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Research Article

Effect of pH on Toxicity of Zinc Sulphate Hydrate Solutions Using the Spirotox Test

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Abstract

Introduction: *Spirostomum ambiguum* is commonly used as a biological indicator due to its high sensitivity to heavy metals. It functions normally across a wide pH range of 4.5 to 8.0. Testing on protozoan organisms provides a general understanding of the impact of pH on the toxicity of zinc in pharmaceuticals. By investigating the toxicity of zinc in environment with varying pH levels, the optimal environment for zinc absorption can be determined.

Objective: The objective of this study is to assess the toxicity of zinc sulphate solutions using different forms of hydrating and at various pH levels, applying the Spirotox method.

Methods: An experiment was conducted using a solution of pharmaceutical substances, specifically zinc sulphate heptahydrate and zinc sulphate monohydrate, with a concentration of 0.01 M. The study aimed to investigate the effect of the solution on the lifespan of *S.ambiguum* within the pH range of 4.8-6.5.

Results: Arrhenius activation energy (Ea linear correlates with LD_{50} oral toxicity for rats) decreases as pH increases from 4.8 to 6.5 for $ZnSO_4{\cdot}7H_2O$ and $ZnSO_4{\cdot}H_2O$ solutions. The values decrease from 140±10 kJ/mol to 55±10 kJ/mol and from 110±2 kJ/mol to 60±10 kJ/mol, respectively.

Conclusion: It was found that the toxicity of zinc sulphate hydrate solution towards *S. ambiguum* increased as the pH value increased. Hydrating of the active pharmaceutical substance was a crucial factor, it is critically important for preparation of solutions of an active pharmaceutical substance.

Keywords: *zinc sulphate, Spirotox test, pH, activation energy, toxicity.*

INTRODUCTION

Zinc is essential for the vital activity of organisms. It is involved in synthesizing and activating various enzymes, regulating cell growth, and supporting growth and development in animals and humans, among other physiological functions [1,2]. Zinc is a crucial trace element for maintaining and developing immune cells in both the innate and adaptive immune systems. Its deficiency can lead to a decrease in the body's immune response, an increased risk of inflammation, and in some cases, even fatal outcome [3]. Zinc also regulates and stimulates the proliferation, differentiation, and secretion of mammary glands during lactation [4]. Additionally, it can be used in combination with other treatments for diabetes [5].

Zinc is an immunomodulator that has multiple effects against a wide range of viral species, particularly RNA viruses. When used in combination with antiviral drugs at low concentrations, zinc inhibits SARS-CoV-2 replication *in vitro* [6]. Mechanism of action of zinc in these processes achieved by inhibiting the elongation stage of RNA transcription. Furthermore, zinc can enhance antiviral immunity by upregulating IFN-α [7,8]. Therefore, the multifunctionality of zinc is of interest for research in the field of pharmaceutical chemistry.

Toxicity evaluation is a significant safety factor of an active pharmaceutical ingredient. This article proposes a method for analyzing the toxicity of heavy metals ions, such as Zn^{2+}, using the Spirotox test and the cell biosensor *S. ambiguum* [9]. It is highly sensitive to a wide range of chemicals, including drugs containing heavy metals ions, antimycotics, adrenoblockers, antiarrhythmics, and neuroleptics [10]. *S. ambiguum* can be stored for several weeks at temperatures ranging from 5 to 28 °C and in a pH range of 5 to 8. This method is highly effective in assessing the toxicity/pH ratio [10]. Previous studies [10,11] have established the dependence of toxicity on the pH value of the solution using the Spirotox test for antidepressants and nitrophenol. Studies have tested the influence of pH on the toxicity of heavy metal drugs on fish, vertebrates [12,13], and microalgae (*Chlorella species, Pseudokirchneriella subcapitata*) [14–16]. However, the relationship between solution pH and the toxicity of zinc ions in pharmaceutical substances with varying degrees of hydrating has not yet been tested on the model *S. ambiguum*.

The objective of this study is to investigate the impact of pH levels ranging from 5.0 to 6.5 on the toxicity of aqueous solutions of various forms of zinc sulphate using the *S. ambiguum*. Additionally, the study aims to compare the activation energies at different pH values, which is crucial in establishing quality standards for zinc-containing pharmaceuticals.

MATERIALS AND METHODS

The toxicity of a 0.01 M solution of $ZnSO_4{\cdot}H_2O$ and $ZnSO_4{\cdot}7H_2O$ was investigated using a *Spirostomum ambigua* cell biosensor (Spirotox test). The test setup comprised deionized water ([D/H] = 140 ppm, purified using Milli-Q, Merck Millipore, USA), a thermostatic 5-cell plate with a set temperature ranging from 22°C to 28°C in 2°C increments (Lauda Alpha 6, Germany), a binocular to observe infusoria behavior, and low-power lamps (10 W). The solution's pH was adjusted using dilute sulfuric acid (H_2SO_4) or potassium hydroxide (KOH) and a laboratory pH meter PB-11 (Sartorius, Germany). Toxicity tests were conducted at five pH values that correspond to normal living conditions of *S. ambiguum*. The results were processed using Origin Pro 9.1 software (OriginLab, USA) and presented as mean ± SD (n=5).

RESULTS

The interaction of *S. ambiguum* with the solution under study is determined by the rapid formation of an intermediate state, which undergoes a slow transition to a stationary state according to Arrhenius kinetics.

Cell death is defined as a transition to a state of either immobility or membrane rupture. When cellular transitions are induced by ligands, the temperature dependence of *S. ambiguum* lifetime can be linearized in Arrhenius coordinates, which follow the form of the Arrhenius equation:

$$k = A \times e^{(-E_a/RT)},$$

where: k is the rate constant of the process; A is the pre-exponential multiplier; R is the universal gas constant; T is the absolute temperature, K.

Zinc is an essential element with a therapeutic function. There are areas of both zinc deficiency and zinc surplus. It has been demonstrated that when the initial solutions of zinc sulphate monohydrate were diluted tenfold, the lifetime of *S. ambiguum* significantly increased (Fig. 1). Therefore, a solution concentration of 0.01 M was deemed most suitable for the study. Higher concentrations are required to optimize the experiment time [17].

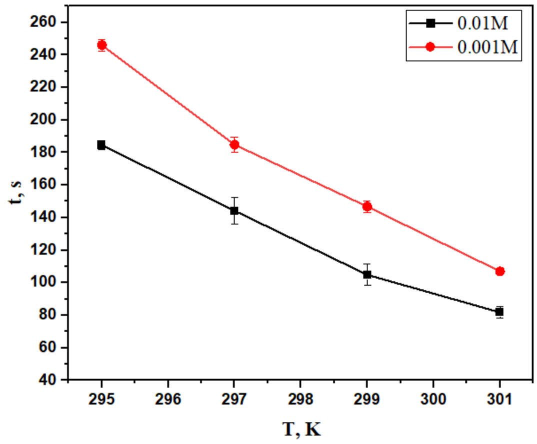

Figure 1: Dependence of *S. ambiguum* lifetime on temperature at different zinc concentrations in $ZnSO_4.H_2O$ solution (n=5)

Figure 2 (A, B) illustrates the dependence of the activation energy of ligand-induced death of *S. ambiguum* on the pH in $ZnSO_4{\cdot}7H_2O$ and $ZnSO_4{\cdot}H_2O$ solutions at a zinc concentration of 0.01 M.

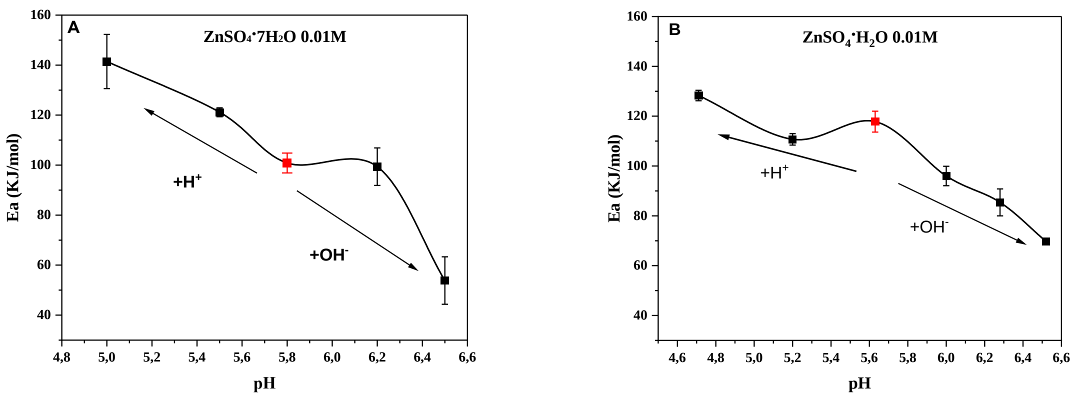

Figure 2: Dependence of activation energy of ligand-induced death of *S.ambiguum* on pH, where A - $ZnSO_4.7H_2O$, B - $ZnSO_4.H_2O$ (mean±SD, n=5)

It was demonstrated that, at the same zinc dosage, the survival time of infusoria decreased gradually as the pH increased. The observed Ea is likely dependent on the sample preparation history. Furthermore, the influence of Zn ions on the formation of ligand-dependent density inhomogeneities, also known as "bubstones", should be considered [18,19]. When the solutions had equal zinc concentrations, the solution prepared from monohydrate showed greater toxicity compared to the solution prepared from zinc sulphate heptahydrate. The pH increased from 5.0 to 6.5, the Ea gradually decreased, indicating an increase in the environment's toxicity for infusoria.

DISCUSSION

It is widely acknowledged that any element can have toxic effects when it reaches certain concentrations in the organism. The most essential elements are included in the composition of dietary supplements, and their quantitative content is not standardized, it is critical to control these preparations for toxicity. Therefore, the daily dose of zinc preparations for an adult range from 10 to 100 mg [20].

The role of pH in the metabolism of zinc sulphate hydrates is significant. The major forms of zinc in solution change from $Zn(OH)_4^{2-}$, $Zn(OH)_3^-$, $Zn(OH)_2$ and $Zn(OH)^+$ to the readily soluble ionic form Zn^{2+} as pH decreases [21]. The ratio of OH^- to Zn^{2+} concentration gradually increases as the pH of the solution increases [22]. Multiple studies have demonstrated that metal toxicity in fish and invertebrates increases as pH decreases, due to the prevalence of free metal ions [12,13]. In our experimental setup, the lifespan of *S. ambiguum* decreased as pH levels approached neutrality. The authors demonstrated an increase in metal toxicity with increasing pH in different studies [14-16]. This effect was due to a decrease in competition between metal ions and H^+ on the membrane surface. According to research [23], a decrease in the concentration of H^+ in solution is believed to be linked to the death of infusoria. This decrease causes an increase in the permeability of the *S.ambiguum* cell membrane, resulting in rapid swelling and cell rupture [23].

The toxicity of zinc sulfate hydrate solution for *S. ambiguum* is affected by pH value. An increase in pH from 4.8 to 6.5 resulted in a 2.6-fold decrease an *Ea* according to the law of Arrhenius kinetics for zinc sulfate heptahydrate, indicating an increase in toxicity. The article considers a control method using the *S. ambiguum* to manage the toxicity of pharmaceutical substances containing zinc sulfate mono- and heptahydrates.

CONCLUSION

In the present study, the biological activity for the Spirotox test was shown to be altered by increasing the pH of solutions of various hydrated forms of zinc sulphate. *S. ambiguum* is known to be stable over a wide pH range. Therefore, the toxic effect on infusoria is not caused by a change in the acidity index of environment, but by the effect of the xenobiotic on their cellular wall. The Spirotox test can be used for the control of the toxicity of zinc sulphate pharmaceutics.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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