Vol.67: e24220892, 2024
https://doi.org/10.1590/1678-4324-2024220892
ISSN 1678-4324 Online Edition

BABT
Brazilian Archives of Biology and Technology

Article - Environmental Sciences

Extraction of Keratin from Chicken Feathers and its Application in the Treatment of Contaminated Water: an Eco-Friendly Approach

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Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Marcos Pileggi

Received: 11-Nov-2022; Accepted: 22-Aug-2023

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HIGHLIGHTS

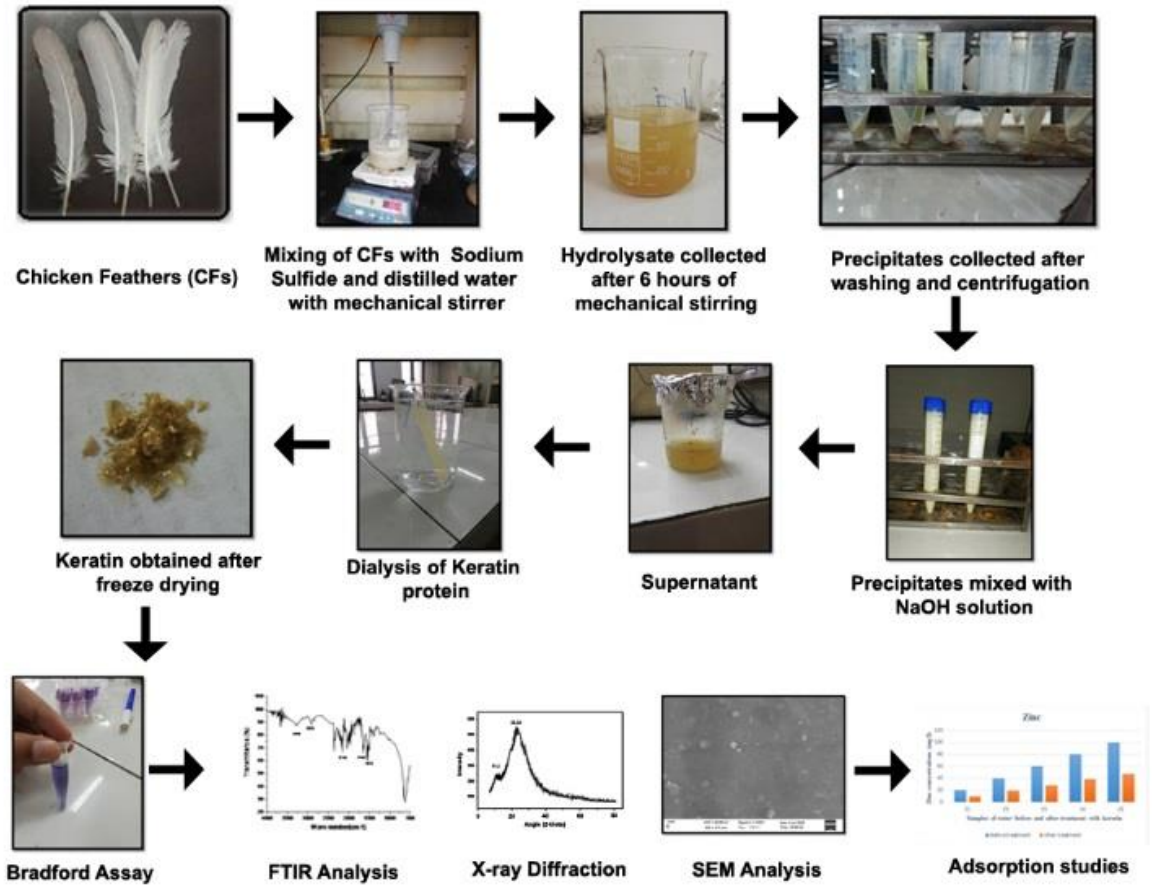
- Extraction of keratin from chicken feathers to reduce environmental burden.
- Characterization of keratin to observe the protein structure.
- Removal of heavy metals from synthetic water with extracted keratin from chicken feathers

Abstract: Chicken feathers that make up 4-6% of total weight of chicken are most influential waste by-product from poultry farm and slaughter. Annual worldwide generation of several tons of feather biomass raise solid waste management concerns. In environmental perspective, the burning or dumping in landfills are not promising approaches. Keratin, tough and fibrous protein, is an important polymer abundant in chicken feathers. The present study aimed at extraction, characterization of keratin from chicken feather waste. Moreover, this study was performed to evaluate the adsorption potential of keratin adsorbent for the treatment of heavy metal contaminated synthetic water. Feathers after collection was treated with sodium sulfide for the extraction of valuable keratin protein. The extracted keratin was dialyzed using cellulose membrane and freeze dried. Adsorption of metals (Zn and Cu) onto extracted keratin has been studied using batch-adsorption studies. The concentration of obtained protein from chicken feather was computed to be 0.95mg/mL. Functional groups of amide I, amide II, tryptophan, stretching C-O and bond of C-N were confirmed through FTIR. XRD analysis confirmed sem-crystalline structure of keratin whereas SEM analysis

showed roughness on surface of keratin due to alkaline hydrolysis. Freundlich isotherm identified ideal parameters for removal of zinc and copper from water as eight hours of contact time, temperature of 25^{0}C. With regard to pH, the optimum level was 5.0 and 4.5 for zinc and copper respectively. After treatment with extracted keratin, the removal of 52% zinc and 69% copper from the synthetic water was observed. Results clearly indicate the potential of keratin from chicken feather for effective, economic and eco-friendly treatment of contaminated water.

Keywords: Chicken feathers; Keratin; Eco-friendly; Bradford method; FTIR; XRD; SEM.

GRAPHICAL ABSTRACT



INTRODUCTION

Chicken feathers are considered as worthless product from poultry industry that makes up four to six percent of the total weight of chicken. Feathers are utilized in ornamentation and packaging, however, in extremely small proportions. Majority of the feathers come from slaughter houses and are a significant source of pollution. According to [1], almost five billion tons of chicken feathers as waste are generated from poultries every year. Most of them are discarded by burning or dumping in landfills occasionally but these disposal methods are not eco-friendly. The storage and handling of feathers in significantly large quantities is a major concern associated with environmental pollution. The emissions generated as a result of burning is a main contributor of air pollution. Moreover, improper disposal of ash complicates the issue [2].

Feather waste is biodegradable in nature comprising of more than 85% of crude protein, 70% of amino acids, vitamins, high-value element and growth factors [3]. Owing to the presence of valuable materials, chicken feathers may be subjected to application as feed [4], fertilizer [5] and biofilm [6]. There is a lack of proper recycling method for chicken feathers [7]. From an environmental and financial perspective, it is, therefore, essential to develop a method for turning wasted feathers into new materials that could prove to be both efficient and cost-effective. The need for environment friendly methods that use renewable resources is increasing in this era. Proteins are a key source of renewable materials. Keratin protein is found in chicken

feathers. Chicken feathers are easily available, one of the unique and economical by-products of poultry industry [8]. Feathers exhibit low density, good mechanical and non-abrasive properties. With the addition of hydrophobic groups in solvents and increase in temperature, the insolubility of feathers in water and organic solvents can be transformed to solubility [9].

Chicken feathers comprise of stable structures owing to copious rigid protein i.e., keratin, the third most abundant material after chitin and cellulose [10], is found naturally in epidermis and epidermal appendages of vertebrates such as nails, skin and feathers. Like other normal proteins, the secondary structure of keratin comprises of α-helix and β-sheet [11]. In β-keratins, β-pleated sheets are normally found, whereas, in α-keratins, α-helix coiled structure are usually present [10]. The variation in the composition of α and β- keratins exist among various organs [12]. For example, α-keratin is present in wool [13], however, both α and β-keratins are present in feathers [14]. Keratin is affluent of disulfide bonds and cysteine residues [15,16]. The stability of keratin is connected with its relatively high mechanical strength owing to the presence of dense polymeric structure in combination to hydrophobic forces and hydrogen bonding [17]. Several attributes including biocompatibility, renewability, sustainability and biodegradability make keratin suitable for a variety of applications [18].

Enormous amount of contaminated water is generated from industries as a result of the usage of synthetic chemicals and dyes [19]. The separation of heavy metals from aqueous media are performed by conventional i.e., chemical, physical and biological methods. During recent decades, most commonly employed removal methods of heavy metals from waste water include ion exchange, chemical precipitation and membrane filtrations (reverse osmosis, ultrafiltration) [20]. In recent years, for the decontamination of waste water, adsorption is considered as economical and ecofriendly. The adsorption method requires the mass transfer that permits the transfer of metallic ions from solution onto solid surface followed by physical or chemical interactions [21]. The efficient removal of pollutants and chemicals from wastewater is paramount for technological advancement [22]. Several sources of sorbents include organic (polymeric resins), minerals (alumina, activated carbons, silica beads) or biological (plant-derived materials, agricultural waste, feather/nails/hairs keratin) [23]. Owing to natural abundance, eco-compatibilities, cost-effectiveness and good adsorption performance, bio-sorbents are extensively used for the removal of heavy metals from waste water [24]. The presence of functional groups i.e., carboxyl, amide, sulfonate, carbonyl, phenol, phosphate, amine, sulfhydryl in bio-sorbents are subjected to the adsorption of metallic ions from wastewater [25].

A study deployed biodegradable material including natural carbohydrate polymeric material of graham flour and rice flour for the effective removal of dye from wastewater [22]. Likewise, for the removal of heavy metals including Pb, Cu, Hg, Co and other elements like dysprosium, lutetium and palladium, composite materials have been utilized [26-33] Chicken feathers can be used as biopolymers for wastewater treatment owing to its unique physical and chemical attributes. The presence of keratin protein in chicken feathers play a significant role in adsorption due to the side chains attached with polypeptide structure of keratin [34]. Keratin-associated proteins contain cysteine side chains that is contemplated to form disulphide bonds cross-linking the intermediate filaments of keratin [35]. In polypeptides, several amino acids with identifying side-chains possess specific chemical structure, charge, reactivity and bonding capability. Such side-chains do not participate in the formation of polypeptide, however, plays a significant role in adsorption. After undergoing through various chemical treatments, their usefulness for the removal of contaminants varies [16]. The enhancement in surface affinity of keratin for wastewater contaminants is attributed to the breakdown of di-sulfide cross-links in native keratin that unfolds and exposes free function groups with excessive potential for the adsorption of trace elements [36-39]. The analysis of the extent of adsorption is employed using adsorption isotherm that outlines the equilibrium function, adsorption mechanism and the association between adsorbent and adsorbate [40]. The commonly used model for multilayer adsorption on heterogenous sites is Fruendlich isotherm [41]. The assumption of isotherm is based on exponential decay in the energy distribution of adsorbed sites [42]. The linearized form of adsorption isotherm is relatively straightforward [43]. Therefore, in current study, the Fruendlich isotherm was utilized.

The efficient treatment of waste-water using biosorbents has become an area of increasing concern nowadays. In light of this, the extracted keratin can be useful as an inexpensive source of protein that can be used in wastewater treatment and other applications including medicines, beauty products and surgeries like bone replacement and grafting. The novelty of current research lies in the optimization, utilization of extracted keratin protein from broiler chicken feathers, a solid waste material, and evaluation of its functionalization in the treatment of contaminated water. Therefore, the current research aimed at extraction of keratin from waste chicken feathers and characterization for their biochemical and structural properties. Moreover, it focused on the evaluation of efficacy of extracted keratin for the removal of heavy metals.

MATERIAL AND METHODS

Sampling and Pre-treatment

Chicken feathers (CFs) were collected from local poultry shop. Dirt and blood attached to feathers were removed by soaking the CFs in water mixed with detergent for half an hour. CFs were rinsed and again washed in detergent water. The process was repeated twice to thrice in order to remove all the unwanted odor, dirt and blood stains from the feathers. After that, feathers were dried in laboratory oven at 40^0C. After drying, feathers were properly stored and sealed in plastic bags [44].

Treatment of chicken feathers

In order to increase the surface area for reaction, the dried CFs were cut into smaller pieces. Hydrolysis using alkaline solution was performed for the extraction of keratin. 25 grams of CFs were mixed in one liter of 0.3M sodium sulfide with continuous stirring for six hours using mechanical stirrer at 400 rpm and a temperature of 60^0C. It caused the dissolution of chicken feathers in sodium sulfide solution. After six hours, the solution was filtered and hydrolysate was collected after separating the insoluble particles from the solution. The solution was then centrifuged at 6037 *g* for 20 minutes followed by collection of supernatant and removal of residues [45].

Purification of protein

Hydrochloric acid (HCl) (2 N), as a precipitant was added drop wise into the solution until the precipitates were formed. In order to achieve maximum precipitation, the solution was left for 24 hours. Precipitates were filtered from the solution and washed with 50mL of distilled water. The precipitation and washing process was repeated twice and the solution underwent centrifugation (Sigma 2-6, Germany) at 6037 *g* for 10 minutes. From this solution, solid particles were collected. 0.2 M NaOH was mixed in 15mL of distilled water and collected solid precipitates were added in that solution. The solution was mixed and again centrifuged for 10 minutes at 6037 *g*. Pellets were formed that were discarded from solution after centrifugation. Supernatant was taken in a small beaker [46].

Protein dialysis

A cellulose membrane tube (4 kDa) was used for dialysis of keratin protein. Cellulose membrane tube was washed with distilled water, knot was made at one side of the tube and the supernatant was filled in the tube. Another knot was made on the other side of the tube to close it. 1 liter distilled water was poured in a beaker and the cellulose membrane tube containing protein solution was placed in it. The dialysis of solution continued for 2 days. The outer distilled water was changed thrice a day that was done to remove impurities from the solution [46].

Freeze drying

The collected solution after dialysis was poured in vials to partial filling. At first, the vials were kept in a freezer to freeze the solution. When the solution was frozen, vials were put in the freeze dryer (Thermo Freeze Dryer PL-6000) that took 24 hours for the sample to freeze dry [46].

Percent yield calculation

The percent yield of keratin was computed using the following equation 1 [47]. The chicken feather contain about 90% the yield [48], therefore, 0.903 represents dry weight content of feathers as decimal fraction [49].

$$Percent\ Yield\ (\%) = \frac{mass\ of\ protein}{0.903 \times mass\ of\ dry\ chicken\ feather} \times 100 \quad (1)$$

Characterization of keratin

Quantification of Keratin Protein

Bradford assay test was employed for the quantification of keratin protein. Bovine serum albumin was used as standard for this test. Stock solution of BSA was prepared from 1mg/mL [50]. Six dilutions (0-1 mg/mL) were made from this stock solution by the addition of distilled water. 1 mL of Bradford reagent was

added to 100 µL of each dilution as well as the solution of keratin. The dilution were made in eppendorf tubes and left for incubation at room temperature for about 20 minutes. After 20 minutes, color of keratin sample was compared with the color of dilutions and absorption of samples was measured using spectrophotometer at a wavelength of 595nm.

FTIR Analysis

FTIR (IR-Tracer-100) analysis was performed in transmission range of 4000-500 cm-1 for the extracted sample of keratin. Functional groups of different kinds were identified using FTIR. Keratin sample was placed and peaks were recorded for the protein sample.

XRD Analysis

In order to identify the crystallinity of extracted keratin, X-ray Diffraction (XRD) patterns were obtained. The contact time of extracted keratin sample for analysis was 20 minutes. 1 gram of sample was used for this purpose.

SEM Analysis

SEM (Scanning Electron Microscopy) was used to study the morphology of surface of the keratin protein. The extracted keratin was directly studied on SEM. It is highly considered that the sample for study should conduct electricity to attain a picture with greater quality.

Application of extracted keratin for the removal of heavy metal containing water

Preparation of synthetic water

The synthetic water was prepared in test tubes in order to check the efficacy of extracted keratin for the removal of heavy metals. The water was spiked with two different metals i.e., copper and zinc. For the preparation of synthetic water, salt of copper sulfate ($CuSO_4$) was used. The solutions were prepared at the concentration of 10, 20, 30, 40, and 50mg/L. In case of preparation of zinc containing synthetic water, zinc sulfate ($ZnSO_4$) was used and the prepared concentration were 20, 40, 60, 80 and 100mg/L.

Batch Sorption studies

0.25 g of extracted keratin from waste chicken feathers was introduced into test tubes containing various concentrations of 50 mL aqueous solutions of zinc or copper ions. The samples were shaken for 200 rpm for 24 hours [51].

Equilibrium experimentation

After the addition of adsorbent at a concentration of 5mg/mL in synthetic water containing copper or zinc, the samples were shaken for 24 hours to achieve equilibrium. The solutions were filtered using Whatman 42 filter paper. The effect of solution pH (3.5-5), temperature (25-50^{0}C) and contact time were studied. The residual concentration of metal ions was determined using Atomic Absorption Spectrophotometer.

Statistical Analysis

Data quantification, analysis and graphical representation was performed using MS Excel 2016. The analyses were perfomed in triplicates and average value was computed.

RESULTS

Protein quantification from Bradford Protein Assay

After 20mins of incubation, absorbance was noted for dilutions of standard and extracted protein sample at 595nm. Slope and intercept were calculated as shown in standard curve (Figure 1). Concentration of keratin was computed to be 0.95mg/mL using equation 2.

$$Concentration\ of\ protein\ =\ (absorbance\ of\ sample - intercept) \div slope \quad (2)$$

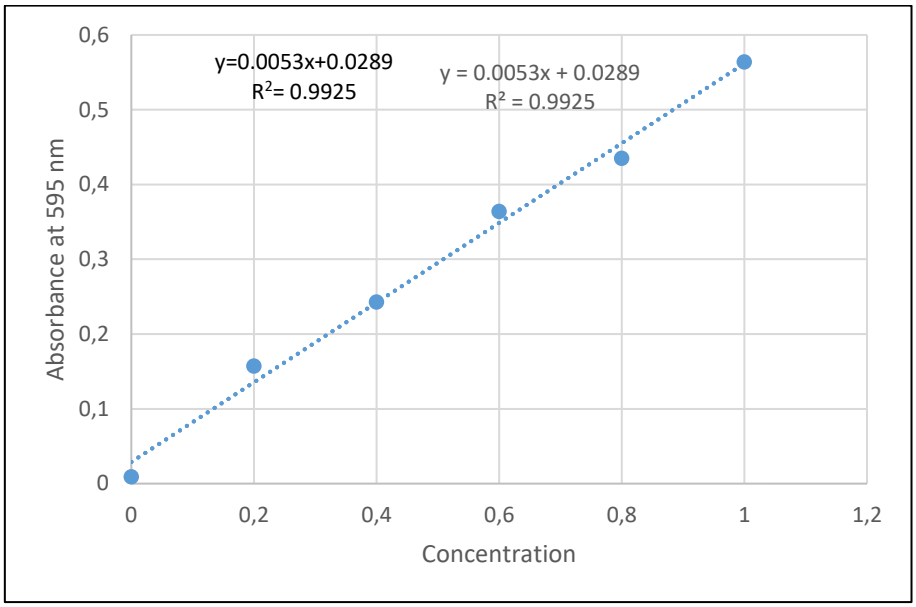


Figure 1. Standard curve for protein between concentration (mg/mL) and absorbance

FTIR Analysis

FTIR analysis was conducted for characterization of keratin. A total of 27 peaks as representative of functional groups were identified in FTIR spectra. Peaks from 1506 to 1558 cm-1 confirmed the presence of amide II. The peak of 1575.8 cm-1 showed tryptophan, whereas, at 1635.63 and 1652 cm-1 amide I group with carbonyl bond were identified. Wavenumbers 1710.85 and 1726.29 cm-1 had stretching C-O group. Seven peaks from 2142.9 to 2243.2 cm-1 showed C-N group. The lowest and highest peaks were at 634.5 cm-1 and 3670.5 cm-1 respectively. These results showed that these peaks exhibit similar characteristics as raw chicken feathers as reported by [52]. In case of raw chicken feather, the dominating peaks representing Amide A, Amide I, II and III were at 1230, 1510, 1630, 2930 and 3280 cm-1. In another study, the absorption peaks in raw chicken feather at 3284.7 and 1680 cm-1 were attributed to O-H stretching of alcohol and amide bond respectively. The C-H and C-N stretching were revealed at 1550 and 1280 cm-1 respectively. Moreover, S-H stretching of thiol appeared at 2574.6 cm-1. In line with current study, the suppression of S-H stretching was dominant in extracted keratin [53]. Figure 2 depicts the result from FTIR data and Table 1 shows the functional groups and observed peaks.

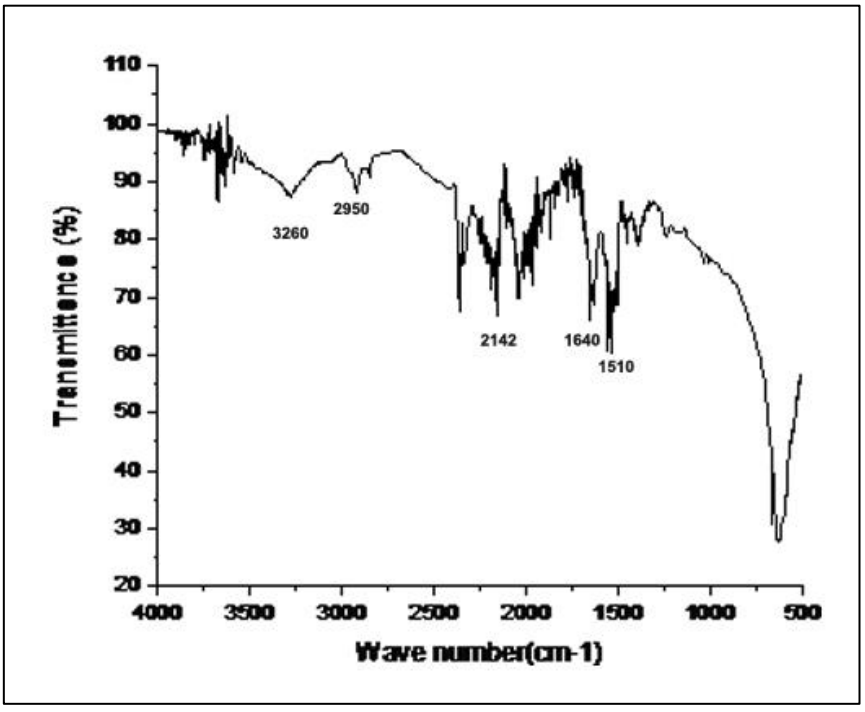


Figure 2. Peaks from FTIR data for extracted keratin protein

Table 1. Identified peaks and functional groups from the FTIR results

Peaks (cm^{-1})	Functional groups
1506 to 1558 cm^{-1}	Amide II
1575.8 cm^{-1}	Tryptophan
1635.63, 1652 cm^{-1}	Amide I
1710.85, 1726.29 cm^{-1}	Stretching C-O
2142.9 to 2243.2 cm^{-1}	C-N

XRD Analysis

Figure 3 displays the XRD diagram of extracted keratin from chicken feathers. Due to the presence of few stacked layers, the diffraction pattern was characterized by highly broad lines. The diffraction pattern showed peaks at 10.2 and 23.25^0 angles. The crystallinity percentage was calculated using equation 3. The percentage of crystallinity was found to be 83%. The peak at 23.25 is relatively intense and sharp that represents high crystallinity. However, the peak at 10.2 is also there i.e., less intense, ultimately, representing low crystallinity and amorphous structure. The overall XRD suggested unorganized pattern that demonstrates the semi-crystalline structure of keratin sample [54].

$$\%Crystallinity = [(area\ under\ the\ crystalline\ peaks) \div (area\ under\ all\ peaks)] \times 100 \quad (3)$$

The size of particles of keratin were found to be 6nm representing small, low-volume pore, wide surface area and its micro porous structure. The porosity affects the ability of keratin to absorb. Its porosity was found to be 1.99%. The size of particles was determined by using the Scherer formula (Equation 4) which is as follow:

$$D = K\lambda \div (\beta \cos\theta) \quad (4)$$

where K is Scherer constant (0.9), λ is wavelength (0.154nm), cos θ is the peak position i.e., 0.996 while β i.e., full width at half maximum capacity (FWHM) was computed to be 0.232.

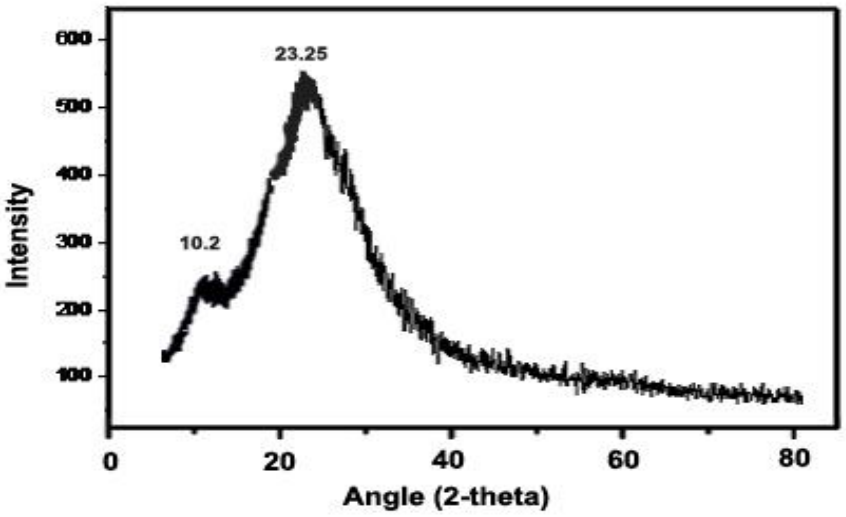


Figure 3. Angle and Intensity of keratin by X-Ray Diffraction (XRD)

SEM Analysis

The SEM images of extracted keratin were investigated to determine the structural morphology of keratin protein. Figure 4 (a) represents the irregularity in structure at magnification of 10kx. There was no smoothness on the surface of protein. A white uneven patch affirms the roughness of the surface. At magnification of 5kx, Figure 4 (b) showed porous structure as holes on the image. Figure 4 (c) and (d) exhibited lighter colored dot-like patches at magnifications 3kx and 949x respectively. The particles were rough with patches at irregular intervals owing to the structure of keratin. A study conducted by [55] also reported the rough texture of chicken feather biochar with irregular-sized particles. It depicts the surface of keratin with breakage of peptide chain bonds as well as denaturation of helical structure.

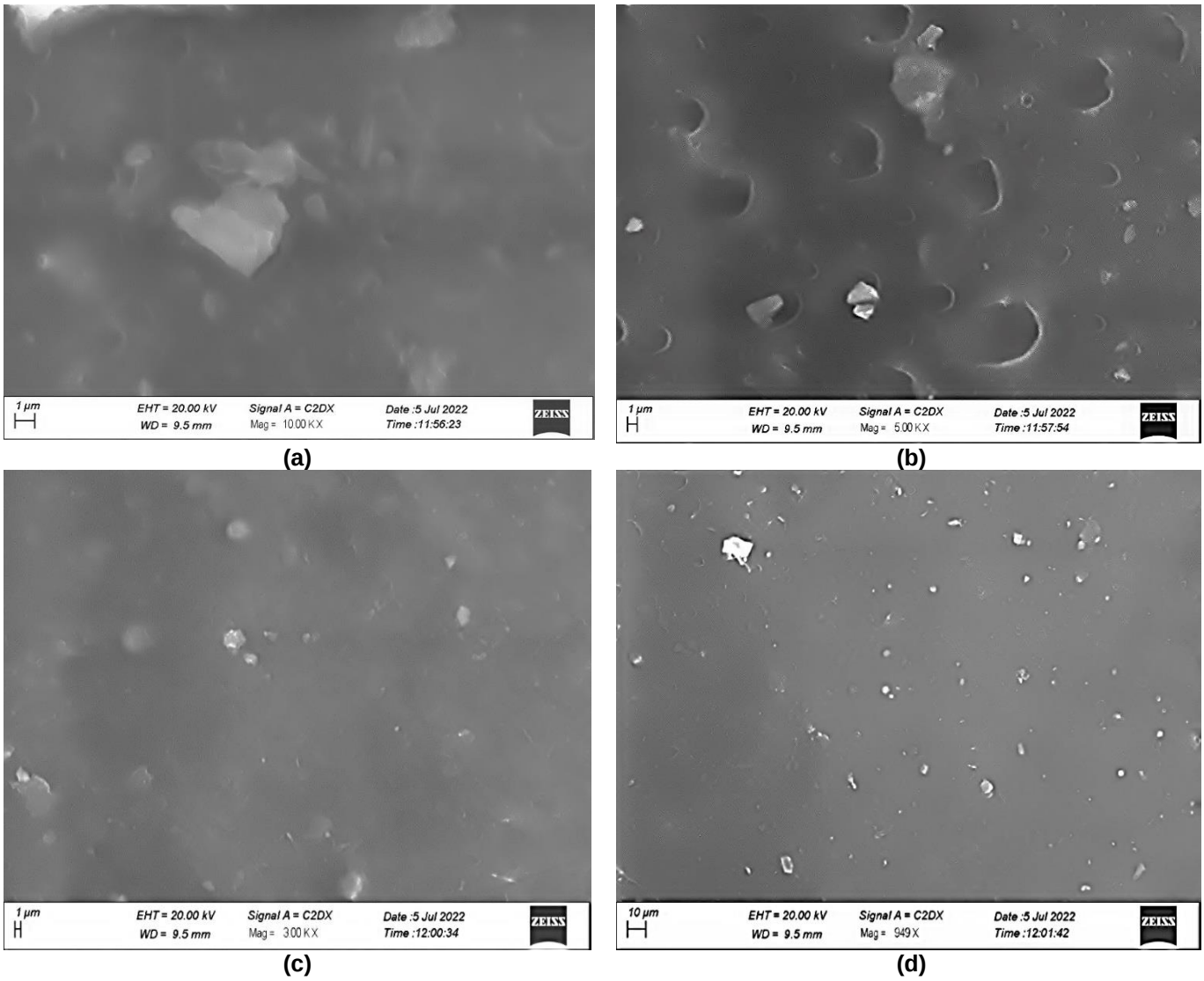


Figure 4. SEM showing keratin surface morphology with magnifications of **(a)** 10kx **(b)** 5kx **(c)** 3kx **(d)** 949x

Batch Sorption Kinetics

Effect of contact time

The effect on contact time on the adsorption of copper and zinc was investigated. The uptake capacity of metals was calculated in mg/g. In order to achieve maximum adsorption of metals, it was found that 8 hours of contact time was required. At first hour of contact, the zinc ion uptake was 2.96, 6.01, 7.65, 9.89 and 11.69 mg/g for initial concentration of zinc at 20, 40, 60, 80, 100mg/L respectively as shown in Figure 5. Highest uptake of zinc that was recorded after achieving equilibrium was 13.62, 10.02, 8.28, 6.12 and 3.50 mg/g for initial concentration of 100, 80, 60, 40 and 20 mg/l respectively. Likewise, at first hour of equilibrium the copper ion uptake was 1.36, 2.21, 4.45, 5.69 and 6.09 mg/g for initial concentrations of copper at 10, 20, 30, 40 and 50mg/L as shown in Figure 6. After achieving equilibrium, the highest uptake of copper was 7.82, 6.95, 5.80, 2.32 and 1.72 mg/g for 50mg/L, 40mg/L, 30 mg/L, 20 mg/L and 10 mg/L of copper concentration respectively.

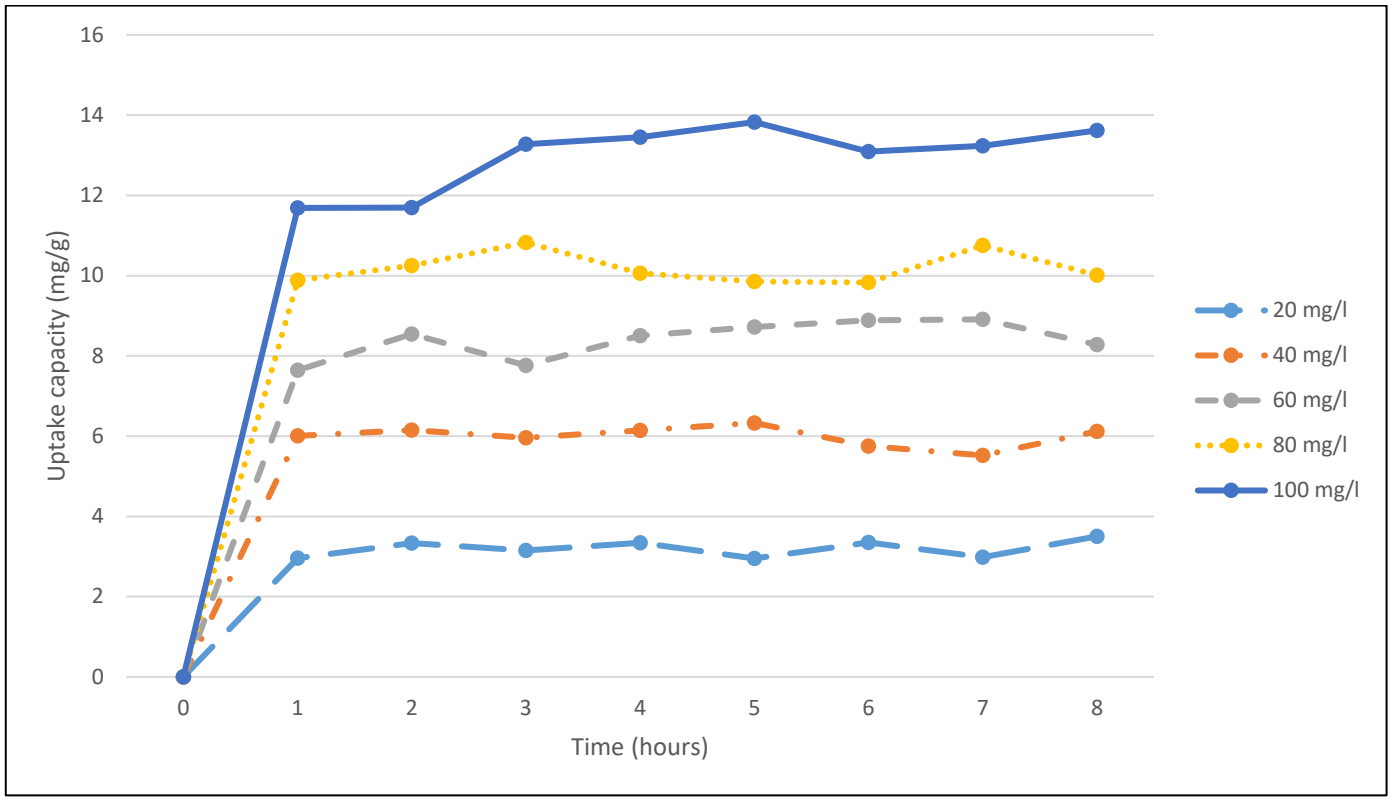


Figure 5. Uptake of zinc ions at different initial concentrations (20-100 mg/L) by keratin adsorbent with its initial concentration of 5 mg/mL

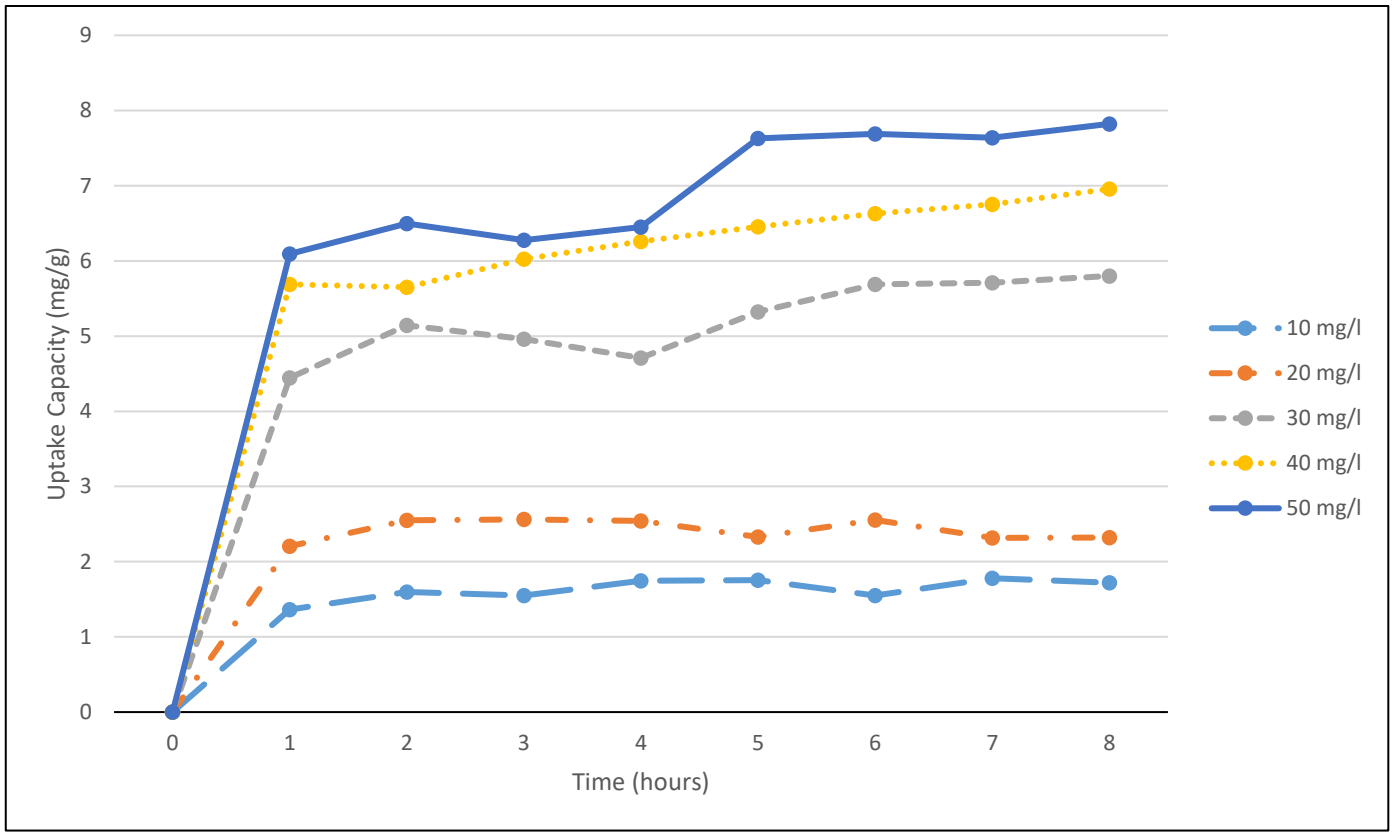


Figure 6. Uptake of copper ions at different initial concentrations (10-50 mg/L) by keratin adsorbent with its initial concentration of 5 mg/mL

Freundlich isotherm of equilibrium

In this study, Freundlich isotherm model was implemented for equilibrium studies. For this purpose, a formula was used to get linear isotherms of zinc and copper uptake (Equation 5).

$$\ln qe = \ln kF + (1/n)\ \ln Ce \quad (5)$$

where qe is the capability of adsorption with the solution containing concentration of metal ions, Ce, while K_f and 1/n are Freundlich constants associated with adsorption capacity and intensity of adsorption respectively.

The constants in Freundlich isotherm were extracted from the results of equilibrium (Table 2). The Freundlich isotherm models indicated that increase in metal ions concentration in the solution led to their increasing uptake by the keratin adsorbent as depicted in Figure 7. The kF value proved that the adsorption capability of keratin was greater for copper ions than that of zinc ions. The reason for this ability was due to difference in size of copper and zinc ions. The ionic radii of copper and zinc ions are 0.57^0A and 0.60^0A respectively [56]. Due to small size, the ions of copper had greater ability to react with the surface of adsorbent. The trend of sorption could be elucidated by ionic radii of metallic ions.The metallic ion with small ionic radii exhibit more adsorption rate [57,58]. But for both metals, the adsorption ability of keratin was satisfying. According to [59], n value ranging between 1 and 10 represents satisfactory adsorption. The value of 1/n less than 1 indicates that adsorption is significant at low concentration [60].

Table 2. Freundlich constants for zinc ion and copper ion adsorption by keratin

Metals	K_f	n	R^2
Zinc	0.0977	1.8975	0.9974
Copper	0.1860	1.2873	0.9972

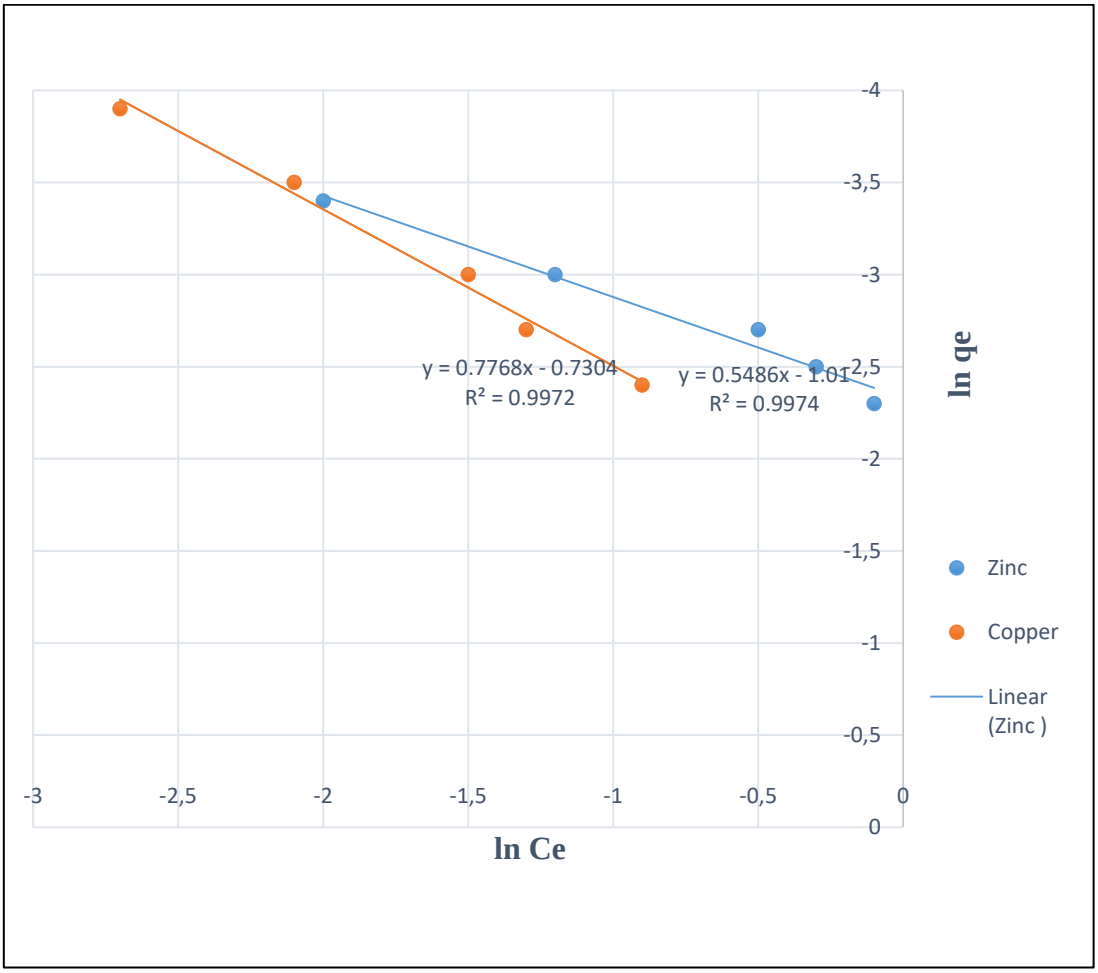


Figure 7. Freundlich isotherm model for zinc and copper ions uptake

Effect of Temperature

In order to check the effect of temperature, the adsorption of copper and zinc ions were studied at 25^{0}C, 40^{0}C and 50^{0}C. The values for Freundlich constants are outlined in Table 3. It was found from the results of linear isotherms that uptake of metal ions of zinc increased with lowering temperatures. For copper ions, similar trend was observed with respect to adsorption capacity that declined with the increase in temperature (Figure 8, 9).

Table 3. Freundlich constants for zinc and copper ion adsorption by keratin at different temperatures

Temperature (°C)	K_f	n	R^2
	Zinc		
25	0.0978	1.8975	0.9974
40	0.0900	1.7492	0.9899
50	0.0775	1.5730	0.9869
	Copper		
25	0.1860	1.2873	0.9972
40	0.0734	3.3967	0.9978
50	0.0672	3.5511	0.9949

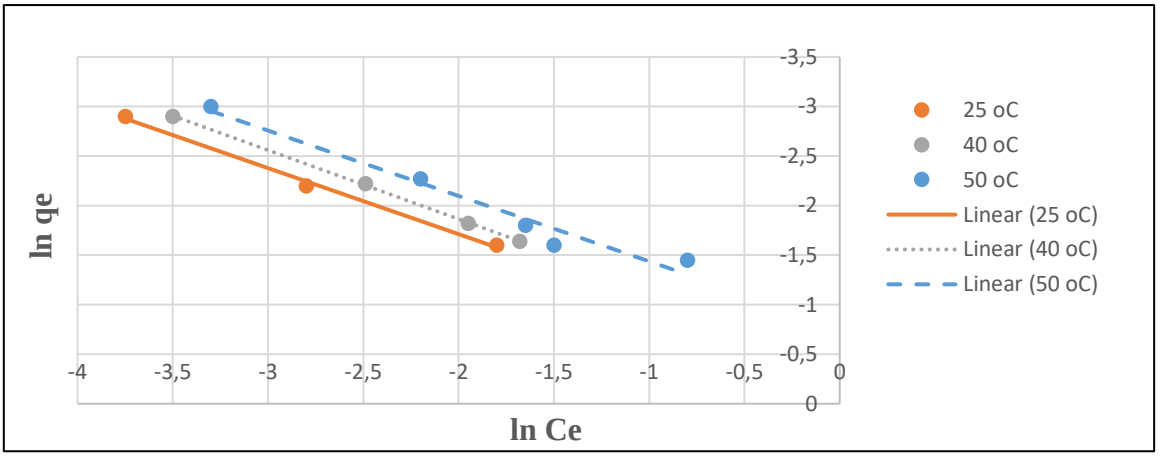


Figure 8. Freundlich isotherms for the removal of zinc ions by keratin at temperatures 25, 40 and 50 °C

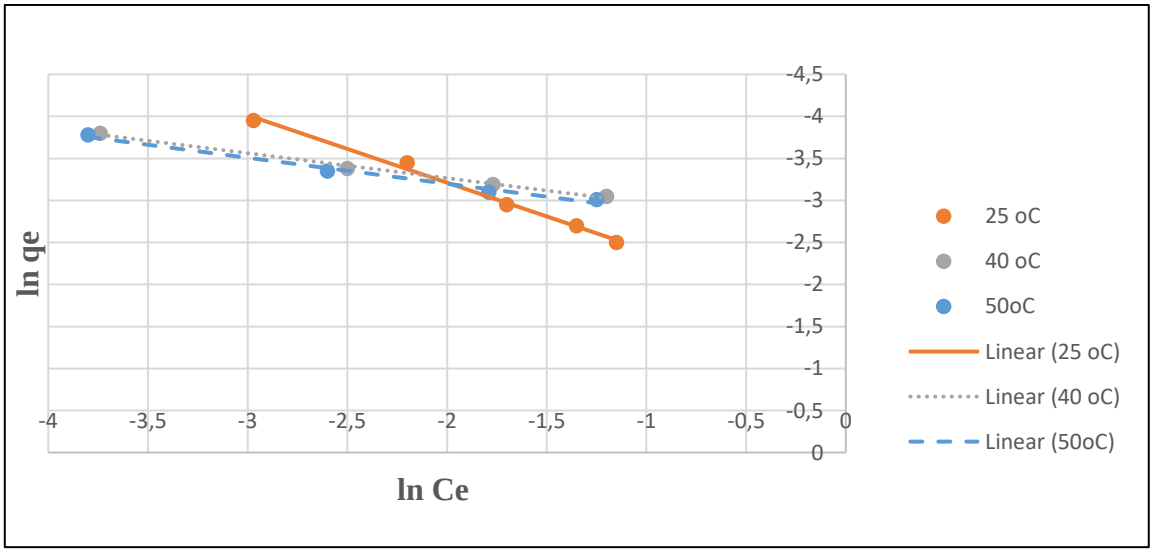


Figure 9. Freundlich isotherms for the removal of copper ions by keratin at temperatures 25, 40 and 50°C

Effect of pH

For the investigation of effect of pH on adsorption of heavy metals, the pH values were selected to be 3.5, 4.0, 4.5 and 5.0. The constants of Freundlich isotherm are represented in Table 4. From the Freundlich isotherm model, it was found that higher level of initial pH resulted in higher uptake of ions of metals (Figure 10, 11). This could be due to charges on the surface. Therefore, it may be concluded that pH of 5.0 and 4.5 were found to be ideal for the uptake of metallic ions of zinc and copper respectively.

Table 4. Freundlich constants for zinc and copper ion adsorption by keratin at different levels of pH

pH	K_f	n	R^2
		Zinc	
3.5	0.1087	1.2203	0.9998
4.0	0.1477	1.3061	0.9960
4.5	0.1503	1.5733	0.9968
5.0	0.1607	1.5389	0.9946
		Copper	
3.5	0.0706	3.3322	0.9975
4.0	0.0774	3.0854	0.9997
4.5	0.0796	3.0684	1.0000
5.0	0.0774	3.6818	0.9996

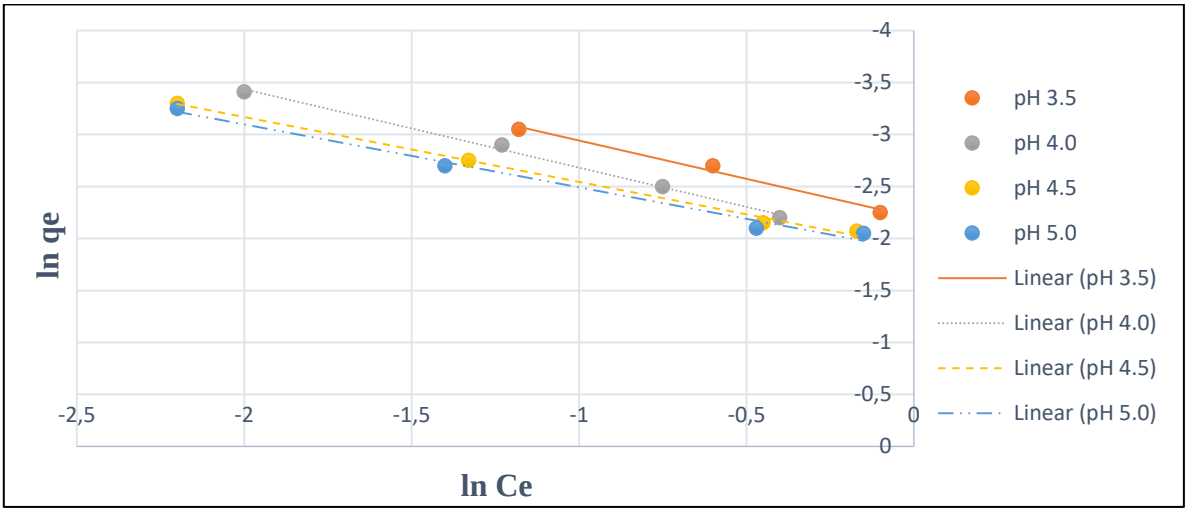


Figure 10. Freundlich isotherms at different pH levels for the removal of zinc ions by keratin

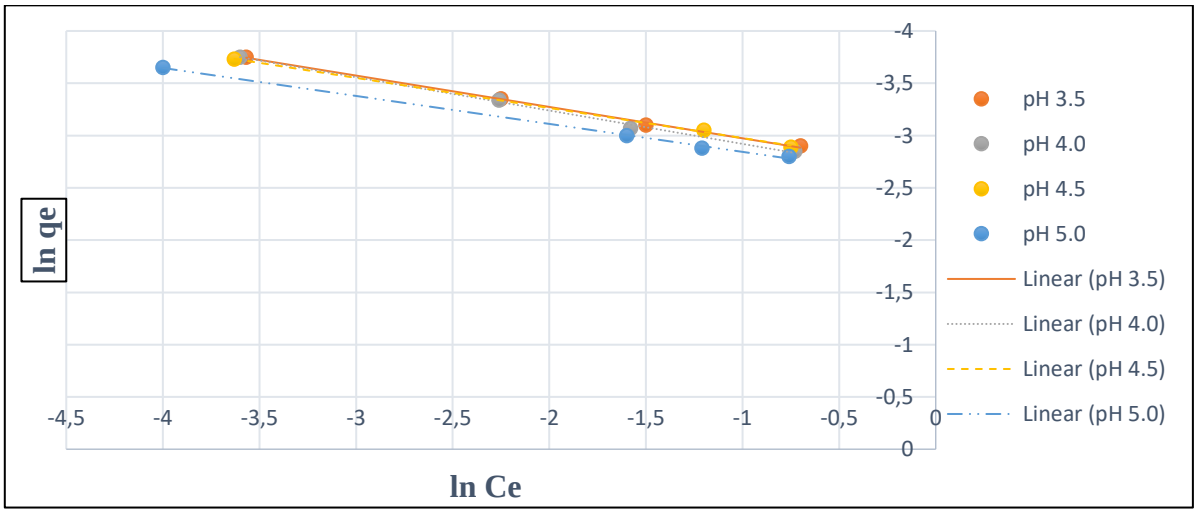


Figure 11. Freundlich isotherms at different pH levels for the removal of copper ions by keratin

Removal of heavy metals from synthetic water

In order to achieve maximum removal efficiency of metals, the ideal conditions i.e., pH 5.0, temp 25^{0}C and 8 hours contact time were given to the samples of synthetic water. Five samples of water including zinc ranging from 20-100mg/l were labelled as Z1, Z2, Z3, Z4 and Z5 for initial concentrations of 20, 40, 60, 80 and 100mg/l respectively. Under set ideal conditions, the concentration reduction is exhibited in Figure 12. The adsorption percentage of keratin in removal of zinc was 52%.

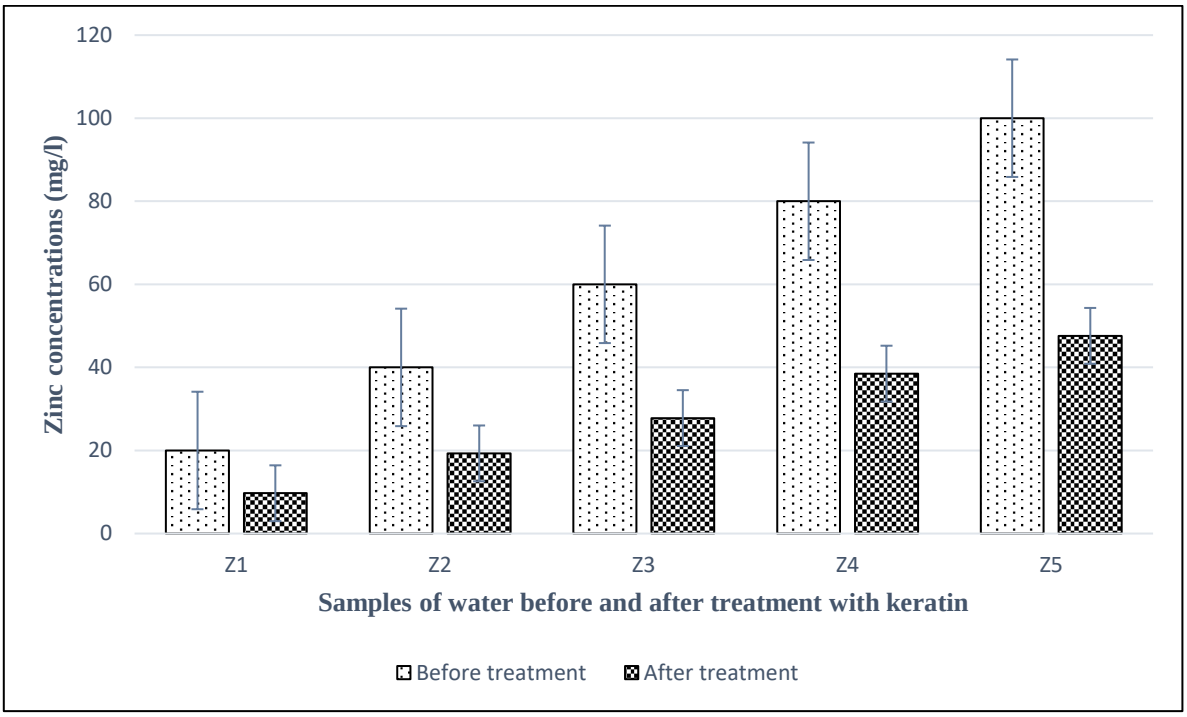


Figure 12. Concentration of Zinc in the samples of water before and after addition of keratin

Five samples of water including copper ranging from 10-50mg/L were labelled as C1, C2, C3, C4 and C5 for initial concentrations of 10, 20, 30, 40 and 50mg/L respectively. The concentration of copper before and after treatment with extracted keratin is shown in Figure 13. The adsorption percentage of keratin for removal of copper was 69%. The removal of metals from water clearly indicates that keratin extracted from chicken feathers may be a good adsorbent for removal of heavy metals from wastewater.

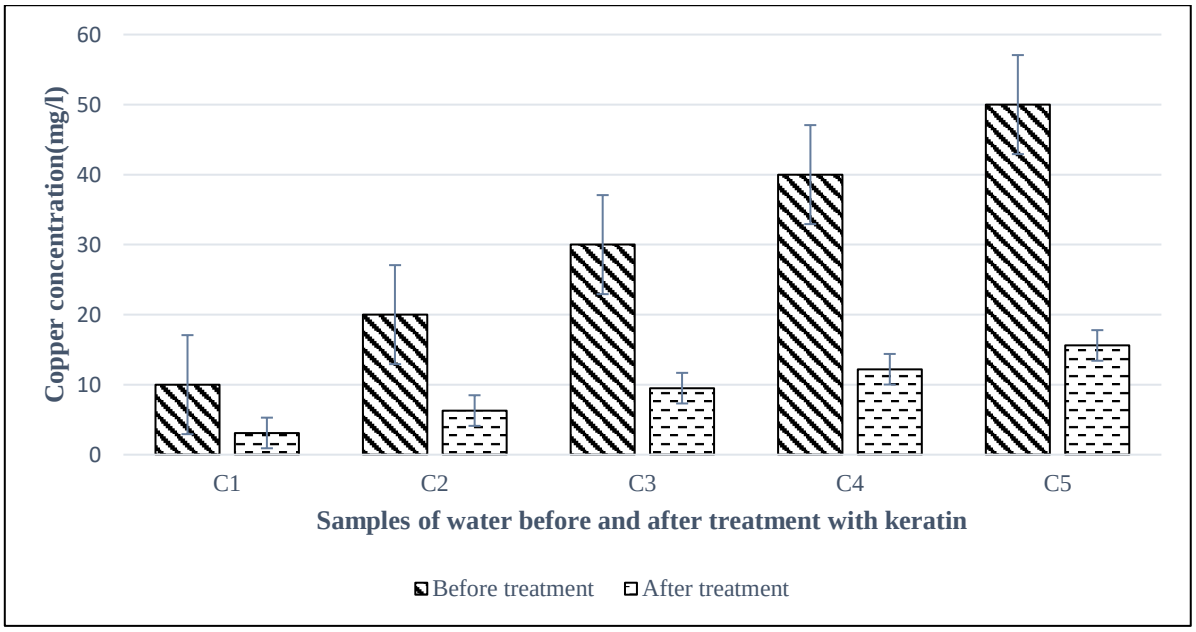


Figure 13. Concentration of Copper in the samples of water before and after addition of keratin

DISCUSSION

In order to avoid end of life incineration or landfilling, chicken feathers can be converted into valuable by-products in an eco-friendly way. Feathers contain high level of protein and amino acids [61]. In light of this, the present study focused on the extraction and characterization of keratin from chicken feathers. The percent yield of protein extracted from chicken feathers was computed to be 66.45%. In a study, the percent yield of keratin was amounted to be 83.8%, 82.4%, 62.9% and 77.6% using various reducing agents i.e., 2-mercaptoethanol, sodium bisulphite, sodium m-bisulphite and dithiothreitol respectively [49]. A research conducted by [62] reported the percentage yield of extracted keratin to be 75% extracted from feathers by 2-mercaptoethanol. Another study measured the protein yield of 62% upon extraction from sodium suphite [63]. Using sodium sulfide and L-cysteine, the yield of extracted keratin was found to be 88% and 66% respectively [64].

It was worth to note that FTIR revealed the presence of amide II at peaks from 1506 to 1558. The peak of 1575.8 cm-1 showed tryptophan, whereas, at 1635.63 and 1652 cm-1 there was amide I group with carbonyl bond. Wavenumbers 1710.85 and 1726.29 had stretching C-O group. Seven peaks from 2142.9 to 2243.2 cm-1 showed C-N group. The lowest peak was 634.5 cm-1, while highest peak was 3670.5 cm-1. A similar study on characterization of keratin was held by [65]. The α and β presence was confirmed and the peaks were visible between 1600 and 1700 cm-1 [66]. There were further peaks that can be seen at 1951cm-1, 1311cm-1, 1556-1587cm-1, and 1228-1251cm-1, which indicate the presence of glutamic acid, cysteine, tryptophan, and amide III, respectively [2]. After comparing these results with our study, it was confirmed that the extracted product from feathers of chicken had protein in it.

The transmission band in the range of 1700-1600 cm-1 and 1580-1540 cm-1 were attributed to Amide I and Amide II respectively [67-69]. The appearance of weak band in the range of 1300-1220 cm-1 is representative of Amide III band that is derived from N-H Bending and C-N stretching [70,71] as well as signals from C-C stretching and C=O bending [72,73]. The impression of Amide I-III bands confirms the presence of proteins and alterations in the protein structure [74]. Amide I signifies the combination of α-helix as well as β-sheet [75,76], and Amide III can be ascribed to β-sheet [77].

The result of XRD analysis confirmed the absence of a uniform geometrical pattern of atoms. The size of particles of keratin and crystallinity were found to be 6nm and 83% respectively. The current study confirmed the semi-crystalline nature of keratin. In a study, most part of the keratin polypeptides compared with raw feathers transpired the amorphous form instead of crystal [78]. Hydrolysis of feathers of chicken lead to broad peaks at 22.44^0 and 11.24^0 while it was shown in our study that broad lines caused peaks at 10.2^0 and 23.25^0. The peaks with this broadness showed that the α-helix and β-sheets that are present in raw feathers of chicken are deformed and broken due to the process of alkaline hydrolysis for keratin extraction. The two strong peaks at $9\text{-}11^0$ and $15\text{-}31^0$ were allocated to α-helix and β-sheet respectively [79-81]. It is evident from a study that the chicken feather and extracted keratin held small amount of α-helix, whereas, significant amount of β-sheet conformation [82].

Moreover, the Scanning Electron Microscopy (SEM) presented the roughness in the structure of keratin protein. Porous structure was visible in the images with pores at some distance. Similar results were observed by [83]. The regenerated keratin had small micro-particles with randomly distributed porous microstructures. These images interpret that there was a loss of smoothness of the surface because of use of alkaline material. There was a white patch with no particular shape and it showed roughness of structure of keratin protein. The particles were rough with patches at irregular intervals which was similar to the results obtained from [36] where the modified keratin showed dissimilar patterns of microstructures and brightness in surface of keratin. These are attributed to the amorphous structure of the keratin. Additionally, it was peculiar to note that that surface morphology of keratin was changed by the treatment and purification processes undergone to extract keratin. The ability of sorption increased because of these surface changes and the breakdown of peptide chain bonds as well as denaturation of helical structure [84] owing to the exposure of more functional groups on the surface [34].

A study conducted by [84] reported the adsorption potential of keratin derived biopolymers (KBP) from chicken feathers in the decontamination of metallic ions from synthetic waste water. KBP-IV manifested the adsorption capacity of 80-85% for Cu and 91% for Cr. Another keratin based polymer i.e., KBP-V showed 60-90% removal efficiency for Zn, Ni and Co. This highlights the potential of keratin from chicken feathers for the treatment of multi-metal contaminated industrial wastewater. In another study, keratin/polyamide blend 6 (90/10) nanofibres manifested 94% and 44% removal efficiency for Cu2+ with an initial concentration of 0.05 and 35mg/L respectively [85].

Batch sorption experiments were conducted to investigate the efficacy of extracted keratin adsorbent to remove zinc and copper. In current study, the optimum pH was found to be 5.0 for the removal of metallic ions. An organic ligand-based composite adsorbent exhibited high functionality at pH 4.0 for the removal of Cu (II) from wastewater in a study conducted by [86]. Several studies reported the high adsorption ability at slightly acidic pH [87-90]. Kinetic studies had shown that 8 hours of contact time was appropriate for equilibrium to be achieved. However, 24 hours were needed to achieve equilibrium for Pb absorption by [91] and most of the sorption took place in the first hour which is similar to our research. After one hour, the rate of adsorption slowed down. It was explained by [33] that the adsorbent surface was filled with metal ions and the capacity for metal sorption slowed down because of saturation of metallic ions on the reactive surface of keratin adsorbent. Likewise, in another study that utilized chitosan-cotton composite for the encapsulation of toxic Remazol Red (RR) reactive dye, it was found that in the commencing stage, the adsorption efficiency was high owing to the accessibility of active site [92].

From the Freundlich isotherm model, it was evident that the adsorption behavior for copper and zinc ions yielded higher regression coefficient (R^2) values. In a study, the Freundlich model best decribed the favorable mechanism for As^{III} adsorption based on high R^2 value [93]. Based on high value of Kf, the Freundlich model describes the better adsorption capability of keratin for copper ions as compared to zinc.For zinc, the Kf and n values were computed to be 0.0977 and 1.8975 respectively. For copper, the values for Kf and n were 0.1860 and 1.2873. A study reported the values of Freundlich constants Kf and n for copper adsorption to be 0.010 and 1.58 respectively [94]. Moreover, the Freundlich model of the linearization showed the higher pH proved in greater uptake of metal ions. Similar was the case for adsorption of lead where increase in pH caused increase in lead uptake by recycled feathers of chicken [90]. Several studies considered the significance of temperature as an intitial factor for the heavy metal removal from aqueous solution thorugh adsorption [95,96]. In order to obtain highest Cu^{2+} adsorption efficiencies, the effect of temperature in the range of 30^0C to 50^0C was studied. The optimum temperature was found to be 30^0C [97]. The current study favored the efficient removal of Cu^{2+} and Zn^{2+} at 25^0C. [84] also treated water solution from keratin and removed 56% zinc and 75% copper. This proves that the keratin extracted from chicken feathers was a suitable source of metal removal from water.

CONCLUSION

This research highlights the potential of extracted keratin from chicken feathers, huge waste from poultry industry for the minimization of environmental burden and its application as an emerging source for the treatment of contaminated water. Keratin was extracted from chicken feathers using sodium sulfide as a reducing agent. The characterization of extracted keratin confirmed the semi-crystalline structure from XRD and SEM. The removal percentage of heavy metals i.e., zinc and copper was computed to be 52% and 69% respectively. For the potential of cost-effectiveness, the elution study of extracted keratin from chicken feathers is recommended.

Funding: This research received no external funding.
Acknowledgments: We are grateful to the chairperson of the Department of Environmental Science for administrative support, and indeed the laboratory staff.
Conflicts of Interest: The authors declare no conflict of interest.

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