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Impact of Zeta Potential on Copper Adsorption of Surface-Modified Hydroxyapatites Derived From Fish Bone Waste

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ABSTRACT

Zeta potential emerges as a crucial parameter in understanding particle surface charges and assessing the stability of colloidal systems. It also serves as a key indicator in determining electrostatic interactions between surfaces and ions. In this study, hydroxyapatite (HA) derived from fish waste was functionalized with histidine (HA_4) and 4-Aminohippuric acid (HA_5), and their surface properties and heavy metal ion (Cu^{2+}) adsorption capacities were investigated. Zeta potential measurements performed after surface modification showed that both modifications induced a negative charge on the surface. The surface modified with histidine exhibited a zeta potential in the range of -3.48 to -5.09 mV, while the surface modified with 4-Aminohippuric acid demonstrated a higher negative charge. Adsorption experiments revealed that HA5 exhibited a superior Cu^{2+} binding capacity of 9.96 mg/g compared to HA_4 (9.52 mg/g). The findings indicate that zeta potential and the presence of functional groups on the surface play a significant role in the retention of heavy metal ions. These results suggest that modified fish bone surfaces can serve as effective and sustainable adsorbents for environmental applications.

INTRODUCTION

Today, increasing industrial production, urbanization, and changing consumption habits result in the generation of large amounts of organic and inorganic waste. The uncontrolled release of these wastes into the environment leads to soil, water, and air pollution. In addition, it poses serious threats to ecosystem balance and human health. Therefore, it is of great importance not only to dispose of these wastes but also to utilize them as secondary raw materials in order to achieve sustainable environmental management (Özkara & Akyıl, 2019; Siddiqua et al., 2022; Hajam et al., 2023; Kurama, 2023; Mishra et al., 2023; Ghulam & Abushammala, 2023; Dehkordi et al., 2024). In recent years, within the framework of the circular economy approach, the reprocessing of waste into high value-added materials has both reduced environmental burden and provided economic benefits. In particular, numerous studies have focused on converting agricultural, animal, and industrial wastes into usable forms for applications such as biosorbents, composites, catalysts, construction materials, and biomedical uses (Wang et al., 2023; Mujtaba et al., 2023; Gherman et al., 2023). Waste materials such as fish bones, shells, and skins obtained from the fish processing industry can be converted into biomaterials like hydroxyapatite (HA) due to their high calcium phosphate content. Hydroxyapatite is a widely used material, especially in fields such as heavy metal adsorption, tissue engineering, and drug delivery systems (Duta et al., 2021; Mondal et al., 2023). Therefore, transforming wastes like fish bones into functional and usable materials through appropriate surface modifications offers environmentally friendly and economically sustainable solutions. Zeta potential is an electrokinetic parameter that describes the electric charge on the surface of a particle and its interaction with the surrounding liquid medium. This potential is a crucial indicator that directly influences the stability of particles in suspension, surface reactivity, and adsorption mechanisms (Danaei et al., 2018; Serrano-Lotina et al., 2023). Zeta potential plays a significant role, especially in the retention of metal ions and organic pollutants on solid surfaces. Adsorption capacity largely depends on the electrostatic attraction forces between the surface and the target ions or molecules. If the surface zeta potential is negative, it facilitates the approach and retention of positively charged metal ions on the surface. Conversely, if the surface is positively charged, it can support the adsorption of negatively charged ions. Therefore, the magnitude and sign of the zeta potential play a fundamental role in determining the binding tendency of ionic pollutants to the surface (Xu et al., 2003; Anielak & Grzegorczuk-Nowacka; 2011; Marzun et al., 2014). The zeta potential value can also vary depending on factors such as the nature of the functional groups on the surface, surface modifications, and the pH of the environment. Therefore, by controlled modification of the surface, adjusting the zeta potential to a desired range can enable selective and efficient adsorption of target pollutants. In this context, characterization of zeta potential is regarded as a fundamental evaluation criterion in the development of high-performance adsorbent materials (Serrano-Lotina et al., 2023; Martins et al., 2025; Khani et al., 2025). Heavy metal pollution, particularly the release of copper (Cu^{2+}) ions into the environment through industrial waste, poses a serious threat to human health and ecosystems. High concentrations of copper cause toxic effects leading to liver damage, neurological disorders, and disruption of environmental balance. The high costs and inefficiencies of traditional treatment methods necessitate the development of low-cost, environmentally friendly, and high-capacity alternative adsorbents. In this context, hydroxyapatite (HA) derived from biological sources is a promising material for heavy metal removal. Hydroxyapatite obtained from fish bones possesses a natural ion exchange capacity due to its calcium phosphate structure and provides effective adsorption, especially through the substitution of Ca^{2+} ions with other metal ions such as Cu^{2+} (Kizilkaya et al., 2010). In this study, the surface of hydroxyapatite derived from fish bones was modified with histidine (HA_4) and 4-Aminohippuric acid (HA_5), and their copper adsorption capacities and zeta potential properties were comparatively investigated. The effects of these modifications on both surface charge (zeta potential) and copper binding mechanisms were examined in detail.

MATERIAL AND METHODS

Functionalization of Bone Surfaces With Organic Acids

In this study, the surface modification of fish bone particles with Histidine (HA_4) and 4-Aminohippuric acid (HA_5) was performed according to our previous work (Tan et al., 2014; Kızılkaya et al., 2015). Briefly, 2.5 g of hydroxyapatite obtained from fish bones was placed in a 50 mL reaction vessel containing 25 mL of acetonitrile solution with 0.1 M histidine (or 4-Aminohippuric acid). An inert gas (e.g., nitrogen) was continuously purged into the reaction medium, and the system was refluxed at boiling temperature under a condenser for 8 hours. After completion of the reaction, the mixture was cooled and left to stand for 12 hours, followed by washing five times with technical water, methanol, and acetonitrile consecutively by centrifugation at 2000 rpm. The obtained modified solid phase was dried in an oven at 45°C and prepared for subsequent analyses.

Determination of Zeta Potentials

The zeta potentials of the modified products were measured using a Malvern Nano-ZS device at the Central Research Laboratory of Bilecik Şeyh Edebali University. Distilled water was used as the dispersant solvent during the measurements. The analysis was conducted under the following conditions: temperature at 25°C, Zeta Run set to 12, measurement position at 2 mm, dispersant refractive index of 1.330, viscosity of 0.8872 cP, and dispersant dielectric constant of 78.5. Approximately 1 mg of the sample was suspended in 10 mL of water, introduced into the measurement cell, and then analyzed.

Copper Removal and Adsorption

Within this scope, the copper removal performance of the obtained materials was investigated. $CuCl_2 \cdot 2H_2O$ (Merck, 1.02733.0250) was used to prepare a solution with a concentration of 50 mg/L. Each material was subjected to adsorption at an adsorbent-to-solution ratio of 1:200 for 30 hours. After adsorption, the liquid phase was filtered through a 0.45 µm syringe filter and analyzed by flame atomic absorption spectroscopy (AAS-Flame) to determine the amount of adsorbed copper, completing the experiment. For copper analysis, a Photron halogen cathode lamp (HGD0599, Australia) was used. Calibration for copper analysis was performed using a Merck multi-element standard solution IV (111355). The copper measurements were conducted at the Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, using a Shimadzu AA-6300 Atomic Absorption Spectrophotometer with flame (AAS-Flame). Acetylene and dry air were used as fuel gases during the AAS-Flame measurements.

RESULTS AND DISCUSSION

Surface modification is an effective method to enhance the chemical and electrostatic properties of adsorbent materials, thereby increasing their selectivity and binding capacity toward target pollutants (Petrovic et al., 2022). Histidine contains both an imidazole ring and amino and carboxyl groups in its structure. This configuration particularly enhances its potential to form coordinative bonds with metal ions. During modification, histidine typically binds to the surface via its carboxyl group, while the imidazole and amino groups remain free, contributing to adsorption capacity (Holeček, 2020). It generally attaches to the surface through its carboxyl group, while the amino groups can form chelate bonds with positively charged metal ions, thereby increasing the surface adsorption efficiency. Both molecules alter the charge balance on the hydroxyapatite surface, playing a decisive role in the zeta potential, and thus have the capacity to create effective adsorbent surfaces where electrostatic attraction and complexation mechanisms work synergistically. The main objective of this study is to elucidate the mechanisms underlying the changes in copper adsorption capacity following the modification of hydroxyapatite derived from fish bones with organic acids. Within this scope, surface charge characterization was performed through zeta potential measurements, adsorption experiments were conducted to determine capacities, and the performances of both modifications were compared. The obtained results are expected to contribute to the development of next-generation adsorbents for wastewater treatment and biomedical applications. Zeta potential, also known as electrokinetic potential, refers to the effective electric charge present on the surface of particles in colloidal dispersions. This value determines the repulsive or attractive forces between particles, providing insight into their dispersion and stability states. Measurement of zeta potential is an important technique for understanding, controlling, and characterizing the surface charge of colloidal and aggregate systems (Serrano-Lotina et al., 2023). Charges on the particle surface attract oppositely charged ions from the surrounding medium, forming a double-layer interface. The potential measured within the slipping plane of this structure is called the zeta potential. A high zeta potential prevents particles from aggregating, thereby increasing the stability of colloidal suspensions. Typically, systems with zeta potential values between 0 and ±5 mV tend to rapidly coagulate, ±10 to ±30 mV indicate instability, ±30 to ±40 mV moderate stability, ±40 to ±60 mV good stability, and values above ±60 mV are considered to have excellent stability (Pochapski et al., 2021; Rodriguez-Loya et al., 2023).

Figure 1 presents the zeta potential distribution spectra of HA_4 and HA_5. The zeta potential distribution obtained after modification of fish bones with histidine reveals significant changes in surface chemistry. Considering that the modification with histidine occurs via acidic groups and that histidine molecules bind to the surface through these groups, it is understood that the other amino and nitrogen groups present in histidine remain free. This structure considerably influences the chemical and functional properties of the surface. Due to the imidazole ring in histidine, it can carry both positive and neutral charges depending on the pH. However, the obtained data indicate that the surface generally acquires a negative charge. This phenomenon may arise from the acidic groups in the histidine structure or the calcium phosphate compounds naturally present on the modified bone surface. Additionally, covalent bonding or electrostatic interactions between histidine and the surface could explain this negative charge balance. However, zeta potential measurements indicate that the surface is strongly negatively charged. This suggests that the free basic groups are insufficient to alter the overall surface charge, and that naturally occurring negatively charged species such as phosphate groups in the bone matrix dominate the system. Consequently, histidine modification creates a surface structure that both maintains a negative charge balance and introduces functional groups, thereby enhancing the material interaction with biological systems and its multifunctional potential. The negative surface charge plays a supportive role in colloidal stability by increasing electrostatic repulsion forces between particles. These findings indicate that histidine-modified fish bones are promising candidates for drug delivery systems, tissue engineering, or other biomaterial applications. While the negative surface charge reduces aggregation tendency and improves stability, the biologically compatible nature of histidine may also support cellular interactions. However, since a high negative charge can differently affect protein adsorption and cellular responses, careful evaluation according to the application field is necessary. In this context, investigating surface charge behavior under varying pH and ionic strength conditions is important for better understanding the system performance in biological environments.

The zeta potential distribution measured after modification of fish bones with 4-Aminohippuric acid (HA_5) indicates significant changes in surface chemistry and colloidal stability. The negative zeta potential values likely arise from the carboxyl groups of 4-Aminohippuric acid and the phosphate groups naturally present in the bone matrix. These groups, especially under physiological or mildly basic pH conditions, become deprotonated and carry negative charges. The negative surface charge enhances electrostatic repulsion forces between particles, thereby strengthening the colloidal stability of the suspension and reducing particle aggregation. Chemically, 4-Aminohippuric acid is a molecule containing both carboxyl and amino functional groups. The charge state of these groups varies depending on the environmental pH. The carboxyl group deprotonates in basic environments to form negatively charged carboxylates, while the amino group protonates in acidic conditions, gaining a positive charge. Therefore, the zeta potential of the surface is highly sensitive to pH changes, and the charge profile of the modified surface may vary accordingly. This surface characteristic offers certain advantages and considerations for practical applications. While the negative surface charge positively influences colloidal stability, positively charged regions can enhance interactions with biological systems. Since cell membranes are negatively charged, positive surface groups may support cell adhesion. Consequently, the fish bone surface modified with 4-Aminohippuric acid acquires a complex structure carrying both negative and positive charges, significantly affecting both the physical stability and biological interaction potential of the surface. Therefore, this type of surface structure can be evaluated for versatile biomedical applications.

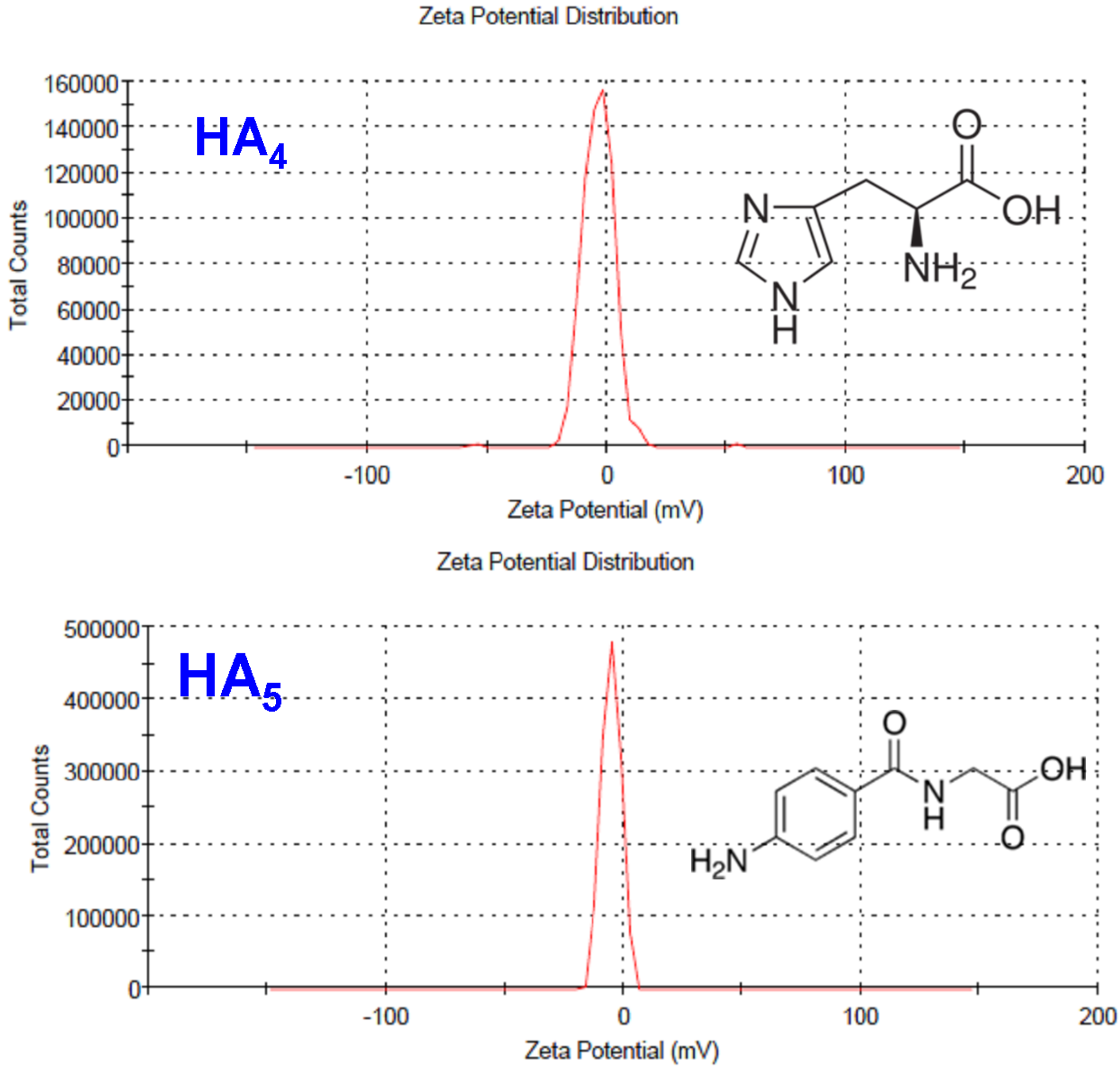

Figure 1. Spectrum of zeta potential distribution of HA_4 and HA_5

Figure 2 presents the zeta potential and surface conductivity results of HA_4 and HA_5. The zeta potential values measured after modification of the hydroxyapatite (HA) surface with histidine (HA_4) and 4-Aminohippuric acid (HA_5) reveal significant differences in surface chemistry and stability. Both modifications induced negative zeta potentials on the surface, indicating the presence of acidic groups and electrostatic repulsion between particles. In the histidine modification, zeta potential values ranged approximately from –3.48 mV to –5.09 mV. Despite histidine being an amino acid that can carry positive or neutral charges depending on pH, these low negative values indicate that the modified surface is generally negatively charged. This effect may result from the interaction of histidine carboxyl group with calcium ions in hydroxyapatite and the dominant influence of phosphate groups on the bone surface. The low negative zeta potential suggests partial neutralization of the surface charge by the modification, leading to moderate colloidal stability. Conversely, the 4-Aminohippuric acid modification generated higher negative zeta potential values on the surface. This can be explained by the presence of both carboxyl and amino groups in 4-Aminohippuric acid and the strong ionic bonding of carboxylate groups with the hydroxyapatite surface. It can be inferred that the 4-Aminohippuric acid modification imparts more negative charge groups on the surface, thereby providing higher colloidal stability compared to histidine. Overall, while histidine modification produces a low and limited negative charge, 4-Aminohippuric acid modification leads to a more pronounced increase in negative charge, contributing to a more stable colloidal system. These differences affect the potential use of the modified surfaces in biomedical applications. In particular, the high negative charge from 4-Aminohippuric acid modification can reduce particle aggregation and improve efficiency in drug delivery systems. Meanwhile, histidine modification, with its lower negative charge, may offer advantages for cell adhesion in certain biological environments. In conclusion, 4-Aminohippuric acid provides a more effective modification of the hydroxyapatite surface than histidine by increasing surface charge and enhancing colloidal stability.

In the adsorption studies, the amount of adsorption is expressed as q_e, which represents the amount of adsorbate adsorbed per gram (g) of adsorbent, calculated in milligrams per gram (mg/g). The adsorption capacity in the experimental studies was calculated using the following equation (1) (Kizilkaya & Tekinay, 2011):

$$q_e = \frac{V \times (Co - Ce)}{W} \times 1000 \qquad (1)$$

In this formula;

q_e: The amount of substance adsorbed per unit adsorbent (mg/g)

V: The volume of the solution (mL)

Co: Initial adsorbate concentration of the solution (mg/L)

Ce: The concentration of adsorbate remaining in the solution after adsorption (mg/L)

W: Adsorbent amount (g)

At the end of the adsorption study, the percentage of the adsorbed substance removed from the solution relative to the initial concentration of the solution was calculated for each material and expressed as R% (percent removal). The percent removal (R%) for each material was calculated using the following equation (2):

$$R(\%) = \frac{(Co - Ce)}{Ce} \times 100 \qquad (2)$$

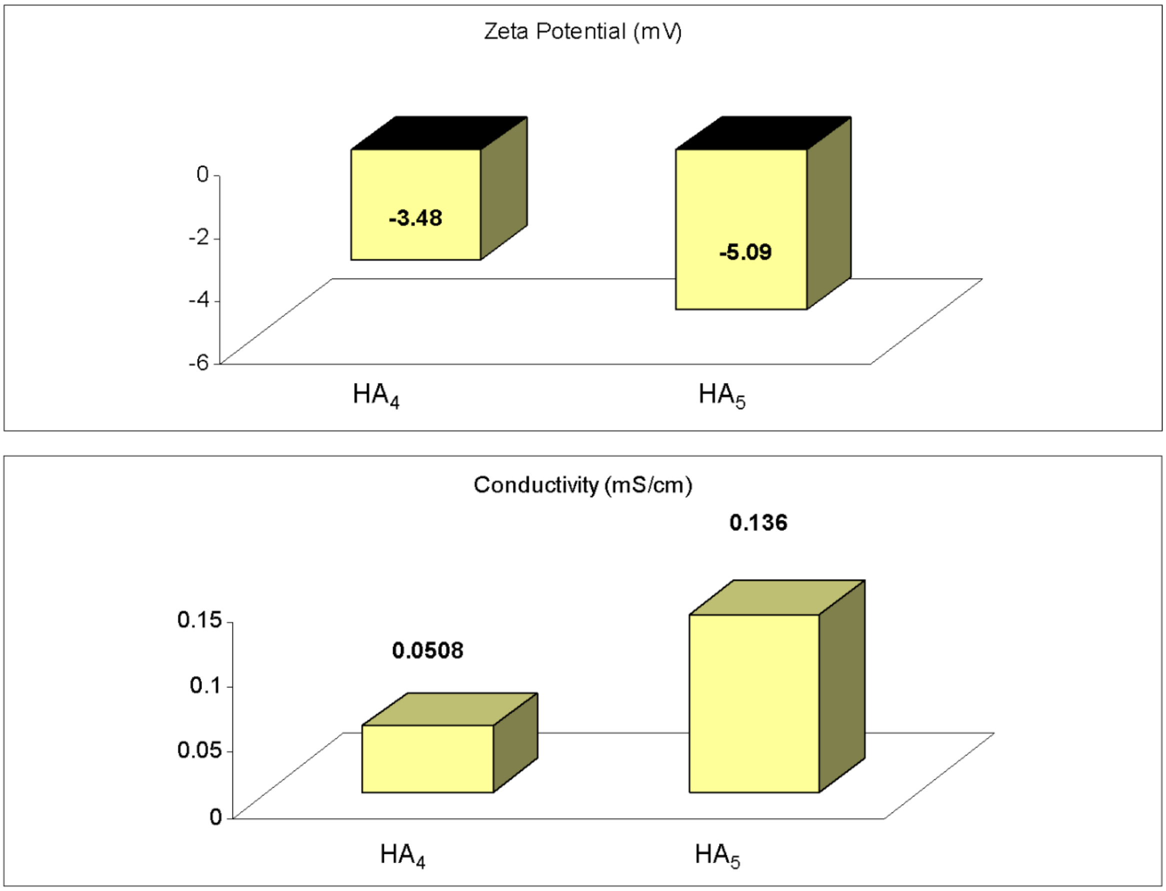

Figure 2. Representation of the zeta potential and surface conductivities of HA_4 and HA_5

The hydroxyapatite structure of fish bones is highly effective in binding heavy metals such as copper (Cu^{2+}). This binding primarily occurs through two mechanisms as ion exchange and chelation. Initially, calcium ions (Ca^{2+}) in the hydroxyapatite structure can be replaced by copper ions. In this process, Cu^{2+} ions substitute for Ca^{2+} ions in the crystal lattice and become incorporated into the structure. This natural ion exchange property plays a significant role in heavy metal retention by fish bones. However, in this study, the copper-binding effect was investigated through surface modification of hydroxyapatite with histidine (HA_4) and 4-Aminohippuric acid (HA_5). Notably, the HA_5 modification exhibited higher performance with an adsorption capacity of 9.96 mg/g compared to HA_4 as 9.52 mg/g (Figure 3). The HA_5-modified surface also exhibited a more negative zeta potential value, which can facilitate stronger electrostatic attraction of positively charged copper ions to the surface. Additionally, the larger molecular structure of HA_5 may create more binding sites on the surface, enhancing copper adsorption capacity. In conclusion, the natural ion exchange capability of hydroxyapatite can be influenced by organic modifications to improve copper binding capacity. Particularly, fish bones modified with 4-Aminohippuric acid can serve as effective adsorbent materials for removing heavy metals like copper from wastewater. Such biomaterials offer a cost-effective and environmentally friendly option for environmental remediation applications.

The effect of histidine-modified fish bone surface on the adsorption of Cu^{2+} ions is closely related to the chemical changes occurring on the surface. The adsorption behavior exhibited by this modification toward Cu^{2+} ions is determined by both the zeta potential values and the presence of free amino and imidazole groups in the histidine molecule. The negative surface charge obtained from the zeta potential measurements indicates that the surface can generate electrostatic attraction forces with positively charged ions, particularly transition metal cations such as Cu^{2+}. This electrostatic attraction facilitates the migration of Cu^{2+} ions toward the modified surface, enabling initial contact. However, adsorption is not limited to electrostatic interactions alone; chemical bonding also plays a significant role. At this point, the structural features of the histidine molecule come into play. During modification, histidine typically binds to the surface via its carboxyl group, leaving the amino group and imidazole ring free on the surface. These groups, particularly, possess the capacity to form coordinative bonds with Cu^{2+} ions. The nitrogen atoms in the imidazole ring and the free amino group can form strong complexes with Cu^{2+} ions. Due to the high coordination tendency of Cu^{2+} ions, they readily bind with such ligands, facilitating the formation of stable complexes. In this context, histidine modification imparts a dual-mechanism adsorption system to the surface, enabling the retention of Cu^{2+} ions through both electrostatic attraction and chemical complexation. This results in high affinity of the surface toward Cu^{2+} ions and enhances adsorption efficiency. Moreover, this structure offers a functional alternative for environmental remediation applications aimed at removing toxic heavy metal ions like Cu^{2+} from water. In conclusion, the negative zeta potential of the histidine-modified fish bone surface allows the attraction of Cu^{2+} ions to the surface, while the free amino and imidazole groups chemically bind these ions, providing stronger and more durable adsorption. This dual interaction improves the effectiveness of the surface in heavy metal removal and highlights such modifications as environmentally friendly and sustainable solutions.

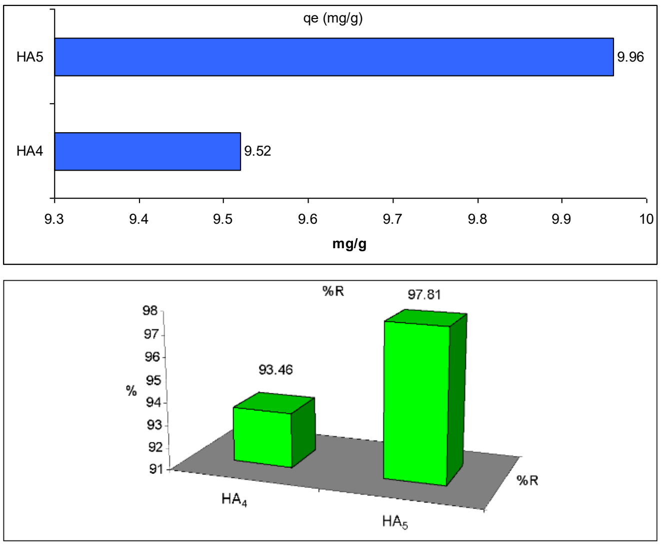

Figure 3. Results of copper adsorption of HA_4 and HA_5

The zeta potential of approximately –5.09 mV for the fish bone surface modified with 4-Aminohippuric acid (HA_5) allows for important inferences regarding the adsorption of Cu^{2+} ions. This slightly negative value indicates that the surface potential to generate electrostatic attraction with positively charged Cu^{2+} ions is limited. Generally, higher negative zeta potential values facilitate stronger electrostatic binding of metal cations to the surface, whereas a charge around –5 mV corresponds to a moderate level of electrostatic interaction. However, the structure of 4-Aminohippuric acid provides a basis for an adsorption process that is not limited to electrostatic interactions alone. While the mildly negative zeta potential supports some degree of Cu^{2+} ion attraction, the primary adsorption mechanism is dominated by the chemical binding capacity of functional groups. As a transition metal, Cu^{2+} ions exhibit a strong tendency to complex with ligands and can form stable chemical bonds with functional groups present on the HA_5-modified surface. This mechanism enables effective adsorption even when electrostatic attraction is weak. Consequently, the –5.09 mV zeta potential value indicates that Cu^{2+} ions bind to the surface not only electrostatically but also through chemical complexation. The 4-Aminohippuric acid modification offers a multifunctional binding environment by presenting diverse functional groups on the surface, resulting in an effective, sustainable, and environmentally friendly adsorbent system for the removal of cationic heavy metals such as Cu^{2+} from aqueous solutions.

CONCLUSION

In this study, the changes in zeta potential and copper (Cu^{2+}) adsorption capacities of hydroxyapatite (HA) surfaces derived from fish bones, modified with histidine (HA_4) and 4-Aminohippuric acid (HA_5), were comparatively evaluated. The analyses revealed that both modifications induced negative zeta potentials on the HA surfaces. The zeta potential of the histidine-modified surface ranged between –3.48 mV and –5.09 mV, indicating a moderate level of negative surface charge. In contrast, the surface modified with 4-Aminohippuric acid exhibited significantly more negative zeta potential values, suggesting a stronger electrostatic attraction capacity. Examination of copper adsorption data showed that the HA_5 modification demonstrated superior performance with an adsorption capacity of 9.96 mg/g compared to 9.52 mg/g for the HA_4 modification. This finding indicates that the higher negative charge generated by HA_5 facilitates the attraction of positively charged Cu^{2+} ions to the surface, thereby enhancing adsorption capacity. Additionally, it is suggested that the carboxyl and amino groups present in 4-Aminohippuric acid synergistically support ion binding. In the case of histidine modification, the presence of imidazole rings and amino groups contributed to some degree of Cu^{2+} ion binding; however, the relatively lower zeta potential limited the overall adsorption capacity. In conclusion, this study demonstrates how functional molecule modifications of fish bone-derived hydroxyapatite surfaces affect their adsorption capabilities toward heavy metal ions. Notably, the 4-Aminohippuric acid modification, with its higher negative surface charge, forms a more effective adsorbent for Cu^{2+} removal, while histidine modification provides a lower but still significant adsorption capacity due to its limited surface charge. These findings suggest that modified fish bone surfaces hold promise for sustainable and environmentally friendly remediation applications.

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Compliance with Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

Generative AI (e.g., ChatGPT 4.0, DeepSeek) was used for grammatical review of the introduction and discussion sections. The author validated all outputs and assume full responsibility for the content.

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