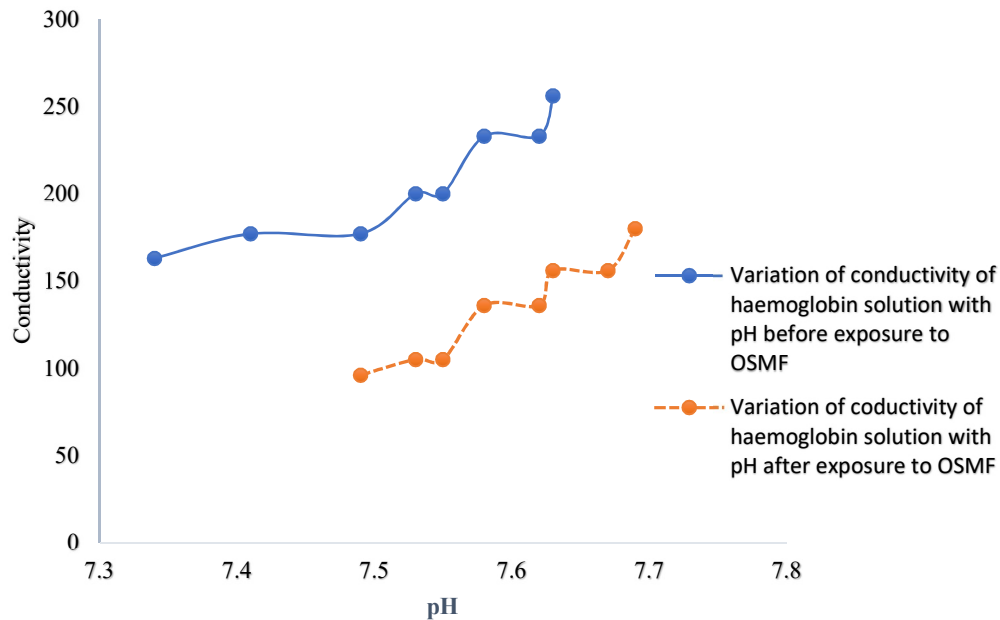


Fig. 3. Curve of variation of conductivity of haemoglobin solution against pH

Upon addition of SDS of different concentrations to the Haemoglobin changes occur in the colour of the solution (as the concentration of the SDS increases the colour of the solution becomes lighter). The obtained results centres on the parameter of conductivity, which gauges the ease of charge flow within a given material. The variability in conductivity across various materials has led to their categorization into lossless, lossy, and conductor classifications.

In the context of conductivity (Table 2 and 3), a material is deemed "lossless" when it exhibits no conductivity. Air and vacuum (space) serve as illustrative examples of such materials. Conversely, materials with a discernible level of conductivity, including non-metallic substances with moderately elevated conductivity, are termed "lossy" materials. Notable instances of lossy materials with conductivity falling within a mid-range value include saltwater and silicon. The final category encompasses materials characterized by exceedingly high conductivity, and these are referred to as conductors. This study aligns with the classification of lossy materials, specifically within the realm of paramagnetic substances (Vardanega et al., 2013). Paramagnetic substances, featuring moderate conductivity, constitute the materials of interest in this investigation. This categorization provides a contextual framework for interpreting the observed variations in conductivity, contributing to a nuanced understanding of the nature of the substances under scrutiny.

4. CONCLUSION:

This study aimed to investigate the effect of the combination of surfactants and sub-acute oscillating magnetic fields (OSMF) on bovine Haemoglobin (Hb) as a function of pH. The experiment involved preparing Haemoglobin solutions at different pH levels, introducing a surfactant (Sodium Dodecyl Sulfate - SDS), and exposing the samples to an OSMF using a solenoid and the findings allow us to draw the following conclusion:

- (a) The results indicate changes in the conductivity of Haemoglobin solutions before and after exposure to OSMF. The conductivity decreased after exposure, suggesting alterations in the ionization of amino acid residues.
- (b) The observed pH values in the presence of OSMF were higher than those in its absence. This increase in pH could be attributed to the exposure of more anionic amino acid residues compared to cationic residues.
- (c) The study suggests that OSMF induces conformational changes in Haemoglobin. The exposure to OSMF resulted in a dimeric conformer at pH 5.9, indicating that the magnetic field promotes the release of ions.
- (d) The addition of SDS to Haemoglobin led to changes in the colour of the solution, becoming lighter with increasing SDS concentration. This observation indicates interactions between Haemoglobin and the surfactant.

In conclusion, this research contributes valuable information to the growing body of knowledge on the effects of magnetic fields on biological systems, specifically on Haemoglobin structure and stability. The findings pave the way for future investigations into the broader implications for

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