

**EGE UNIVERSITY GRADUATE SCHOOL OF APPLIED AND
NATURAL SCIENCES
(MASTER OF SCIENCE THESIS)**

**REMOVAL OF HEAVY METAL IONS
FROM AQUEOUS SOLUTIONS
BY KERATIN**

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Chemical Engineering
603.01.00

August, 2008

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Bornova, Izmir

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Saniye KOCATÜRK tarafından **Yüksel Lisans** tezi olarak sunulan **“Removal of Heavy Metal Ions from Aqueous Solutions by Keratin”** başlıklı bu çalışma, E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve **20.08.2008** tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

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ÖZET**SULU ÇÖZELTİLERDEN AĞIR METAL İYONLARININ
KERATİN İLE UZAKLAŞTIRILMASI**

KOCATÜRK, Saniye

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Ağustos, 2008, 126 sayfa

Bu çalışmada tavuk tüyünün ağır metal iyonlarını (bakır, çinko, nikel ve kadmiyum) atık sulardan uzaklaştırma kapasitesi araştırılmıştır. Bu kapasitenin artırılması için sodyum hidroksit (NaOH), asetik asit (AA) veya sitrik asit (SA) ile modifiye edilen veya ısıtılma tabii tutularan tüyün adsorplama kapasitesii ölçülmüştür. Elde edilen tavuk tüyü örnekleri arasında adsorpsiyon kapasitesinde en yüksek artış SA ile modifikasyonda gözlenmiştir. Langmuir, Freundlich ve DKR izoterm modelleri incelendiğinde tüm örnekler için DKR modelinin uygun olduğu görülmüştür.

Ağır metal iyonlarının SA ile modifiye edilmiş tavuk tüyleri üzerine adsorpsiyonunda, adsorpsiyon mekanizmasını ortaya çıkartmak için kinetik çalışmaları sözde birinci mertebe, sözde ikinci mertebe ve Weber-Morris partikül içi difüzyon modelleriyle araştırılmış ve sözde ikinci mertebe modelinin en uygun model olduğu görülmüştür.

VI

ATR-FTIR analizleri yapılarak protein yüzeyinde modifikasyon sonucunda oluşan değişiklikler tespit edilmeye çalışılmıştır.

Yapılan analizler sonunda protein yapısında keratinden oluşan tüyün, karboksil, amin, amid gibi fonksiyonel gruplar içerdiği görülmüştür. Metal iyonları bu fonksiyonel gruplar ile etkileşmektedirler. İşlem görmemiş, sadece NaOH ile işlem görmüş, AA ile ve SA ile işlem görmüş tavuk tüyleri için sırasıyla kadmiyum ile 1; 3,5; 4,5; 6,5 mg/g, bakır iyonu ile 1,8; 3,2; 4,0; 6,5 mg/g, nikel iyonu ile 0,7; 2,0; 2,5; 3,6 mg/g ve çinko iyonu ile 0,7; 2,0; 2,8; 3,4 mg/g adsorplama kapasitesi elde edilmiştir. Bütün metal iyonları için en yüksek adsorplama kapasitesi SA ile işlem görmüş TT ile ulaşılmıştır. İşlem görmemiş tavuk tüyleriyle kıyaslandığında yaklaşık olarak Cd^{+2} için %85, Cu^{+2} için %72, Ni^{+2} için %80 ve Zn^{+2} için %79 kapasite artışı görülmüştür.

Anahtar Sözcükler: Ağır metal iyonları, protein, keratin, tavuk tüyü, adsorpsiyon, ATR-FTIR.

ABSTRACT

**REMOVAL OF HEAVY METAL IONS FROM AQUEOUS
SOLUTIONS BY KERATIN**

KOCATÜRK, Saniye

Master Science Thesis, Department of Chemical Engineering

Supervisor: Assoc. Prof. Dr. Günseli ÖZDEMİR

August, 2008, 126 pages

The aim of this study is to investigate the capacity of chicken feathers (CF) in the removal of heavy metal ions from waste water. For this aim, untreated and treated CF were used in the removal of cadmium, nickel, zinc and copper ions from their dilute aqueous solutions. Chicken feathers were modified with sodium hydroxide (NaOH), acetic acid (AA) and citric acid (CA) to increase the uptake of the heavy metal ions. The highest uptake capacity was reached for all heavy metal ions with CF modified with CA. Langmuir, Freundlich and DKR isotherm models were studied and results indicated that the adsorption equilibrium data fitted well to DKR isotherm model.

In this investigation, pseudo-first order, pseudo-second order and Weber-Morris intra-particle diffusion kinetics models were studied to evaluate the adsorption kinetics mechanism. The results showed that the sorption can be approximated more appropriately by the pseudo-second order kinetic model for the sorption of metal ions onto CF modified with CA.

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SEM photographs and ATR-FTIR spectra of chicken feathers (CF) (unmodified, modified with NaOH, AA and CA) were taken.

For CF which is composed of fibrous proteinaceous material known as keratin, coordination was shown to play an important role in metal binding with COO^- , SH, aromatic side chains and $-\text{NH}_2$ groups. These bands were observed by ATR-FTIR analysis. By interaction of these groups, for untreated CF, CF modified with NaOH, AA and CA, respectively, for Cd^{2+} , 1, 3.5, 4.5, 6.5 mg/g, for Cu^{2+} 1.8; 3.2; 4.0; 6.5 mg/g, for Ni^{2+} , 0.7; 2.0; 2.5; 3.6 mg/g and for Zn^{2+} 0.7; 2.0; 2.8; 3.4 mg/g adsorption capacities were obtained. For all heavy metal ions the highest uptake capacities were reached with CF modified with CA. Comparing with unmodified CF, the uptake capacities were increased by 85% for Cd^{2+} , 72% for Cu^{2+} , 80% for Ni^{2+} and 79% for Zn^{2+} when CF was modified with CA.

Key words: Heavy metal ions, protein, keratin, chicken feather, adsorption, FTIR/ ATR.

ACKNOWLEDGEMENT

I would like to thank my supervisor, Assoc. Prof. Dr. Günseli ÖZDEMİR for suggesting me to investigate such an interesting and necessary subject. I am deeply indebted to her for valuable suggestions and encouragement that helped me during the time of research, experimental study and writing of this thesis.

I would like to give my special thanks to my family, whose patience and support enabled me to complete this work

I also would like to thank Mehmet Akçay for AAS analysis, Gönül Yavuz for ATR-FTIR analysis and Ege University for financing the project 06-MÜH-023.

Saniye Kocatürk

August 2008, Bornova

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NOMENCLATURE

C_{in}	:the initial metal ion concentration (mg/L)
C_{out}	: effluent concentration(mg/L)
E	:free energy(kJ/mol)
I	:thickness of the boundary layer
$k_{1,2}$	:kinetic rate constants
K_F	: Freundlich adsorption constant
K_{id}	:kinetic constant for intra particle diffusion model(mg.g ⁻¹ .min ^{-1/2}).
K_L	:Langmuir adsorption constant
M_w	:molecular weight (g/mol)
n	:Freundlich adsorption constant
pK_a	:hydrolysis constants
pK_{sp}	:solubility product constants
q_e	: concentration in the equilibrium at any time (mg/g)
q_{max}	: maximum uptake per g of adsorbent in the batch system(mg/g)
R	:the gas constant (kJ/ mol.K)
r^2	:correlation coefficients
T	:the absolute temperature (K)
t	:the shaking timetime(min.)
α	:the initial adsorption rate (mg/g.min)
β	:(polanyi potential)

1. INTRODUCTION

Heavy metal pollution of water is an extremely serious environmental problem which has emerged in recent years due to the development of industries that transmit heavy metal ions to water (Banat et al., 2002). Removal of heavy metals from aqueous solutions is necessary because of the frequent appearance of heavy metals [Zn^{2+} , Cu^{2+} , Cd^{2+} , Ni^{2+} etc.] in waste waters from many industries, including electroplating, metallurgical, chemical manufacturing, mining and battery manufacturing industries (Markovska et al., 2006). It has been well established that excess cadmium exposure produces adverse health effects on human beings. For virtually all chemicals, adverse health effects are noted at sufficiently high total exposures. For certain elements such as copper and zinc which are essential to human life, a deficiency as well as an excess can cause adverse health effects (http://www.cadmium.org/env_exp.html).

High exposure to cadmium, particularly if it is inhaled, has been linked to a range of health problems, including certain lung diseases (http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/Pollution_air?OpenDocument). Copper is particularly toxic, complexing with enzymes and other metabolic agents connected with respiration and rendering them inactive. Copper is an irritant to skin causing itching and dermatitis, and may result in keratinization of the hand and soles of the feet (Banat et al., 2002). Zinc toxicity (in doses > 80 mg/day) may also decrease levels of HDL-cholesterol and white blood cells. Impaired cholesterol metabolism may also result from excess intake of zinc supplements (<http://www.advancehealth.com/zinc.html#Zinc%20Toxicity>). Certain nickel-containing substances may be toxic under

certain conditions. Two principal adverse health effects have been associated with nickel under certain circumstances, one is skin allergy and the other is lung or nasal cancer ([http://www.nidi.org/index.cfm/ci_id/101/la_id/1.htm# Nickel % 20Use](http://www.nidi.org/index.cfm/ci_id/101/la_id/1.htm#Nickel%20Use)). Because of the hazardous effect of heavy metals, waste water that contain heavy metal ions should be treated to reduce such contents to acceptable levels before being discharged to the environment (Banat et al., 2002).

Many physicochemical methods have been developed for heavy metal removal from aqueous solution; including, chemical coagulation, adsorption, extraction, ion exchange and membrane separation process (Markovska et al., 2006). These technologies either have the inherent problem of sludge handling and disposal and/or are best suited for solutions having high metal concentrations (Kar et al., 2004). Among these methods, adsorption is a highly popular one and has been widely practiced in industrial waste water treatment processes. The major advantages of an adsorption system for water pollution control are less investment in terms of initial cost and land, simple design and easy operation, and no effect of toxic substances compared to conventional biological treatment processes (Markovska et al., 2006). However, since conventional water treatment materials are relatively expensive and require complex operational set-up and safety precautions, there is an increasing interest in the use of biomass for removal of dissolved metals from aqueous solutions. A variety of low cost biomass has been developed and commercialized for controlling pollution from diverse sources. Examples for such low-cost adsorbents are clay, activated carbon, tea leaves, agricultural waste, cellulose, wool fiber, fly ash, peanut hull pellets, animal bones, pine barks and immobilized fungal biomass (Kar et al., 2004).

The aim of this study is to remove heavy metal ions from waste water by using chicken feathers. Chicken feathers are composed of fibrous proteinaceous material known as keratin. Keratin has a complicated structure that exhibits a large surface area (Banat et al., 2002). Moreover, the keratin fibers' properties, like high tensile strength and water insolubility, make it more attractive for biosorption.

As chicken consumption in the world keeps growing, feather generation, as a by-product, will also grow. Figuring out what to do with all these feathers can be a real headache. Most farmers, at present, throw them away or grind them up and mix them into animal feed to add protein to animal diets. The success in using chicken feathers in decontamination of waste water would add value to this by-product. Chicken feathers were capable of adsorbing precious metal ions selectively from their dilute aqueous solutions with high yield in short contact time. The uptake of metal ions was found to be pH dependent. Nonetheless, little work has been done using biosorbents for the removal of organic pollutants such as phenol from aqueous and non-aqueous solutions (Banat et al., 2000).

Therefore, in the present work, chicken feather and treated chicken feather was used as an adsorbent to improve the removal of cadmium, nickel, zinc and copper ions from their dilute solutions. In the study the chicken feathers washed, dried and cut into small filaments with a length of 1 mm were modified with different chemicals such as sodium hydroxide (NaOH), acetic acid (AA) and citric acid (CA) to increase the uptake of the heavy metal ions. The treated chicken feathers were repeatedly washed with deionized water before adsorption experiments were accomplished.

Heavy metal stock solutions were prepared using their nitrate salts, 1-19 mg/L Cu^{2+} , 1-31 mg/L Ni^{2+} , 1-15 mg/L Zn^{2+} and 1-11 mg/L Cd^{2+} solutions obtained by dilution. 0.05 g of feathers was put in contact with 25 ml of solutions and batch equilibrium adsorption tests and kinetic studies were conducted in conical flasks with the desired adsorbent to adsorbate ratio. The suspensions were agitated by shaking in a water bath shaker at 175 revolutions per minute (rpm). The conditioning temperature was adjusted and maintained at the desired level. The conditioned solutions were filtered after a desired time and analyzed by AAS.

SEM photographs and ATR-FTIR spectra of chicken feathers (CF) (unmodified, modified with NaOH, AA and CA) were taken.

2. LITERATURE REVIEW

Heavy metals are on the forefront of academic and regulatory concern, since millions of tons of water containing toxic heavy metals are generated annually from several metal processing industries and discharged into the environment. Metals discharged into water bodies are not biodegraded but undergoes chemical or microbial transformations, creating large impact on the environment and public health. Therefore, increasing awareness is rapidly growing worldwide and one of the offshoots of it is treatment and removal of heavy metals from such effluents to permissible limits before discharging into natural streams and waters (Joshi, 2003).

Kar et al., (2004) studied separation of heavy metals from water using nano-porous keratin fibers over a wide range of pH. They used down feathers of chicken with pores of size in the range of 0.05–0.10 μm . Keratin fibers extracted from down feathers of chicken were used for enhanced metal uptake after ultrasonically processed and size reduced.

Ultrasonic treatment was carried out in batch and continuous methods. They concluded that:

-Keratin protein fiber has demonstrated a good capacity as a medium for purification of copper, lead, chromium, mercury and uranium from solutions having low concentrations of contaminant. In the natural pH range (pH 5.0–7.0) the keratin protein fibers have unique selectivity for lead and copper, and at acidic pH for removal of chromium and mercury. Its stability and high tensile strength rendered it reusable,

applicable in a broad pH range and adaptable to pre-processing for improved uptake.

-The surface hydrophobicity of keratin protein fibers made mixing difficult in aqueous solutions, thereby limiting adsorbate–adsorbent contact and the metal uptake. The metal uptake was increased significantly by the column mode due to the nonexistence of difficulties such as mixing.

-Alkaline ultrasonic processing, by the cavitation effect, increased the surface area. It was also believed that due to processing, hydroxyl ions became embedded in the surface and caused surface precipitation of metal cations, thereby improving uptake.

-Though it was difficult to reduce particle size, grinding increased the surface area and exposed the surface functional groups responsible for sorption, thereby increasing metal uptake.

-Adsorption of heavy metals like copper and lead was steadily improved with increasing temperature.

Augustine et al., (2007) studied adsorption kinetics and modeling of Cu^{2+} ion sorption from aqueous solution by mercaptoacetic acid modified cassava (*manihot sculenta cranz*) wastes. They studied the rate of removal of Cu^{2+} ions from aqueous solution by 0.5M and 1.0M mercaptoacetic acid modified cassava fibre wastes (0.5 MCF and 1.0 MCF) in batch conditions. They claimed that the rate of sorption of copper was rapid initially within 5-15 minutes and reached a maximum in 30 minutes. Kinetic modeling analysis of the Elovich, pseudo-first order, pseudo-second order, intra-particle diffusion, mass transfer and

intra-particle diffusivity equations using the correlation coefficients, r^2 values, showed that the pseudo-second order equation was the most appropriate model for the description of Cu^{2+} transport. Thus the sorption of Cu^{2+} ion followed a pseudo-second order model, with chemical sorption as its rate limiting step. The initial adsorption rate values were: 2.94×10^{-1} and $2.60 \times 10^{-1} \text{ mg.g}^{-1}.\text{min}^{-1}$ for 0.5 MCF and 1.0 MCF adsorbents, respectively.

Douglas et al., (2006) studied adsorption of Cu^{2+} ion from aqueous solution using soybean hulls. Their aim was to study the efficiency of removing Cu^{2+} ions from CuCl_2 solution using soybean hulls. The experiment was divided into two parts. In the first part, soybean hulls were modified with citric acid by placing the soybean hulls first in 0.1N NaOH and then in 0.6 M citric acid. Then they have determined the time to heat the modified soybean hulls. In the second part the factors affecting copper ion adsorption by soybean hulls were determined as initial concentration and pH of the solution, ratio of soybean hulls to copper chloride concentration in solution and size of the soybean hulls. From the results of the experiments they have obtained that the optimum conditions for heating was 90 minutes at an initial solution pH of 4.8. The ratio of soybean hulls to solution was set 10 grams per 1 liter. The size of soybean hulls was 602 micrometers and the initial concentration of copper chloride solution was 50 ppm. The copper ion removal efficiency using soybean hulls was 97.68 % under these conditions and the concentration of copper ion has been reduced below the industrial standard of 2.0 (mg/L).

Mc Sweeny et al. (2006) studied the effect of citric acid modification of aspen wood on sorption of copper ion. Milled aspen wood was thermochemically modified with citric acid for the purpose of

improving the Cu^{2+} ion sorption capacity of the wood when tested in 24-hour equilibrium batch tests. They claimed that the wood-citric acid adducts provided additional carboxyl groups to those in the native wood and substantially increased Cu^{2+} ion uptake of the modified wood compared with that of the unmodified wood. Sorption capacity (q_e) measured with an unbuffered standard solution increased to a maximum of 7.9 mg Cu^{2+} ion/g of wood (treated) from 1.3 mg Cu^{2+} ion/g wood (untreated). When measured with a buffered standard solution, the q_e increased to a maximum of 13.8 mg Cu^{2+} ion/g of wood (treated) from 4.1 mg Cu^{2+} ion/g wood (untreated). The treatment necessary for maximum q_e was 2 hours at 130°C. Modification treatments included three time periods (2, 4, 6 hours) and three temperature regimes (110, 120, 130°C). To further evaluate the efficacy of modification treatments, weight change after treatment was monitored as an indirect measure of bound citric acid. It was found that increases to the original mass of greater than about 30% were associated with no further increase or a decline in q_e . The contribution of citric acid ester linkages to increasing mass at longer reaction times was monitored with ATR-FTIR.

Sheffield et al., (2005) studied the uptake of Cu^{2+} ions by wool. The uptake of Cu^{2+} onto wool from aqueous solutions of Cu^{2+} sulfate was investigated as a function of treatment time and temperature, amount of Cu^{2+} ions applied, and pH. They found that longer heating times and higher temperatures lead to increased uptake: 1 hour near the boiling temperature gave optimal results. Increasing the amount of applied Cu^{2+} ions increased the total uptake, but the fraction of applied Cu^{2+} ions taken up decreased with the amount applied. As pH was increased, Cu^{2+} uptake increased linearly with pH up to a pH of approximately 6; above pH 6, solid copper salts were precipitated. The possibility of specific buffer interference with Cu^{2+} uptake was discussed.

Tonin et al. (2004) studied thermoanalytical characterization of modified keratin fibers. Sheep's wool was chemically treated with reductive agents such as sodium bisulphite, then stretched and set. They claimed that treatment led to the modifications of the original protein structure causing changes in thermal behavior, dyeing, coloristic and wet resistance properties. A multidisciplinary approach was used to investigate treated and untreated wools, with the aim of exploiting the nature of the structural changes. SEM and TEM revealed changes on the cuticle and cortical cell morphology; structure modifications were studied by FT-IR and DSC.

Taddei et al. (2003) studied binding of Co^{2+} and Cu^{2+} ions to chemically modified wool fibers by IR analysis. Wool fibers were modified by treatment with tannic acid (TA) solution or by acylation with ethylenediamine tetraacetic (EDTA) dianhydride. The unmodified and modified fibers were subsequently treated with Cu^{2+} and Co^{2+} solutions, at alkaline pH, and analyzed by ATR-FTIR spectroscopy to evaluate the changes induced in the structure of the fiber by metal binding. The spectral changes were correlated to metal adsorption results obtained by Inductive Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). The IR results were discussed in relation to their previous findings on the metal binding mode of *Bombyx mori* and Tussah silk fibers; the changes observed in the spectra were explained by considering the different affinity of the fibers for the modifying reagent and the amount of the metal adsorbed. More relevant spectral changes were observed upon Cu^{2+} ion complexation rather than Co^{2+} ion complexation, according to the metal adsorption results. The most relevant changes were observed for the EDTA modified wool sample treated with Cu^{2+} ions, according to the higher affinity of wool for EDTA. The IR spectra were quantitatively evaluated by the intensity ratio between the Amide I and Amide II bands

(Intensity of Amide I peak/Intensity of Amide II peak) and its trend as a function of metal adsorption was reported. The investigation demonstrated that the interaction between fiber and metal and the subsequent fiber modification depended on the chemical nature of the fiber, the metal ion and the modifying reagent.

Banat et al. (2002) studied the different keratin-composed biosorbents for the removal of heavy metal ions from aqueous solutions. They examined and compared the ability of chicken feathers, human hair and animal horns, as keratin-composed biosorbents, for the removal of Zn^{2+} and Cu^{2+} ions from single metal ion aqueous solutions under different operating conditions. The three biosorbents they studied were all capable of adsorbing Zn^{2+} and Cu^{2+} ions from aqueous solutions. The biosorbent showing the highest uptake of Zn^{2+} and Cu^{2+} ions was animal horns. Chicken feathers showed a higher Cu^{2+} ion uptake compared to Zn^{2+} ions, which showed a higher uptake to human hair. Increasing the initial concentration of Zn^{2+} or Cu^{2+} ions, or increasing the initial pH value, increased the metal ion uptake. They have further found that such uptake decreased when the temperature was raised from 25 °C to 50 °C for all adsorbent/metal ion combinations except for Zn^{2+} ion / human hair where the uptake increased with temperature. It was demonstrated that the addition of NaCl salt to the metal ion solution depressed the metal ion uptake. The Freundlich isotherm model was found to be applicable to the adsorption equilibrium data for Cu^{2+} and Zn^{2+} ions.

Balköse et al. (1992) studied adsorption kinetics of heavy metal ions from aqueous solutions by wool fibers. Adsorption of heavy metal ions by sheep's wool from aqueous NiCl_2 , CuCl_2 , CdCl_2 , HgCl_2 and $\text{Pb}(\text{NO}_3)_2$ solutions at 25 °C and 50 °C was investigated using atomic absorption spectroscopy (AAS). A fiber diffusion controlled adsorption

rate model was used to predict the effective diffusion coefficients of metal ions in wool. It had been shown that wool was a potential adsorbent for removing toxic metal ions from contaminated water. The apparent diffusion coefficients for the initial adsorption stage of metal ions in wool at 50 °C were reported for Cu^{2+} , Zn^{2+} , and Ni^{2+} ions as $0.25 \times 10^{-14} \text{ m}^2\text{s}^{-1}$, $1.2 \times 10^{-14} \text{ m}^2\text{s}^{-1}$ and $1.0 \times 10^{-14} \text{ m}^2\text{s}^{-1}$, respectively.

Miura et al. (1985) studied selective preconcentration of Cu^{2+} ions from aqueous solution by using keratine gel. The gel was prepared by partial oxidation of keratine which was obtained by reducing the disulfide bonds of wool fiber. Cu^{2+} was selectively adsorbed on the gel at pH 3.5. It was eluted with 3M hydrochloric acid and was determined by AAS. That method had been applied to the determination of Cu^{2+} ions in river water. They showed that the Cu^{2+} uptake increased with increasing amount of Cu^{2+} ions added. Less than 80 μg (1.26 μmole) of Cu^{2+} ions in 25 ml of aqueous phase was completely recovered by 20 mg (4.28 $\mu\text{mole-SH}$) of the gel.

Gutherie et al. (1968) studied the binding of Cu^{2+} to mohair keratin. Measurements of the uptake of Cu^{2+} ions by insoluble keratin, mohair, as a function of Cu^{2+} ion concentration and of pH, between 3 and 6, were given. From these and other data on Cu^{2+} ion uptake and its effects the conclusion was drawn that carboxyl groups were the primary, but not the sole sites of Cu^{2+} ions binding in mohair keratin below pH 5. The data presented showed the side-chain carboxyl groups to be the main binding-sites for Cu^{2+} ions in keratins.

3. THEORY

3.1. Water pollution

Water covers 71 % of the planet's surface and exists mainly in the form of salty water in the oceans. It is a vital substance for supporting life on Earth. For example, a tree contains 60% water; most animals are composed of about 65% water, while our bodies contain around 55% water. Everyone needs fresh water everyday to cover the daily demand in food, domestic use, etc. Fresh water is used in agriculture, construction, transport, chemical industry, and numerous other activities of human beings. The use of abstracted water in Europe is presented in Figure 3.1.

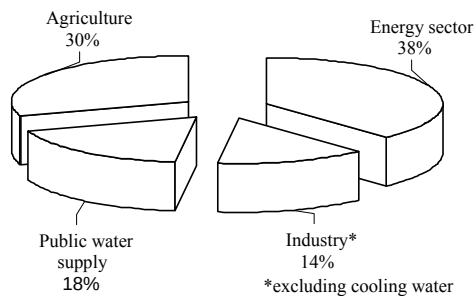


Figure 3.1: The use of abstracted water in Europe (Inglezakis et al., 2006).

Despite the fact that most of the planet is covered by water, only a small amount of this water is available as fresh water. Almost 97.5% of the total is in oceans in the form of salty water and is not suitable for drinking, watering, or industrial use as is. The remaining 2.5% is fresh

water. However, not even that small amount is easily accessible or exploited, because it is stored as ice on the poles and on mountain tops. Furthermore, a significant amount of the rest lies so deep in the ground that it is very difficult to extract. In Figure 3.2, the distribution of water on Earth is presented.

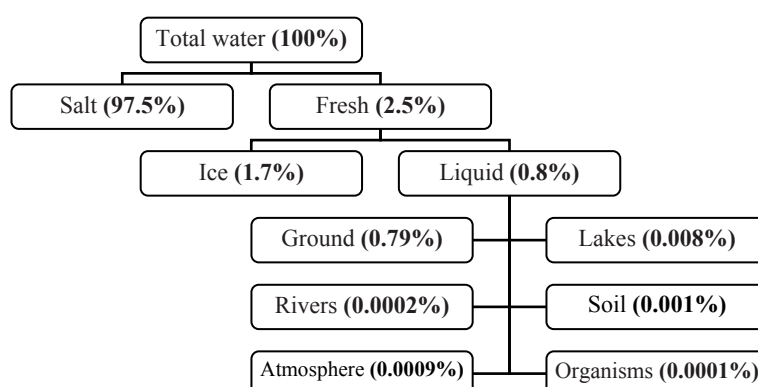


Figure 3.2: Water on earth (Inglezakis et al., 2006).

According to the European Community (EC), less than 1% of the planet's water is available for human consumption and more than 1.2 billion people in the world have no access to safe drinking water. Fresh water is purified and reallocated through the hydrological cycle in nature. Nowadays, this natural process is inadequate due to human activities, and specifically because of the thoughtless wasting of water and discharge of various pollutants into the aquatic environment. These activities of human beings threaten not only the fresh water supply but also marine life. Moreover, with an ever-increasing world population, the situation is expected to worsen in the near future, especially in densely populated or industrial areas. These areas consume large amounts of fresh water, and at the same time produce and release large amounts of waste water into

the environment. Water-quality deterioration can be attributed to water pollution or contamination. Water pollution is generally defined as any physical, chemical, or biological alteration in water quality that has a negative impact on living organisms. In the stricter sense, pollution can be defined as the transfer of any substance to the environment. However, there is a tolerance limit for each pollutant, since zero-level pollution is economically and technically unpractical. The most important kinds of water quality deterioration are the following.

- Thermal pollution
- Biological pollution by oxygen-demanding wastes
- Pollution by persistent organic chemicals (POPs)
- Eutrophication by nitrates and phosphorus
- Inorganic pollutants

In Table 3.1, the direct releases of the most important pollutants into water are presented in association with the corresponding main industrial source. For purposes of comparison, it is useful here to recall that road transport and combustion installations, mainly of the energy sector, are the most important sources of air pollutants. In the case of water bodies, it is obvious that agriculture and the metal industry, plus the activities in the production of inorganic chemicals and fertilizers, constitute the major polluters.

Table 3.1: The releases of the main pollutants charged directly into water from the industrial sector in Europe 2001 (Inglezakis et al., 2006).

Compound	t/yr	Main source
Phenols	1,419,344	Basic inorganic chemicals or fertilizers (47%)
Total organic carbon	246,524	Industrial plants for pulp from timber or other fibrous materials, and paper or board production (70%)
Nitrogen	22,317	Basic inorganic chemicals or fertilizers (29%)
Phosphorus	1662	Basic inorganic chemicals or fertilizers (25%)
Chromium	864	Metal industry (87%)
BTEX	82.5	Basic organic chemicals (56.1%)
Nickel	71.5	Metal industry (45%)
Copper	45.8	Metal industry (23%)
Lead	41.7	Metal industry (40%)
Polycyclic aromatics	10.3	Metal industry (74%) hydrocarbons
Cadmium	8.1	Metal industry (74%) hydrocarbons
Arsenic	5.1	Metal industry (22%)
Mercury	0.5	Metal industry (23%)

3.1.1 Inorganic Pollutants

Metals, nonmetals, and acids/bases released by human activities severely deteriorate water quality, since they are toxic even at concentrations of parts per million. It has to be noted that heavy metals are extremely dangerous to human health and aquatic life. But what is worse is that there is no cycle of natural treatment of these substances.

Inevitably, heavy metals remain intact in the environment and finally, they are accumulated in the food chain (bioaccumulation). Unless it has been taken the right course of action, problems associated with both quality and quantity of water are going to be encountered, even in regions that seem to have sufficient amounts of clean water today. After the disputes and wars over the possession of oil in the past, water may be the next conflict territory between adjacent countries, even in Europe. According to the EC, 20% of all surface water in the European Union is seriously threatened with pollution. Furthermore, water is far from being evenly distributed in Europe and this is one major reason for resource problems (Kraemer et al., 2001).

The major amount of waste water in the chemical industry does not come from chemical reaction steps, but from the subsequent physicochemical processing of the final reaction mixture.

Heavy metal ions are among the most important pollutants of water. They are generally considered to be those whose density exceeds 5 g/cm^3 . Characteristic examples of toxic heavy metal ions are cadmium (Cd^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), mercury (Hg^{2+}), nickel (Ni^{2+}) and zinc (Zn^{2+}) etc. (Green et al., 2003). Heavy metals are found in the environment due to general waste-disposal and industrial activities, including the mining industry, metal industry, coating/electroplating industry, production and deposition of batteries, burning of fossil fuels, the use of phosphate fertilizers, waste incineration, leaching from waste deposits, etc. Heavy metals can also be found naturally in the environment. Heavy metal contents of different water quality are listed on the Table 3.2.

Table 3.2: The quality parameters of surface water sources (inorganic pollution parameters) (Güler et al., 1997).

Water Quality Classes				
	high quality water	low contaminated water	wastewater	highly contaminated water
Water Quality Parameters	I	II	III	IV
Mercury (µg/L)	0.1	0.5	2	2
Cadmium (µg/L)	3	5	10	10
Lead (µg/L)	10	20	50	50
Arsenic (µg/L)	20	50	100	100
Copper (µg/L)	20	50	200	200
Chromium (Total) (µg/L)	20	50	200	200
Chromium (Cr ⁺⁶) (µg/L)	in trace amount	20	50	50
Cobalt (µg/L)	10	20	200	200
Nickel (µg /L)	20	50	200	200
Zinc (µg /L)	200	500	2000	2000
Cyanide (Total) (µg/L)	10	50	100	100
Fluoride (µg /L)	1000	1500	2000	2000
Free chlorine (Cl ₂) (µg /L)	10	10	50	50
Sulfide (S ⁻²) (µg /L)	2	2	10	10
Iron (µg /L)	300	1000	5000	5000
Manganese (µg /L)	100	500	3000	3000
Boron (µg /L)	1000	1000	1000	1000
Selenium (µg /L)	10	10	20	20
Barium (µg /L)	1000	2000	2000	2000
Aluminum (µg /L)	0.3	0.3	1	1
Radioactivity (pCi/L)				
alfa-activity	1	10	10	10
beta-activity	10	100	100	100

The metal industry is responsible for 41.68 t Pb, 8.13 t Cd, 864.25 t Cr, 45.77 t Cu, 71.55 t Ni, and 5.09 t as releases directly into water, annually.

Impact of heavy metals is immediate threat to aquatic life and human population (Inglezakis et al., 2006).

3.2. Heavy metal toxicities

The toxic heavy metals like copper, mercury, chromium, lead, zinc, nickel and cadmium cause serious impact on the aqueous environment, animals and humans (Arıca et al., 2005).

Metals and their "free radicals" are highly reactive attacking other cellular structures. The ability of metals to disrupt the function of essential biological molecules, such as protein, enzyme and DNA is major cause of their toxicity. Displacement of certain metals essential for cell by a similar metal is another cause of toxicity (Joshi, 2003).

3.2.1. Copper (Cu^{2+})

Copper is among heavy metals found in municipal and industrial waste waters. Copper is particularly toxic, complexing with enzymes and other metabolic agents connected with respiration and rendering them inactive. In addition copper is an irritant to skin causing itching and dermatitis, and may result in keratinization of the hand and soles of the feet (Banat et al., 2002).

Cu is an essential micronutrient and a component of several enzymes mainly participating in electron flow and catalyzing the redox reactions. But it becomes toxic at high concentrations (Hou et al., 2007).

Cu in addition to binding to aromatic amino acid residues in enzyme molecules, can cause oxidative damage of proteins by induction of oxidative stress associated with the production of reactive oxygen species like hydroxyl or superoxide radicals (Baldrian, 2003).

If allowed to enter the environment Cu^{2+} can cause serious potential health issues. Even at low concentrations Cu^{2+} may be harmful to humans. It has been found that absorption of excess copper results in “Wilson’s disease” where Cu^{2+} is deposited in the brain, skin, liver, pancreas and myocardium (Murphy et al., 2007).

3.2.2. Cadmium (Cd^{2+})

Cadmium is released into the environment by human activities such as phosphate fertilizers, disposal of household, municipal and industrial wastes such as pigments, batteries, plastics and metal coatings (Ünyayar et al., 2006, Jiang et al., 2007).

Cadmium nitrate is used in photographic emulsions, to color glass and porcelain, in nuclear reactors, and to produce cadmium hydroxide for use in alkaline batteries. These sources may cause enhanced soil and hence crop Cd^{2+} levels, which may lead to increases in Cd^{2+} exposure. Cd^{2+} is a particularly dangerous pollutant due to its high toxicity and great solubility in water. At low concentrations, Cd^{2+} is not toxic to plants but at higher concentrations it is toxic. Exposures to high Cd^{2+}

concentration have been found to be carcinogenic, mutagenic and teratogenic for a large number of animal species, including human beings. In several studies, it is indicated that Cd^{2+} damaged the nucleolar structure, DNA and RNA in both animal and plant cells. According to the International Agency for Research on Cancer classified Cd^{2+} is suspected as co-mutagen and human carcinogen (Ünyayar et al., 2006).

The largest sources of environmental Cd exposure are contaminated food and cigarettes. It has been observed that cadmium levels in the bodies of Alzheimer's disease (AD) patients were significantly elevated, especially in liver, plasma and brain tissues, when compared with the 'normal'. In recent years, mounting evidence is demonstrating roles for the aberrant metal homeostasis in Alzheimer's disease pathogenesis (Jiang et al., 2007).

Cd^{2+} ions have a strong affinity for sulphydryl-containing ligands such as the amino acids cysteine and histidine and have toxic effects on proteins and ion channels. In humans, Cd^{2+} produces toxic effects on the human kidney and was thought to cause Japanese Itai-itai disease, replacement of Ca^{2+} ions on bones with Cd^{2+} (Thawley et al., 2004).

3.2.3. Nickel (Ni^{2+})

Nickel is an important metal in many industries such as electroplating, nickel-based alloys and nickel-cadmium battery manufacture. In the blood circulation, it is transported bound to albumin. It is then distributed to the kidney and further into other tissues (Chaiyasith et al., 2006).

Ni holds a special place among the heavy metals. Unlike Cd, Pb, Hg, Ag, and several other metals that are not the components of plant enzymes, Ni is a constituent of urease, and small quantities of Ni (0.01 to 5 µg/g dry wt) are essential for some plant species. On the other hand, Ni is not as important for plant metabolism as Zn and Cu (Seregin et al., 2006).

Ni compounds are well-known carcinogens to humans. Several types of cellular damage have been identified that may contribute to their carcinogenic potentials, such as the induction of oxidative DNA damage and epigenetic alterations such as gene silencing by changes in DNA methylation patterns, as shown for particulate Ni compounds (Hartwig et al., 2002).

3.2.4. Zinc (Zn²⁺)

Zinc is transition metal and is essential, being found in every body cell as a constituent of proteins. Zn is essential for a number of cellular processes, including DNA synthesis, transcription, and translation, but its excess can be toxic (Thawley et al., 2004, Smalinskienė et al., 2005).

Zn toxicity (in doses > 80 mg/day) may also decrease levels of HDL-cholesterol and white blood cells. Impaired cholesterol metabolism may also result from excess intake of zinc supplements (<http://www.advance-health.com/zinc.html#Zinc%20Toxicity>).

Intraperitoneal administration of Zn²⁺ may result in increase in liver mass due to hypertrophy of the hepatocytes. Toxic effect of Zn²⁺ ions in the lung can be caused by their inhibitory action on the pathways

of RNA and protein synthesis. On the other hand, Zn^{2+} is ubiquitous element essential for normal functioning of a number of enzymes in various metabolic pathways. Concentration of Zn^{2+} ions required for enzyme activation is under tight control in cells. Metal-binding proteins known as metallo-thioneins are involved in this control (Smalinskienė et al., 2005).

3.3. Heavy Metal Removal Methods

The most commonly used treatment methods for heavy metal-containing waste include chemical precipitation, adsorption, ion exchange and bioremediation. Other methods that are less widely used include reverse osmosis, electrolysis, cementation, irradiation, membrane processes, and ion flotation (Roundhill, 2004).

However, these techniques have certain disadvantages such as incomplete removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require disposal (Arica et al., 2005).

The major advantages of an adsorption system for water pollution control are less investment in terms of initial cost and land, simple design and easy operation, and no effect of toxic substances compared to conventional biological treatment processes (Markovska et al., 2006).

For these reasons, in recent years, many researchers studied the behavior of natural organic and inorganic materials having high adsorption capacity and which are particularly abundant and inexpensive in order to use them as low-cost effective sorbents for the removal of

heavy metals from various effluents. Some of these low-cost sorbents include peat, bark, lignin, agricultural wastes, fly ash, clay minerals, activated carbon, tea leaves, cellulose, wool fiber, peanut hull pellets, animal bones and immobilized fungal biomass, etc. (Kar et al., 2004, Malandrino et al., 2006). However, in many cases this type of adsorbents does not allow to reach the heavy metal level within the legal limits imposed for waste water (0.1-3.0 mg/L for most metals) (Malandrino et al., 2006).

Chicken feathers have the ability of heavy metal removal at very low ion concentrations and help to bring heavy metal level below the imposed limits in waste waters.

3.3.1. Fundamentals of Adsorption Equilibria

The phenomenon of adsorption - the accumulation of a species at a surface is essentially an attraction of adsorbate molecules (a gaseous or liquid component) to an adsorbent surface (a porous solid). Interaction between adsorbate and adsorbent consists of molecular forces embracing permanent dipole, induced dipole and quadrupole electrostatic effects, otherwise known as van der Waal's forces. The preferential concentration of molecules in the proximity of a surface arises because the surface forces of an adsorbent solid are unsaturated. Both short range (repulsive) and longer range (attractive) forces between adsorbate and adsorbent become balanced when adsorption occurs. Adsorption is nearly always an exothermic process. The heat evolved on adsorption of a solute onto a solid from a liquid, however, is strongly dependent on the source and history of the solid adsorbent. Nevertheless the heat evolved when a porous solid is immersed in a liquid solvent containing an adsorbable

solute is of the same order of magnitude as the heat of adsorption of a saturated vapor onto a porous solid (Thomas et al., 1998).

3.3.2. Forces and Energetics of Adsorption

When a molecule having three degrees of freedom of translation approaches an unsaturated surface, at least one degree of freedom of translation is lost as a consequence of its attraction to the surface, and it is constrained to movement across the adsorbent surface. Potential energy equation incorporates a term arising from molecular attractive forces (inversely proportional to the sixth power of the separation distance between molecules) and a repulsive force (inversely proportional to the twelfth power of the separation distance). Constants multiplying each of these terms are derived from molecular susceptibilities and polarizabilities deduced from spectroscopic data (Thomas et al., 1998).

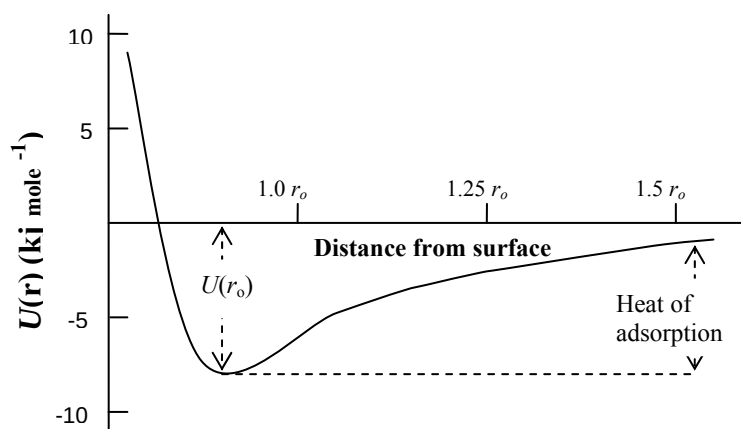


Figure 3.3: Potential energy diagram for adsorption (Thomas et al., 1998).

Clearly, when an adsorbate molecule approaches a solid adsorbent surface, the molecule interacts with a large assemblage of atoms in the crystal lattice of the adsorbent simultaneously. It is instructive to see from Figure 3.3 how the potential energy curves of an adsorbate-adsorbent system relate to experimental heats of adsorption. The potential energy function $U(r)$ (the sum of all interactions between an adsorbate molecule and molecules in the lattice of the adsorbent) passes through a minimum known as the potential well, the depth $U(r_0)$ of which is the energy of adsorption at a temperature of absolute zero. The depth corresponds to several kilojoules per mole. For a given adsorbate-adsorbent system $U(r_0)$ equates closely with measured heats of adsorption. Such heats of adsorption can be measured from calorimetric experiments or adsorption isotherms and isobars. Physical adsorption is an exothermic process and heat is always released when adsorption occurs. That this is always the case may be justified thermodynamically (Thomas et al., 1998).

During a sorption process, solute molecules or ions are essentially transferred from the solvent phase to the sorbent phase. As the binding of a solute takes place at the sorption site, the rotational and translational freedom of the solute are reduced. Hence, entropy change (ΔS) during sorption is negative. In order for the sorption to be favorable, Gibbs free energy change (ΔG) must be negative, which in turn requires the enthalpy change (ΔH) to be negative because

$$\Delta G = \Delta H - T\Delta S$$

In general, all favorable sorption processes (including ion exchange) conform to decrease in entropy. Figure 3.4 illustrates such enthalpy-driven sorption processes (Pingli et al., 2004).

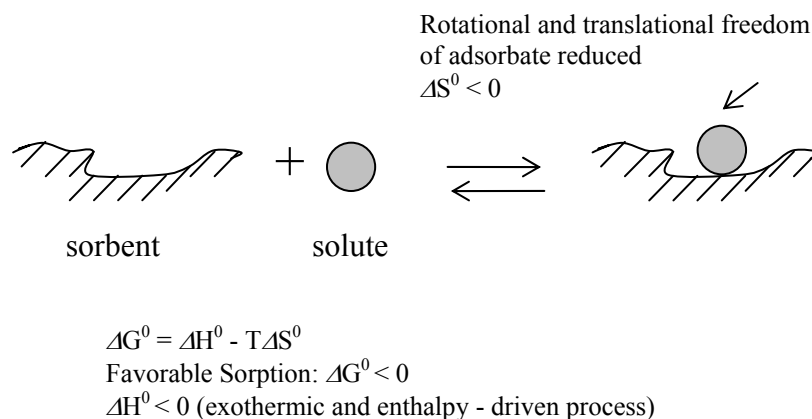


Figure 3.4: A schematic drawing illustrating an exothermic and enthalpy-driven sorption process (Pingli et al., 2004).

3.3.3. Adsorption Equilibria Models

Models are used to interpret and generalize experimental adsorption data. However, in using adsorption models for interpretative purposes, it is well to consider the admonition that the adherence of experimental adsorption data to a particular adsorption equation provides no evidence of the actual mechanism of an adsorption process. The Freundlich and Langmuir equations have historically been used, and are still being used, by investigators in many fields. Surface complexation models have recently become widely used in many scientific disciplines. All these models either explicitly or implicitly invoke the mass action law ; hence, all of these models are inherently related (Jenne, 1998).

The analysis of the adsorption equilibria begins with the classification of the isotherms. This classification is important for the modeling of adsorption phenomena and for a number of practical

reasons. For example, it is important for measuring surface area of adsorbents. The IUPAC classification scheme has six types of isotherms as shown in Fig. 3.5 (Sangwichien et al, 2002).

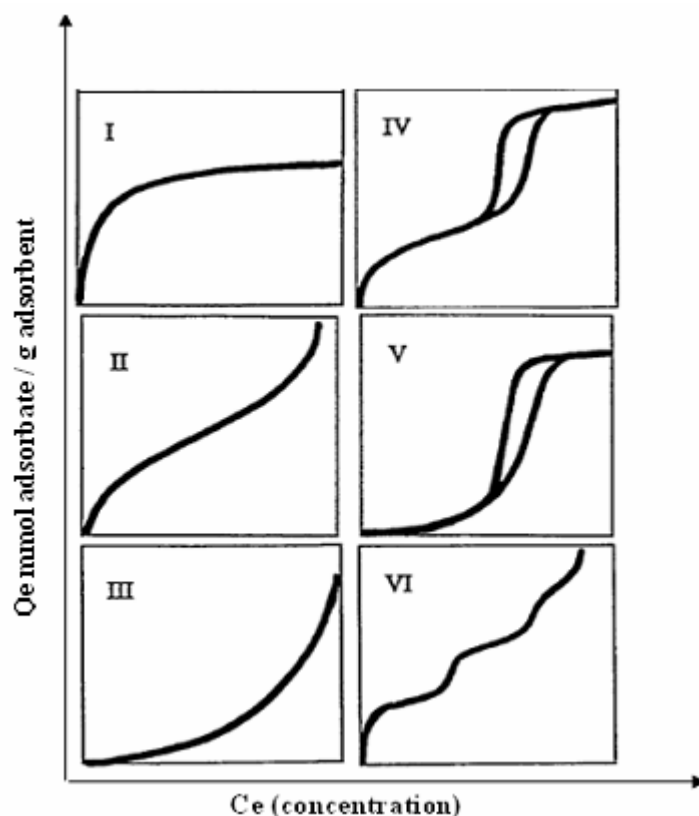


Figure 3.5: Types of different adsorption isotherms

Type I isotherm ‘approaches a limiting value’ and usually is used to describe adsorption on microporous adsorbents. Types II and III isotherms describe adsorption on macroporous adsorbents with strong and weak adsorbate-adsorbent interactions respectively. Types IV and V isotherms represent mono- and multilayer adsorption plus capillary

condensation. Type VI isotherm illustrates that the adsorption isotherms can have one or more steps.

3.3.3.1. Langmuir Isotherm Model

The Langmuir model is a monolayer adsorption model for the adsorption of adsorbate on the homogeneous surfaces.

Langmuir equation is given as

$$q_e = \frac{q_{\max} K_L C_e}{1 + K_L C_e} \quad (3.1)$$

q_e , adsorbate amount that is adsorbed on the unit adsorbent amount, (mmol/g); C_e , the concentration of excess material in the solution at equilibrium, (mmol/L); K_L , a constant related with the adsorption net enthalpy; $q_{\max}$, adsorbate amount need to form monolayer on the surface when it is adsorbed on the unit adsorbent amount, (mmol/g).

The equation obtained by linearizing the Langmuir equation;

$$\frac{1}{q_e} = \frac{1}{q_{\max}} + \left(\frac{1}{q_{\max} K_L} \right) \frac{1}{C_e} \quad (3.2)$$

3.3.3.2. Freundlich Isotherm Model

Freundlich equation considered for ideal adsorptions on heterogeneous and polluted solid surfaces, and it is a semi-empirical equation.

$$q_e = K_F C_e^{1/n} \quad (3.3)$$

where; q_e is adsorbate amount adsorbed on the solid surface, (mg/g); C_e , the equilibrium concentration of adsorbate in the liquid solution, (mg/L); K_F (constant), a measure of the capacity of the adsorbent; $1/n$ (constant), shows the intensity of the adsorption.

The equation obtained by linearizing the Freundlich equation

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (3.4)$$

3.3.3.3. Dubinin-Kaganer-Radushkevich (DKR) Model

The Dubinin–Kaganer-Radushkevich (DKR) isotherm is more general than the Langmuir isotherm because it does not assume a homogeneous surface or constant adsorption potential. It was applied to distinguish between the physical and chemical adsorption of heavy metal ions. The linear form of (DKR) isotherm equation is;

$$\ln q_e = \ln q_m - \beta \varepsilon^2 \quad (3.5)$$

where q_e is the metal ions adsorbed per unit weight of adsorbent (mg/g), q_m is the maximum adsorption capacity, β is the activity coefficient related with sorption energy and ε is the Polanyi potential, that can be calculated as follows:

$$\varepsilon = RT \ln \left(1 + \frac{1}{C_e} \right) \quad (3.6)$$

where R is the gas constant ($\text{kJ mol}^{-1} \text{K}^{-1}$) and T is the absolute temperature (K). The sorption energy can also be evaluated by the following expression:

$$E = \frac{1}{(2\beta)^{1/2}} \quad (3.7)$$

with β value of the model we can calculate the mean free energy E (kJ/mol) of adsorption per molecule of the adsorbate. This means the amount of E calculated gives us if the binding to the surface is in electrostatic nature or occurs by complexation or by van der Waals forces etc (Ma et al, 2007).

3.3.4. Adsorption Kinetics

The study of sorption kinetics describes the adsorbate uptake rate and evidently this rate controls the residence time of adsorbate at the solid liquid interface. Different kinetic models are: the pseudo-first order, pseudo-second order, Elovich, mass transfer, intraparticle diffusion, and intraparticle diffusivity (Augustine et al., 2007).

3.3.4.1. The Pseudo-First Order Model

The pseudo-first order equation is generally expressed as:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (3.8)$$

where q_e and q_t are the sorption capacities at equilibrium and at time t , respectively (mg/g) and k_1 is the rate constant of pseudo-first order

sorption (L/min). After integration and applying boundary conditions $t=0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$, the integrated form of equation (3.8) becomes:

$$\log(q_e - q_t) = \log q_e - k_1 t \quad (3.9)$$

When the values of $\log (q_e - q_t)$ are linearly correlated with t , the plot of $\log (q_e - q_t)$ versus t will give a linear relationship from which k_1 and q_e can be determined from the slope and intercept of the graph respectively.

3.3.4.2. The Pseudo- Second Order Model

The pseudo-second order chemisorption kinetic equation is expressed as Eqn 3.10:

$$\frac{dq_t}{dt} = k_2 (q_e - q_t)^2 \quad (3.10)$$

where q_e and q_t are the sorption capacity at equilibrium and at time t , (mg/g) respectively and k_2 is the rate constant of the pseudo-second order sorption (g/(mg min)). For the boundary conditions $t = 0$ to $t = t$ and $q_t=0$ to $q_t = q_t$, the integrated form of Eqn. (3.10) becomes:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \quad (3.11)$$

which is the integrated rate law for a pseudo-second order reaction. Eqn. (3.11) can be rearranged to obtain:

$$q_t = \frac{1}{\frac{1}{k_2 q_e^2} + \frac{t}{q_e}} \quad (3.12)$$

which has a linear form:

$$\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (3.13)$$

where h (mg/(g.min)) can be regarded as the initial sorption rate as $q_t/t \rightarrow 0$. Hence

$$h = k_2 q_e^2 \quad (3.14)$$

Furthermore Eqn. (3.13) can be written as:

$$\frac{t}{q_t} = \frac{1}{h} + \frac{t}{q_e} \quad (3.15)$$

If the pseudo-second order kinetics is applicable to the experimental data, the plot of t/q_t versus t of Eqn. (3.15) should give a linear relationship from which q_e , k and h can be determined from the slope and intercept of the plot respectively.

3.3.4.3. The Elovich Kinetic Model

The Elovich equation is generally expressed as:

$$\frac{dq_t}{dt} = \alpha e^{-\beta q_t} \quad (3.16)$$

where q_t is the sorption capacity at time t (mg/g), α is the initial adsorption rate (mg/(g.min)) and, β is the desorption constant (mg/(g.min)) during any one experiment. To simplify the Elovich equation assumed $\alpha \beta t \gg 1$ and by applying boundary conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ and $t = t$, Eqn. (3.16) becomes:

$$q_t = \frac{\ln(\alpha\beta)}{\beta} + \frac{\ln t}{\beta} \quad (3.17)$$

Thus, if a plot of q_t versus $\ln t$ is linearly correlated, the constants α and β can be computed from the slope and intercept of the graph.

3.3.4.4. Mass Transfer Model

The mass transfer equation is generally expressed as:

$$c_{in} - c_t = De^{K_o t} \quad (3.18)$$

where C_{in} is the initial metal ion concentration (mg/dm³), C_t is the metal ion concentration at time t , t is the shaking time (min), D is a fitting parameter, K_o is the adsorption constant which is related to the mass transfer adsorption coefficient. A linearised form of Eqn. (3.18) is:

$$\ln(c_o - c_t) = \ln D + K_o t \quad (3.19)$$

If the sorption of ions on the two adsorbent is depicted by the mass transfer model, then a plot of $\ln (C_o - C_t)$ versus time should give a linear relationship from where the constants $\ln D$ and K_o can be determined from the slope and intercept of the plot respectively.

3.3.4.5. Weber-Morris Intra-Particle Diffusion Model

Adsorption kinetics are usually controlled by different mechanisms, of which the most limiting are the diffusion mechanisms, including the initial curved portion, attributed to rapid external diffusion or boundary layer diffusion and surface adsorption, and the linear portion, a gradual adsorption stage due to intra-particle diffusion, followed by a plateau to equilibrium where the intra-particle diffusion starts to decrease due to the low concentration in solution as well as fewer available adsorption sites (Ong et al., 2007). Weber and Morris (1963) intra-particle diffusion was characterized using the relationship between specific sorption (q_t) and the square root of time ($t^{1/2}$) as shown below:

$$q_t = K_{id} t^{1/2} + I \quad (3.20)$$

where q_t is the amount adsorbed per unit mass of adsorbent (mg/g) at time t and K_{id} the intra-particle diffusion rate constant ($\text{mg.g}^{-1}.\text{min}^{-1/2}$). The value of I gives an idea about the thickness of the boundary layer, i.e. the larger the intercept the greater the boundary layer effect. If the intra-particle diffusion is involved in the adsorption process, then a plot of the amount of heavy metal adsorbed per unit mass of adsorbent (q_t) against square root of time ($t^{1/2}$) gave a straight line and the particle diffusion would be the controlling step if this line passed through the origin (Singh et al., 2007).

3.4. Amino Acids: The Building Blocks of Proteins

Despite enormous functional diversity all proteins consist of a linear arrangement of amino acid residues assembled together into a polypeptide chain. Amino acids are the ‘building blocks’ of proteins. All amino acids contain *carbon, hydrogen, nitrogen* and *oxygen* with two of the 20 amino acids also containing sulfur (Whitford, 2005).

Table 3.3: The properties of amino acids and their frequencies with which amino acid residues occur in proteins (Whitford, 2005, proteins).

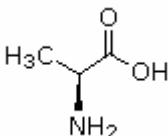
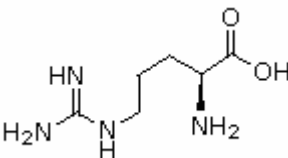
Name, abbreviation	Property of individual amino	Formula
Alanine A Ala M _r 71.09	acid residues Small side chain volume. Van der Waals volume = 67 Å ³ * Frequency in proteins = 7.7% Surface area = 115 Å ² Unreactive side chain	
Arginine R Arg M _r 156.19	Positively charged side chain at pH 7.0. pK for guanidino group in proteins ~12.0 Van der Waals volume = 167 Å ³ Frequency in proteins = 5.1% Surface area = 225 Å ² Participates in ionic interactions with negatively charged groups	

Table 3.3: continued

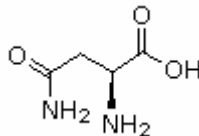
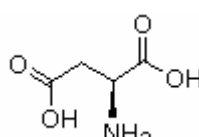
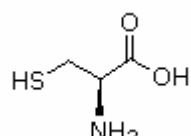
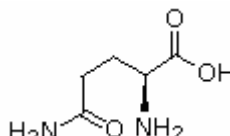
Asparagine	Polar, but uncharged, side chain	
N	Van der Waals volume = $148 \text{ \AA}^3$	
Asn	Frequency in proteins = 4.3%	
M _r 114.11	Surface area = $160 \text{ \AA}^2$ Polar side chain will hydrogen bond Relatively small side chain volume leads to this residue being found relatively frequently in turns	
Aspartate	Negatively charged side chain	
D	pK for side chain of ~4.0	
Asp	Van der Waals volume = $67 \text{ \AA}^3$	
M _r 115.09	Frequency in proteins = 5.2% Surface area = $150 \text{ \AA}^2$ Charged side chain exhibits electrostatic interactions with positively charged groups.	
Cysteine	Side chain contains thiol (SH) group.	
C	Van der Waals volume = $86 \text{ \AA}^3$	
Cys	Frequency in proteins = 2.0%	
M _r 103.15	Surface area = $135 \text{ \AA}^2$ Thiol side chain has pK in isolated amino acid of ~8.5 but in proteins varies 5–10 Thiol group is very reactive	
Glutamine	Polar but uncharged side chain	
Q	Van der Waals volume = $114 \text{ \AA}^3$	
Gln	Frequency in proteins = 4.1%	
M _r 128.12	Surface area = $180 \text{ \AA}^2$ Polar side chain can hydrogen bond	

Table 3.3: continued

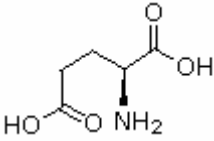
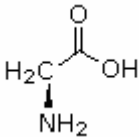
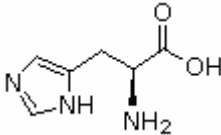
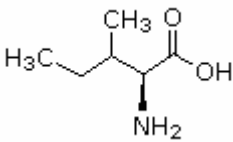
Glutamate	Negatively charged side chain.	
E	Van der Waals volume = $109 \text{ \AA}^3$	
Glu	Frequency in proteins = 6.2%	
M_r 129.12	Surface area = $190 \text{ \AA}^2$ Side chain has pK of ~ 4.5 .	
Glycine	Uncharged, small side chain.	
G	Often found in turn regions of proteins or	
Gly	regions of conformational flexibility	
M_r 57.05	No chiral centre; due to two hydrogens attached to C_α centre Van der Waals volume = $48 \text{ \AA}^3$ Frequency in proteins = 7.4% Surface area = $75 \text{ \AA}^2$	
Histidine	Imidazole side chain	
H	Van der Waals volume = $118 \text{ \AA}^3$	
His	Frequency in proteins = 2.3%	
M_r 137.14	Surface area = $195 \text{ \AA}^2$ The side chain exhibits a pK ~ 6.0 in model peptides but in proteins can vary from 4–10	
Isoleucine	Hydrophobic side chain exhibiting non-polar based interactions but generally unreactive	
I	Van der Waals volume = $124 \text{ \AA}^3$	
Ile	Frequency in proteins = 5.3%	
M_r 113.16	Surface area = $175 \text{ \AA}^2$	

Table 3.3: continued

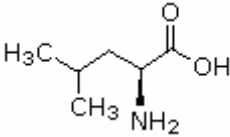
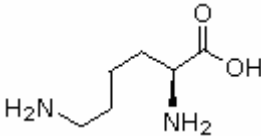
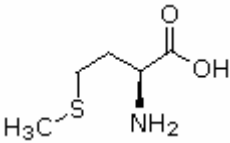
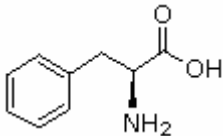
Leucine L Leu M _r 113.16	Hydrophobic side chain Van der Waals volume = 124 Å ³ Frequency in proteins = 8.5% Surface area = 170 Å ²	
Lysine K Lys M _r 128.17	Positively charged side chain Van der Waals volume = 135 Å ³ Frequency in proteins = 5.9% Surface area = 200 Å ² Side chain is basic with pK of ~10.5. Shows ionic interactions	
Methionine M Met M _r 131.19	Sulfur containing hydrophobic side chain The sulfur is unreactive especially when compared with thiol group of cysteine Van der Waals volume = 124 Å ³ Frequency in proteins = 2.4% Surface area = 185 Å ²	
Phenylalanine F Phe M _r 147.18	Hydrophobic, aromatic side chain Phenyl ring is chemically unreactive in proteins. Exhibits weak optical absorbance around 280 nm Van der Waals volume = 135 Å ³ Frequency in proteins = 4.0% Surface area = 210 Å ²	

Table 3.3: continued

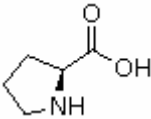
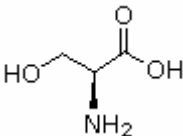
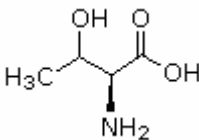
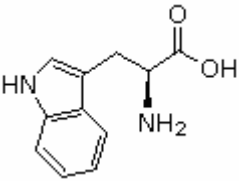
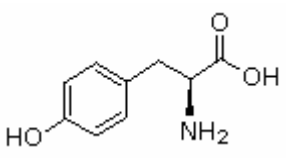
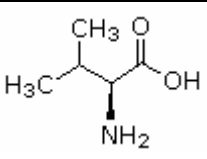
Proline P	Cyclic ring forming hydrophobic side chain The cyclic ring limits conformational flexibility around N-C α bond	
Pro M _r 97.12	In a polypeptide chain lacks amide hydrogen and cannot form backbone hydrogen bonds Van der Waals volume = 90 Å ³ Frequency in proteins = 5.1% Surface area = 145 Å ²	
Serine S Ser M _r 87.08	Polar but uncharged side chain. Contains hydroxyl group (–OH) that hydrogen bonds Oxygen atom can act as potent nucleophile in some enzymes Van der Waals volume = 73 Å ³ Frequency in proteins = 6.9% Surface area = 115 Å ²	
Threonine T Thr M _r 101.11	Polar but uncharged side chain. Contains hydroxyl group (–OH) Hydrogen bonding side chain Van der Waals volume = 93 Å ³ Frequency in proteins = 5.9% Surface area = 140 Å ²	
Tryptophan W Trp M _r 186.21	Large, hydrophobic and aromatic side chain Almost all reactivity is based around the indole ring nitrogen Responsible for majority of near UV absorbance in proteins at 280 nm Van der Waals volume = 163 Å ³ Frequency in proteins = 1.4% Surface area = 255 Å ²	

Table 3.3: continued

Tyrosine	Aromatic side chain	
Y	Van der Waals volume = $141 \text{ \AA}^3$	
Tyr	Frequency in proteins = 3.2%	
M_r 163.18	Surface area = $230 \text{ \AA}^2$	
	Phenolic hydroxyl group ionizes at pH values around pH 10 Aromatic ring more easily substituted than that of phenylalanine	
Valine	Hydrophobic side chain	
V	Van der Waals volume = $105 \text{ \AA}^3$	
Val	Frequency in proteins = 6.6%	
M_r 99.14	Surface area = $155 \text{ \AA}^2$	

3.4.1. Zwitterionic Character, pK and pI of Amino Acids

As amino acids contain a basic and an acidic functional group, they are amphoteric. The carboxyl group of an amino acid has a pK_a between 1.8 and 2.5, the amino group has a pK_a between 8.7 and 10.7. At the pH found under physiological conditions, pH 6 to 7, the amino group is ionised to $-\text{NH}_3^+$ (http://www.worldscibooks.com/chemistry/etextbook/p297/p297_chap1_1.pdf).

Amino acids exist at zwitterions ("twin ions") at physiological pH as shown in the Figure 3.6.

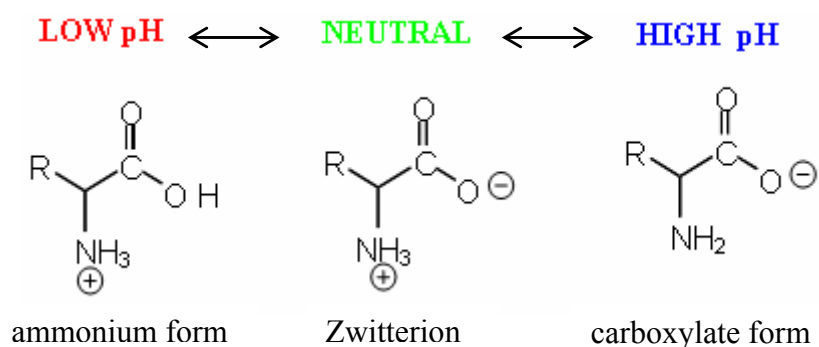


Figure 3.6: Zwitterionic character of amino acids

The pK_a of an acid or base is defined as the pH at which half of the molecules are charged. A compound which can act as both acid and base (like an amino acid) has another important property: The isoelectric point pI , which is the pH at which the number of positive charges on the molecule is the same as the number of negative charges. At the pI the molecule would therefore appear uncharged. At this pH the molecules ability to interact with water is lowest, and therefore its solubility (Buxbaum, 2007).

The pI of feather keratin ($\text{Mw:} = 10,500 \text{ g/mol}$) is 5.6 (Barone et al., 2007).

Table 3.4.: pI values of natural amino acids. (http://www.worldscibooks.com/chemistry/etextbook/p297/p297_chap1_1.pdf).

Amino acids Non-polar chain	pI	Amino acids Polar chain	pI	Amino acids Charged chain	pI
Alanine	6.02	Glycine	5.97	Lysine	9.74
Valine	5.97	Asparagine	5.41	Histidine	7.58
Leucine	5.98	Glutamine	5.65	Arginine	10.76
Isoleucine	6.02	Tyrosine	5.65	Aspartic acid	2.87
Phenylalanine	5.98	Cysteine	5.02	Glutamic acid	3.22
Tryptophan	5.88	Serine	5.68		
Methionine	5.75	Threonine	6.53		
Proline	6.1				

3.4.2. The Acidic Residues: Aspartate and Glutamate

Aspartic acid and glutamic acid have side chains with a carboxyl group (Figure 3.7). This leads to the side chain having a negative charge under physiological conditions and hence to their description as acidic side chains. For this reason the residues are normally referred to as aspartate and glutamate reflecting the ionized and charged status under most cellular conditions. The two side chains differ by one methylene group (CH_2) but are characterized by similar pK values (range 3.8–4.5). The side chains of aspartate and glutamate are potent chelators of divalent metal ions and biology exploits this property to bind important ions such as Ca^{2+} in proteins such as calmodulin or Zn^{2+} in enzymes such as carboxypeptidase.

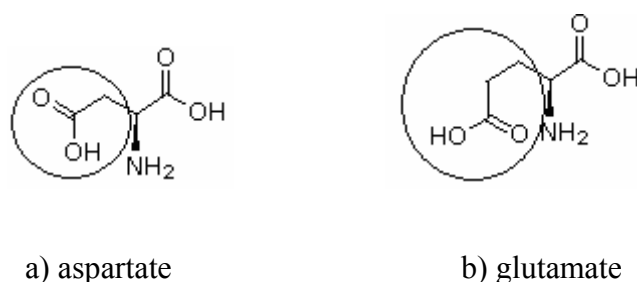


Figure 3.7: The acidic side chains of aspartate and glutamate (Whitford, 2005).

3.4.3. The Sulfur-Containing Residues: Cysteine and Methionine

Sulfur occurs in two of the 20 amino acids: cysteine and methionine. In cysteine the sulfur is part of a reactive thiol group whilst in methionine sulfur is found as part of a long, generally unreactive, side chain. The side chain of methionine is non-polar (Figure 3.8) and is larger than that of valine or leucine although it is unbranched. Its properties are dominated by the presence of the sulfur atom, a potent nucleophile under certain conditions. The sulfur atom is readily methylated using methyl iodide in a reaction that is often used to introduce a 'label' onto methionine residue via the use of ¹³C labelled reactant.

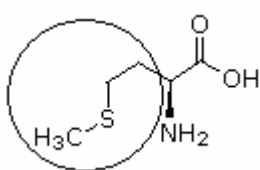


Figure 3.8: The side chain of methionine (Whitford, 2005).

In addition the sulfur of methionine interacts with heavy metal complexes particularly those involving mercury and platinum such as K_2PtCl_4 or HgCl_2 and these have proved extremely useful in the formation of isomorphous heavy atom derivatives in protein crystallography. The sulfur atom of methionine can be oxidized to form first a sulfoxide and finally a sulfone derivative (Figure 3.9).

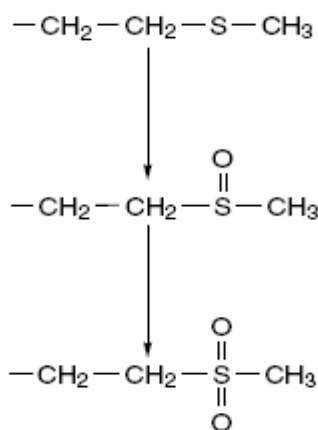


Figure 3.9: Oxidation of methionine side chains by strong oxidizing agents (Whitford, 2005).

This form of oxidative damage is known to occur in proteins and the reaction scheme involves progressive addition of oxygen atoms. One of the most important reactions of methionine involves cyanogen bromide – a reagent that breaks the polypeptide chain on the C-terminal side of methionine residues by sequestering the carbonyl group of the next peptide bond in a reaction involving water and leading to formation of a homoserine lactone (Figure 3.9). This reaction is used to split polypeptide chains onto smaller fragments for protein sequencing. When compared with cysteine, however, the side chain of methionine is less

reactive undergoing comparatively few important chemical reactions. The functional group of cysteine is the thiol ($-\text{SH}$) group, sometimes called the sulfhydryl or mercapto group. It is the most reactive side chain found amongst the 20 naturally occurring amino acids undergoing many chemical reactions with diverse reagents (Figure 3.10).

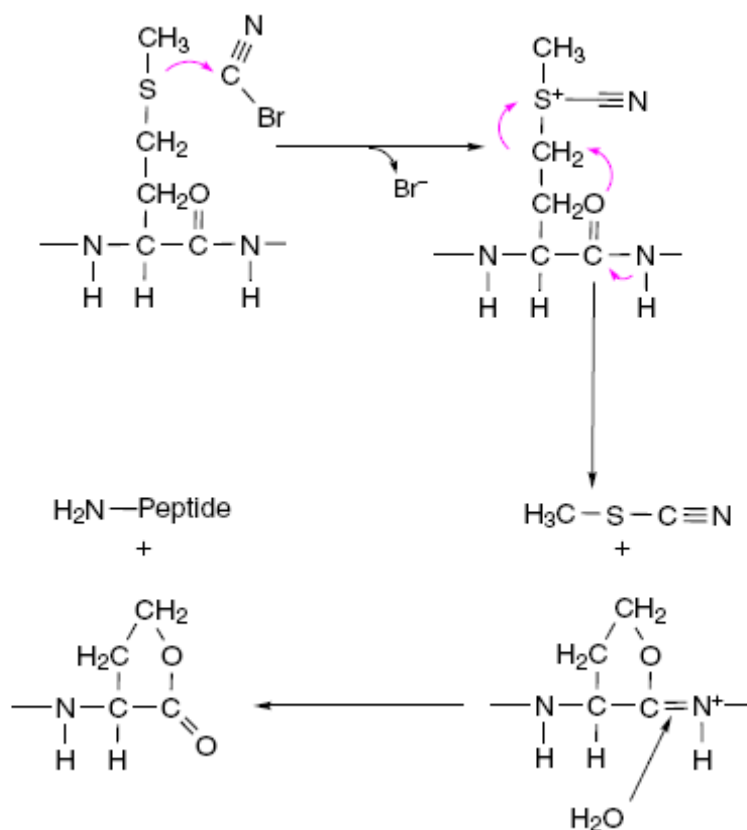


Figure 3.10: The reaction of cyanogen bromide with methionine residues (Whitford, 2005).

Some enzymes exploit reactivity by using a conserved cysteine residue at their active sites that participates directly in enzyme-catalysed reactions. The large sulfur atom as part of the thiol group influences side chain properties significantly, with disulfide bonds forming between cysteine residues that are close together in space. This forms a strong covalent bond and exercises considerable conformational restraint on the structure adopted in solution by polypeptides. Formation of a disulfide bridge is another example of post-translational modification (Figure 3.12).

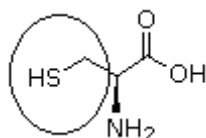


Figure 3.11: The thiol group in the side chain of cysteine (Whitford, 2005).

The thiol group ionizes at alkaline pH values (~pH 8.5) to form a reactive thiolate anion (S^-). The thiolate anion reacts rapidly with many compounds but the most important includes other thiols or disulfides in exchange type reactions occurring at neutral to alkaline pH values.

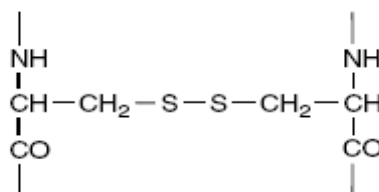


Figure 3.12: Formation of a disulfide bridge between two thiol side chains (Whitford, 2005).

A general reaction scheme for thioldisulfide exchange is given in Figure 3.13. A common reaction of this type is the reaction between cysteine and Ellman's reagent (dithionitrobenzoic acid, DTNB; Figure 3.14).

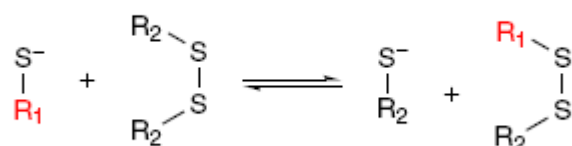


Figure 3.13: The formation of mixed disulfides via the interaction of thiolate groups. A thiolate anion reacts with other symmetrical disulfides to form a mixed disulfide formed between R1 and R2 (Whitford, 2005).

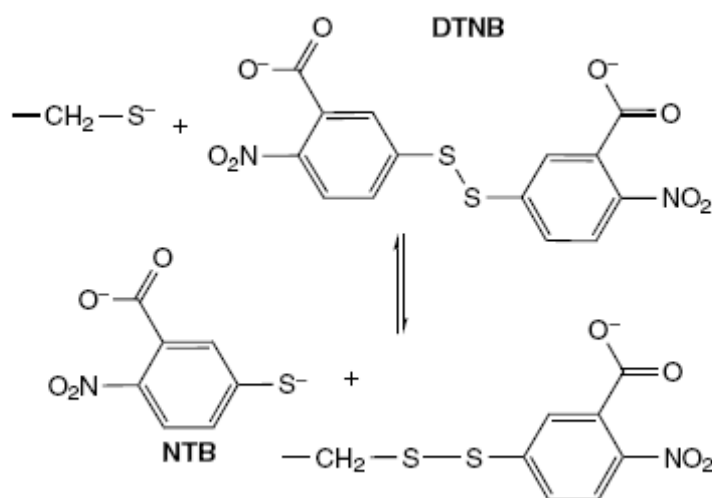


Figure 3.14: The reaction between the thiolate anion of cysteine and Ellman's reagent (Whitford, 2005).

The aromatic disulfide undergoes exchange with reactive thiolate anions forming a colored aromatic thiol–nitrothiobenzoate. The benzoate anion absorbs UV light intensely at 416 nm allowing the concentration of free thiol groups to be accurately estimated in biological systems. Thiols are also oxidized by molecular oxygen in reactions catalysed by trace amounts of transition metals, including Cu and Fe. More potent oxidants such as performic acid oxidize the thiol group to a sulfonate (SO_3^{2-}) and this reaction has been exploited as a method of irreversibly breaking disulfide bridges to form two cysteic acid residues. More frequently the disulfide bridge between cysteine residues is broken by reducing agents that include other thiols such as mercaptoethanol or dithiothreitol (Figure 3.15) as well as more conventional reductants such as sodium borohydride or molecular hydrogen.

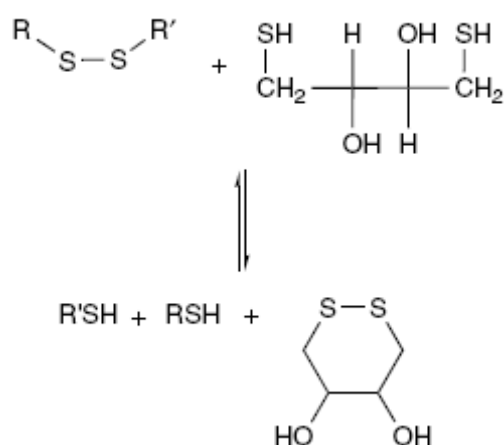


Figure 3.15: The reaction between dithiothreitol and disulfide groups leads to reduction of the disulfide and the formation of two thiols (Whitford, 2005).

Dithiothreitol, sometimes called Cleland's reagent, reacts with disulfide groups to form initially a mixed disulfide intermediate but this rapidly rearranges to yield a stable six-membered ring and free thiol groups (-SH). The equilibrium constant for the reaction lies over to the right and is largely driven by the rapid formation of cyclic disulfide and its inherent stability (Whitford, 2005).

Beyond the iron-sulfur proteins, many other metal cofactors in enzymes are bound to the thiolate substituent of cysteinyl residues. The thiol group has a high affinity for heavy metals, so that proteins containing cysteine will bind metals such as mercury, lead, and cadmium tightly. (<http://en.wikipedia.org/wiki/Cysteine>)

3.4.4. Metal-binding Properties of Amino Acids and Peptides

Amino acids are bidentate ligands for several transition metal ions. Consequently, Cu^{2+} ions can be used to form complexes in order to block functional groups selectively. The —CONH— groups in peptides also complex with Cu^{2+} ions and this forms the basis of the classical biuret reaction for peptides and proteins. Some of the side-chains in peptides and proteins, especially those of Glu and His, are good ligands for transition metal ions. Several enzymes such as collagenase, stromelysin, carboxypeptidase and carbonic anhydrase contain Zn^{2+} ions that are complexed with functional groups in the side-chains of amino acids such as His and Glu (Barrett et al., 2004).

Metal ions play essential roles in many metabolic path-ways, and in trace amounts these ions are required for cellular health. Problems arise

when cells are confronted with much higher concentrations of these essential ions or ions of related metals that can poison essential biochemical reactions. The group of elements often referred to as the heavy metals and metalloids are of the most practical importance because of their high level of toxicity and the extensive contamination of the environment by ions of these elements. As a consequence of the chemical reactivity of many toxic metals with thiols, thiol-mediated defense mechanisms are often involved. The main molecules responsible for the cytoplasmic sequestration of metals include glutathione, small metal binding peptides known as phytochelatins, and the metallothionein family, small cysteine-rich protein metallothioneins (Perego et al., 1997, Jonak et al, 2004).

The toxicity of heavy metals may depend on their binding to various ligands; among such ligands in the biological systems, carboxylate ion, imidazole, sulfhydryl group, and aliphatic amine are the most important. To illustrate, heavy metal binding to various functional groups of proteins, primarily SH-groups, would modify protein conformation and result in the loss of activity in many enzymes comprising SH-groups in their activity centers. Besides, metal binding to SH-groups of the physiologically active compounds of low molecular weight would also interfere with cell metabolism. Thus, binding to SH-groups alone would already give rise to diverse metabolic disturbance. Heavy metals differ in their affinity for oxygen-, nitrogen- and sulfur-containing ligands, and these differences depend on the physical and chemical properties of heavy metal ions. The metal cations are classified into hard and soft Lewis acids. According to this classification, the ions of hard acids, such as Li^+ , Sr^{2+} etc., would rather interact with hard bases, whereas the ions of soft acids, such as Pb^{2+} , Hg^{2+} , Ag^+ etc., with soft bases. Cd^{2+} , Cu^{2+} , Ni^{2+} , and several other ions come into an intermediate

class, with Ni^{2+} shifting towards hard and Cd^{2+} , soft class. In the biological chemistry, there are four major classes of donor atoms in ligands. Among these, oxygen and aliphatic nitrogen are classified as hard, aromatic nitrogen as intermediate, and sulfur as soft. It follows that Ni^{2+} would rather bind to aromatic nitrogen, and Cd^{2+} , Hg^{2+} , and Ag^+ , to SH-groups. The rate of ligand exchange within and beyond the coordination sphere of the particular metal ion is an important parameter of its reactions. For example, Ni^{2+} and Zn^{2+} practically do not differ in several physical and chemical properties, such as the size of the ion. However, Zn^{2+} is a constituent of numerous enzymes, whereas Ni^{2+} is found in few plant enzymes, probably because in the former case the rate of ligand exchange is higher by three orders of magnitude. Thus, diverse effects of the metals of similar properties apparently depend on the different rates of ligand exchange, and their physiological properties are immediately derived from their physical and chemical parameters. Because of the competition between various metals in the course of their uptake by roots, some metals are absorbed in insufficient quantities, whereas the uptake of other metals is excessive. Such situation would indirectly predetermine the effects of heavy metals on the various facets of metabolism, such as photosynthesis, respiration, etc.. It also seems important to compare the mechanisms of heavy metal accumulation, transport, toxicity, and detoxification in susceptible and tolerant plant species and populations (Seregin et al., 2006).

3.5. Peptides and Proteins

Peptides and proteins are macromolecules made up from long chains of amino acids joined head-to-tail via peptide bonds (Figure 3.16). They are formed by the condensation of carboxylic and amino group with the elimination of water. Two amino acids connected via a peptide bond are called a dipeptide, three acids a tripeptide and so on. With an increasing number of acids in sequence, the molecules are referred to as oligopeptides and polypeptides. The C-N bond cannot rotate due to its partial double bond character. Hence, the peptide unit NH-CO is rigid. The bonds to neighboring α C-atoms can rotate within steric constraints (Figure 3.17) and play an important part in folding of the protein. The peptide units together with the tetrahedral C-atoms form the backbone of a protein, while the R substituents are referred to as side chains. (http://www.worldscibooks.com/chemistry/etextbook/p297/p297_chap1_1.pdf).

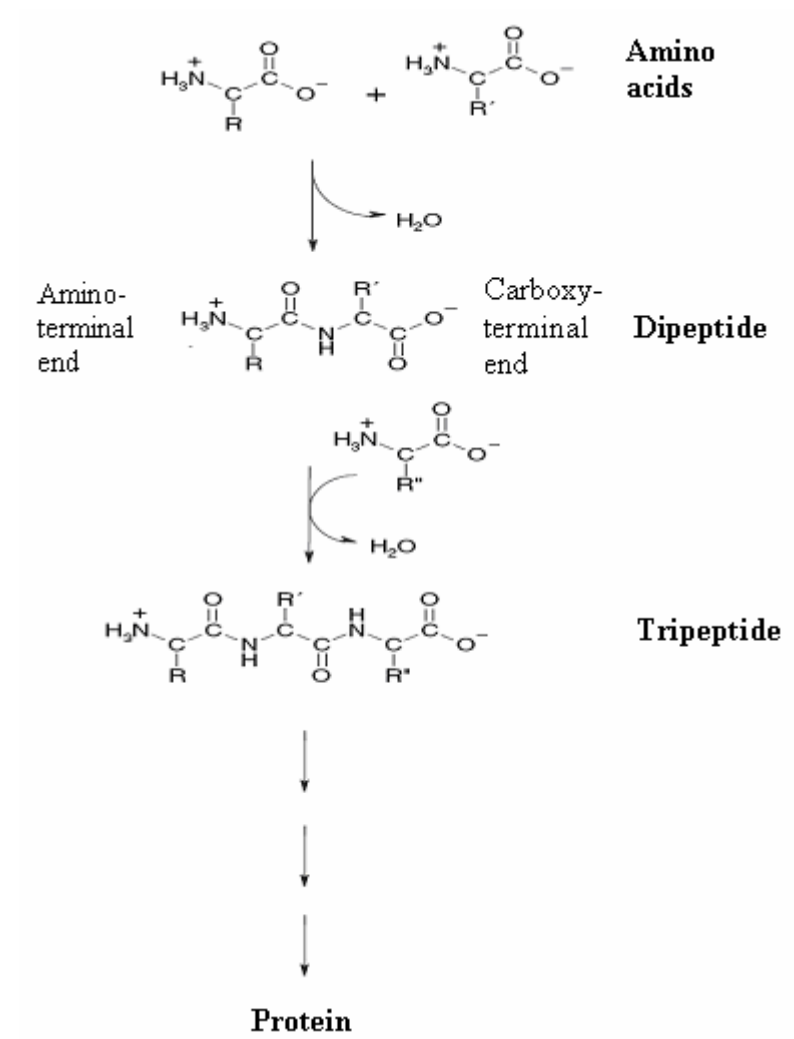


Figure 3.16: The peptide bond formation (Buxbaum, 2007).

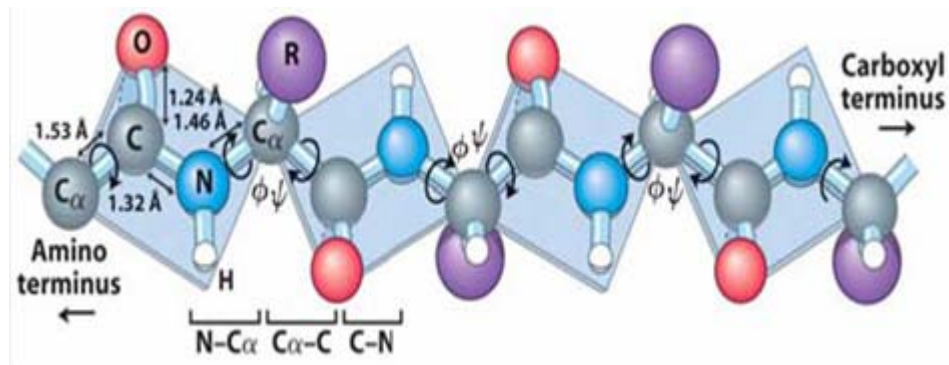


Figure 3.17: The C-N bond is rigid due to partial double bond character, rotation is possible within steric constraints around the bonds to the α C-atoms.

Only *non-planar* bonds rotate and each α -carbon has a Φ and Ψ rotation angle, which affect the conformation of the proteins.

3.5.1. Structure of Proteins

Protein structure is described in terms of four levels. Primary, secondary and tertiary refer to the molecules in a single polypeptide chain, and the fourth (quaternary) refers to the interaction of several polypeptide chains to form a multi-chained protein (Valstar, 2000).

3.5.1.1. Primary structure

The sequence of amino acids determines the primary structure of a protein (Figure 3.18). Changing just a single amino acid in a critical position of the protein can significantly alter its activity and function and be the cause of disease and disorders.

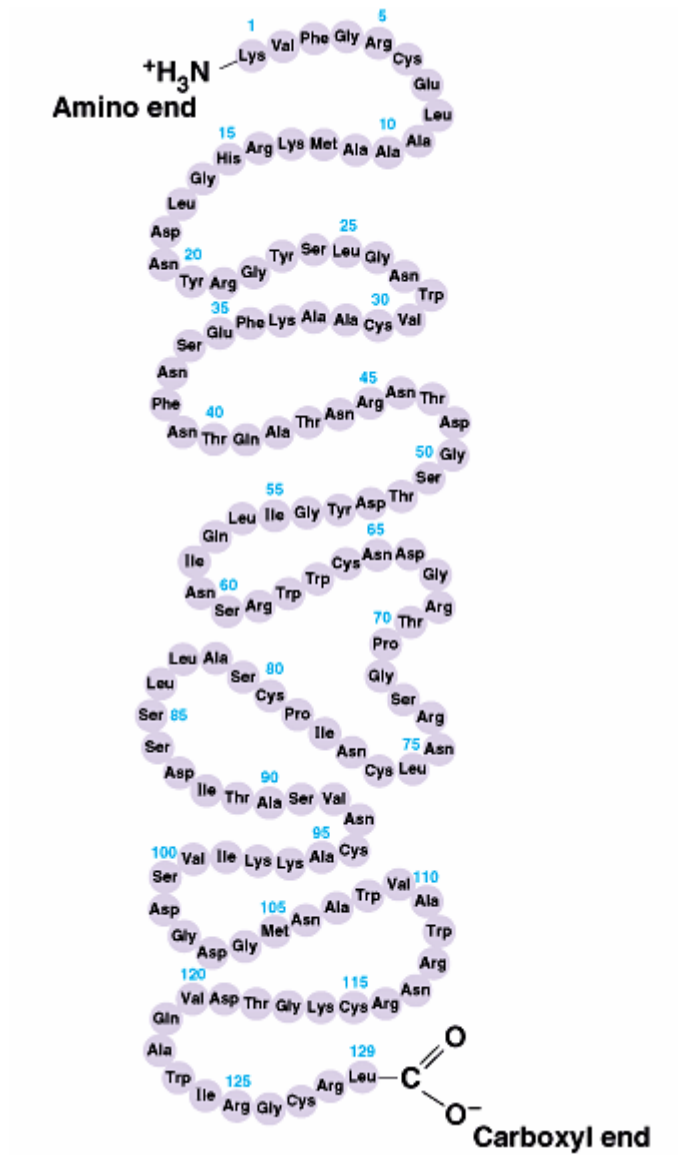


Figure 3.18: The primary structure of proteins.

3.5.1.2. Secondary Structure

Secondary structures are regular elements such as α helices and β -pleated sheets, which are formed between relatively small parts of the protein sequence. These structural domains are determined by the conformation of the peptide backbone, the influence of side-chains is not taken into account for secondary structures.

An α -helix is a coil (Figure 3.19), which is held together by hydrogen bonding between a-CO group of an n^{th} amino acid residue in the sequence and the -NH group of the $n+4^{\text{th}}$ amino acid residue. The coiling is such that the -R groups are pointing outwards perpendicular to the axis of the coil. α -helices are important in structural proteins like keratin. Not all amino acids favor α -helix formation due to steric hindrance. The secondary amino acid proline, for example, is likely to disrupt the formation of a helix.

In a β -pleated sheet (Figure 3.19), two polypeptide backbones are folded and aligned next to each other. They are connected via hydrogen bonds. The amino acid substituents R are pointing outwards to the top or bottom of the sheet. Adjacent chains can be aligned either in the same direction (parallel β -folding) or in opposite directions (anti-parallel β -folding), β -folding often occurs with amino acids carrying small non-charged side chains.

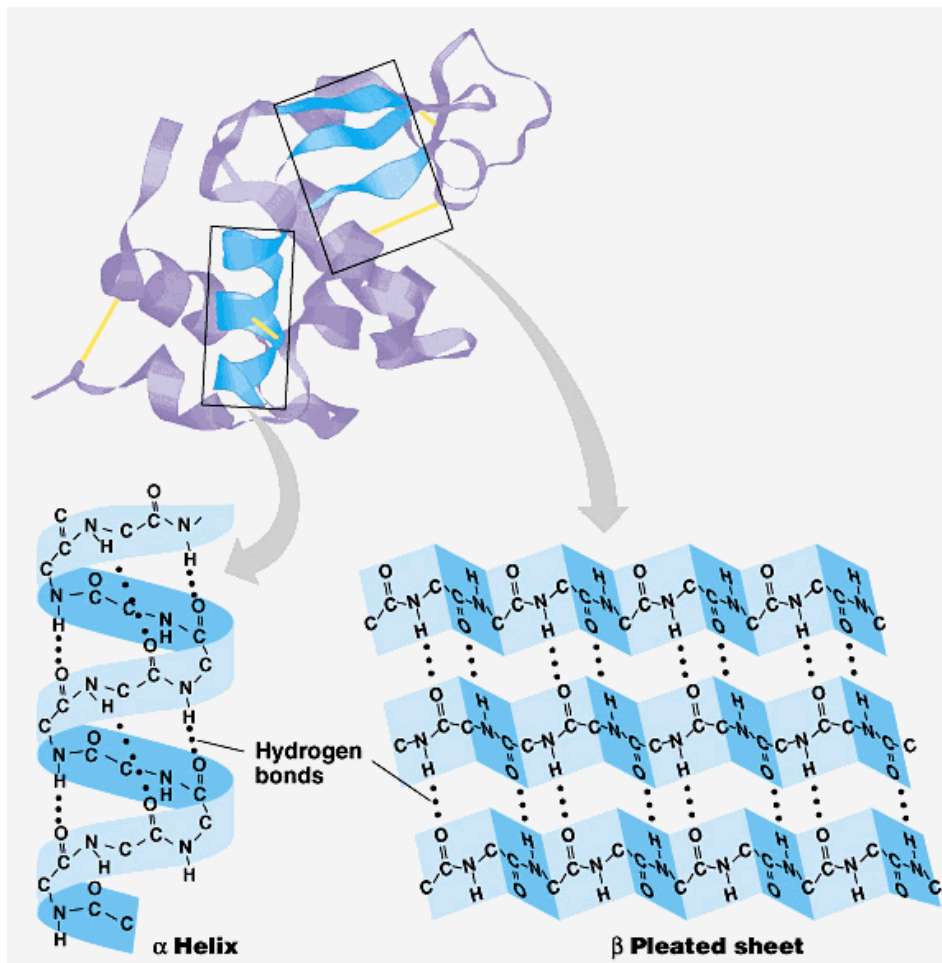


Figure 3.19: The secondary structure of proteins.

3.5.1.3. Tertiary Structure

The tertiary structure describes the complete three-dimensional structure of the whole polypeptide chain. It includes the relationship of different domains formed by the protein's secondary structure and interactions of amino acid substituent $-R$ groups (Figure 3.20). The specific folding of a protein is only thermodynamically stable within a

restricted range of environmental parameters, i.e. the right temperature, pH and ionic strength. Outside of this range, the protein could unfold and lose its activity.

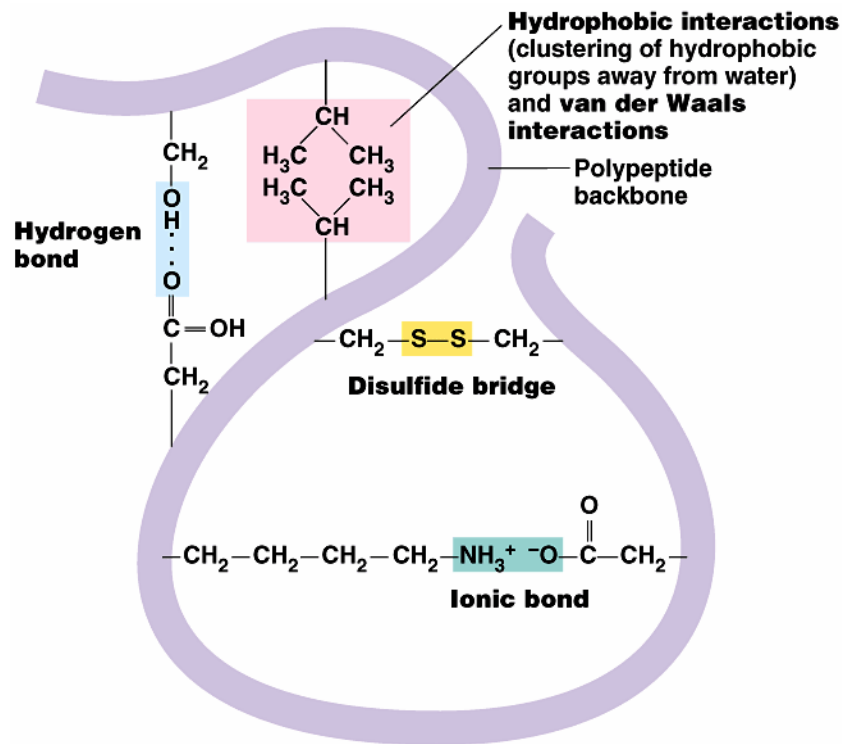


Figure 3.20: The tertiary structure of proteins.

3.5.1.4. Quaternary Structure

A protein can consist of two or more separate polypeptide chains linked together. Other, non-amino acid components such as minerals, lipids and carbohydrates can also be part of a protein. The quaternary structure describes how these different chains and components interact and connect to each other by hydrogen bonding, electrostatic attraction

and sulphide bridge. Such sulphide bridges are formed by oxidation of the –SH groups of cysteine. The product of this reaction is covalently bonded dipeptide called Cystin.

The hormone insulin, which produced in the pancreas, contains two different polypeptide chains, A and B. Sulfide bridges can occur within a chain as well as between the two chains.

The separate polypeptide chains forming a protein can be identical (homogenic protein) or, as in the case of insulin, different (heterogenic protein).

This last level of organization is simply taking 2 or more 3-D (tertiary proteins) and sticking them together to form a larger protein. Many enzymes and transport proteins are made of two or more parts (ex.: Hemoglobin: an oxygen carrying protein in red blood cells) (Figure 3.21)

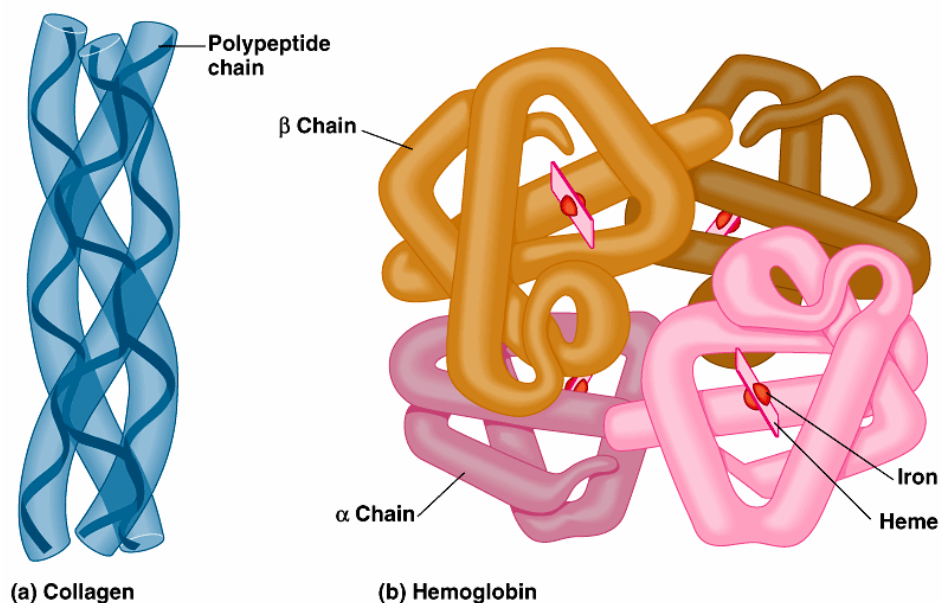


Figure 3.21: Quaternary structure of proteins

3.6. The Biological Diversity of Proteins

Proteins are joined covalently and non-covalently with other biomolecules including lipids, carbohydrates, nucleic acids, phosphate groups, flavins, heme groups and metal ions. Components such as hemes or metal ions are often called prosthetic groups. Complexes formed between lipids and proteins are lipoproteins, those with carbohydrates are called glycoproteins, whilst complexes with metal ions lead to metalloproteins, and so on. The complexes formed between metal ions and proteins increases the involvement of elements of the periodic table beyond that expected of typical organic molecules (namely carbon, hydrogen, nitrogen and oxygen). The involvement of some elements is contentious tungsten and cadmium are claimed to be associated with

proteins yet these elements are also known to be toxic (Whitford, 2005). Inspection of the periodic table (Figure 3.22) shows that at least 20 elements have been implicated directly in the structure and function of proteins (Table 3.5) (Whitford, 2005).

Table 3.5: The involvement of trace elements in the structure and function of proteins (Whitford, 2005).

Element	Functional role
Sodium	Principal intracellular ion, osmotic balance.
Potassium	Principal intracellular ion, osmotic balance.
Magnesium	Bound to ATP/GTP in nucleotide binding proteins, found as structural component of hydrolase and isomerase enzymes.
Calcium	Activator of calcium binding proteins such as calmodulin.
Vanadium	Bound to enzymes such as chloroperoxidase.
Manganese	Bound to pterin co-factor in enzymes such as xanthine oxidase or sulphite oxidase. Also found in nitrogenase and as component of water splitting enzyme in higher plants.
Iron	Important catalytic component of heme enzymes involved in oxygen transport as well as electron transfer. Important examples are haemoglobin, cytochrome oxidase and catalase.
Cobalt	Metal component of vitamin B12 found in many enzymes.
Nickel	Co-factor found in hydrogenase enzymes.
Copper	Involved as co-factor in oxygen transport systems and electron transfer proteins such as haemocyanin and plastocyanin.
Zinc	Catalytic component of enzymes such as carbonic anhydrase and superoxide dismutase.
Chlorine	Principal intracellular anion, osmotic balance.
Iodine	Iodinated tyrosine residues form part of hormone thyroxine and bound to proteins.
Selenium	Bound at active centre of glutathione peroxidase.

Periodic table of the chemical elements and their involvement with proteins

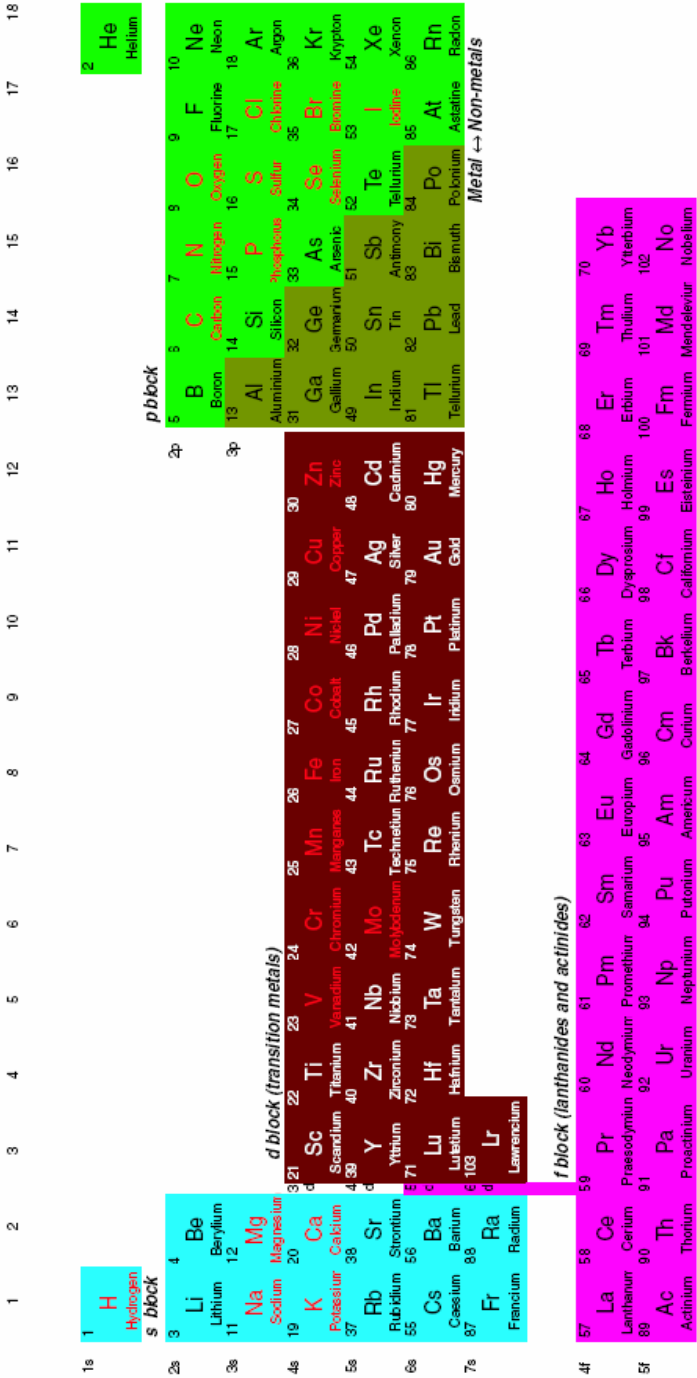


Figure 3.22: The elements to have involvement in structure and/or function of proteins (highlighted in grey)

Surprisingly elements such as aluminum and silicon that are very abundant in the Earth's crust (8.1 and 25.7 percent by weight, respectively) do not occur in high concentration within cells. Aluminum is rarely, if ever, found as part of proteins whilst the role of silicon is confined to biomineralization where it is the core component of shells. The involvement of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur is clear although the role of other elements, particularly transition metals, has been difficult to establish. Where transition metals occur in proteins there is frequently only one metal atom per mole of protein and led in the past to a failure to detect metal. Other elements have an inferred involvement from growth studies showing that depletion from the diet leads to an inhibition of normal cellular function. For metalloproteins the absence of the metal can lead to a loss of structure and function. Metals such as Mo, Co and Fe are often found associated with organic co-factors found in proteins such as pterin, flavins, cobalamin and porphyrin. These co-factors are pterin, the isoalloxine ring found as part of flavin in FAD and FMN, the pyridine ring of NAD and its close analogue NADP and the porphyrin skeletons of heme and chlorophyll. R represents the remaining part of the co-factor whilst M and V signify methyl and vinyl side chains (Figure 3.23.) (Whitford, 2005).

These organic ligands hold metal centers and are often tightly associated to proteins (Whitford, 2005).

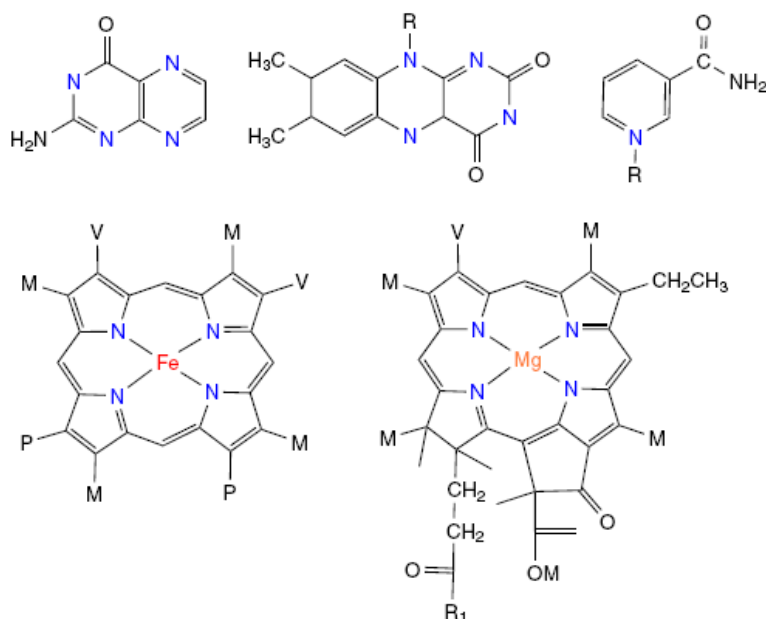


Figure 3.23: Organic co-factors found in proteins (pterin, flavins, cobalamin and porphyrin) (Whitford, 2005).

3.7. The Biological Function of Proteins

In general, there are two types of protein structures: (1) fibrous, elongated proteins which are not soluble in water and provide structural support and (2) globular, spherical proteins which are water soluble and have specific functions in the immune system and metabolism.

Globular proteins have a compact, spherical structure with very characteristic grooves and peaks on their surface. Analogous to a key fitting into a lock, other molecules fit into these grooves and peaks. This makes globular proteins specific when it comes to interacting with or

recognizing other molecules. Enzymes are an example of such specific proteins (http://www.worldscibooks.com/chemistry/etextbook/p297/p297_chap1_1.pdf).

In *fibrous proteins* at least three different structural plans or designs have been recognized in construction. These designs include: (i) structure composed of ‘coiled-coils’ of α helices and represented by the α keratins; (ii) structures made up of extended antiparallel β sheets and exemplified by silk fibroin a collection of proteins made by spiders or silkworms; and (iii) structures based on a triple helical arrangement of polypeptide chains and shown by the collagen family of proteins (Whitford, 2005).

3.8. Keratin Structure

Keratins are the major class of proteins found in hair, feathers, scales, nails or hooves of animals. In general the keratin classes of proteins are mechanically strong, designed to be unreactive and resistant to most forms of stress encountered by animals. At least two major groups of keratins can be identified; the α -keratins are typically found in mammals and occur as a large number of variants whilst β -keratins are found in birds and reptiles as part of feathers and scales containing a significantly higher proportion of β sheet. The β -keratins are analogous to the silk fibroin structures produced by spiders and silkworms.

The α -keratins are a subset of a much larger group of filamentous proteins based on coiled-coils called intermediate filaments (IF). The distribution of intermediate filaments is not restricted to mammals but appears to extend to most animal cells as major components of cytoskeletal structures (Whitford, 2005).

3.9. Chicken Feather Structure

Feathers are an important waste disposal of poultry industry and are composed basically of structural proteins, the keratins. It is known that the stability of the keratin in the solid state is due to cross-linking produced by the formation of cystine disulphide bonds, hydrogen bonds and salt linkages. The addition of reagents that break these bonds decreases the strength of the fibers. Therefore, it is possible to dissolve keratin using solvents that break the –S–S– bonds and the hydrogen bonds. Thus, it is possible to develop biodegradable materials based on keratin, such as films, by casting. Ideally, the regeneration of disulfide bonds in these materials during the drying of film-forming dispersion may be used as a method to impart water insolubility and good mechanical properties to resultant keratin materials (Plácido Moore et al., 2006).

Chicken feathers are approximately 91% protein (keratin), 1% lipids, and 8% water. The amino acid sequence of a chicken feather is very similar to that of other feathers and also has a great deal in common with reptilian keratins from claws (Rao et al., 2007). Table 3.6 shows the amino acid composition of keratin protein.

Chicken feathers are a waste product from the processing of chickens for food. Chicken feathers consist of approximately half fiber and half quill (by weight).

Table 3.6: The amino acid composition of keratin protein (g amino acid / 100g of protein) (Plácido Moore et al., 2006).

Amino acids	Concentration (%)	side chain	
Alanine	3.56	Unreactive side chain	Neutral Non-polar
Arginine	5.36	Positively charged side chain	Basic Polar
Aspartic acid	4.73	Negatively charged side chain	Acidic Polar
Cysteine	7.63	Side chain contains thiol (SH) group.	Neutral Slightly Polar
Glutamic acid	7.65	Negatively charged side chain.	Acidic Polar
Glycine	6.18	Uncharged, small side chain.	Neutral Non-polar
Histidine	0.43	Imidazole side chain	Basic Polar
Isoleucine	4.28	Hydrophobic side chain exhibiting non-polar	Neutral Non-polar
Leucine	7.04	Hydrophobic side chain	Neutral Non-polar
Lysine	0.53	Positively charged side chain	Basic Polar
Methionine	1.3	Sulfur containing hydrophobic side chain	Neutral Non-polar
Phenylalanine	4.2	Hydrophobic, aromatic side chain	Neutral Non-polar
Proline	8.77	Cyclic ring forming hydrophobic side chain	Neutral Non-polar
Serine	9.31	Polar but uncharged side chain.	Neutral Polar
Threonine	3.5	Polar but uncharged side chain.	Neutral Polar
Tyrosine	1.96	Aromatic side chain	Neutral Polar
Valine	6.94	Hydrophobic side chain	Neutral Non-polar



Figure 3.24: Parts of a feather (1. Vane 2. Rachis 3. Barb 4. Afterfeather 5. Hollow shaft, calamus (<http://en.wikipedia.org/wiki/Feather>).

Both the fiber and quill consist of hydrophobic keratin, a protein with strength similar to that of nylon (Winandy et al., 2007).

In the dry conditions, thermal transition occurs in a markedly higher temperature region close to 170-200°C for feather keratin (Takahashi et al., 2004).

4. EXPERIMENTAL STUDY

4.1. Materials

4.1.1. Raw materials

Chicken feather: Feathers are received from a farm.

Petroleum ether: Boiling point range: 40-60 °C.

Water: Deionized water.

Filter paper: Whatman filter (No:3).

Acetic acid: CH_3COOH ; MW: 60 g/mol (MERCK extra pure with acidimetric 99%).

Sodium hydroxide: NaOH ; MW: 40.5 g/mol (MERCK with assay $\geq 97\%$).

Citric Acid: $\text{C}_6\text{H}_8\text{O}_7 + \text{H}_2\text{O}$ MW: 210 g/mol (MERCK).

Zinc Nitrate: $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (BAKER Co. with assay $\geq 99,101\%$).

Copper Nitrate: $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (MERCK extra pure with iodometric assay $\geq 99,0\%$).

Cadmium Nitrate: $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (ACROS ORGANICS with assay $\geq 99\%$).

Nickel Nitrate: $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (MERCK with Complexometric assay $\geq 99,0\%$).

All chemicals used for preparation of metal solutions were of analytical grade, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$. All metal solutions were prepared using deionized water. The petroleum ether (40-60°C) was used for extraction to remove grease from chicken feathers.

4.2. Methods

4.2.1. Chicken Feather Preparation

4.2.1.1. Cleaning and Preparation of Chicken Feather

The feathers used in this study were received from a farm. Before extraction with petroleum ether, feathers were washed many times with deionized water and with detergent addition until all the dirt and impurities attached to the CF were removed. They were dried in an oven at 40 °C and the barb and after feather parts of the feathers were cut into short segments. 10g of short filaments of CF were Soxhlet extracted for 12 h with petroleum ether to remove grease. After treatment with petroleum ether, feathers were dried and stored at room temperature.

4.2.1.2. Untreated Chicken Feather

Degreased with Soxhlet extraction and dried chicken feathers were used as untreated chicken feathers in the experiments.

4.2.1.3. Chicken Feather Treatment with NaOH

Soxhlet extracted chicken feathers were treated with sodium hydroxide in order to remove the lipid layer surrounding the feather fiber surface. 1.5 g of chicken feather were stirred with 100ml 0.1 N NaOH solution (prepared by dissolving 1 g of NaOH pellets in 250 ml of deionized water) for an hour, washed and filtered with adding 100ml of deionized water and dried in an oven at 105°C. Dried feathers were stirred with 150 ml of deionized water for half an hour, filtered and dried at 105°C and dried feathers were stored at room temperature.

4.2.1.4. Chicken Feather Treatment with Acetic Acid

1.5 g of NaOH treated and dried feathers were reacted with 25 ml of 0.6 M acetic acid at 50°C in an oven over night. Then they were washed with deionized water several times and dried in an oven at 105°C. After feathers were dried, they were washed with 0.05 N 100 ml of NaOH solution and washed several times with deionized water, filtered and dried at 105°C and stored at room temperature.

4.2.1.5. Chicken Feather Treatment with Citric Acid

1.5 g of NaOH treated and dried feathers were reacted with 25 ml of 0.6 M citric acid (prepared by dissolving 12.6 g of citric acid powder in 250 ml of deionized water) at 50°C in an oven over night. Feathers reacted with citric acid were washed then with deionized water several times and dried in an oven at 105°C. After feathers were dried, they were washed with 0.05 N 100 ml of NaOH solution, washed several times with deionized water, filtered and dried at 105°C and stored at room temperature.

4.2.2. Analysis of Chicken Feathers

4.2.2.1. ATR-FTIR Analysis

Untreated and treated chicken feather samples were dried in an oven at 95°C for 4h before Fourier Transform Infrared - Attenuated Total Reflectance spectra were taken. The ATR-FTIR spectra were obtained by Perkin Elmer Spectrum 100 Spectrofotometer, setting a representative amount of chicken feather at force 35. Spectra were acquired at 8 cm⁻¹ resolution in the spectral range 4000 – 700 cm⁻¹ using an OPD velocity of 2 cm s⁻¹ and strong Beer-Norton apodization. Data were acquired using Spectrum for Windows software (Perkin Elmer Corp., Norwalk, CT, USA). Four scans were co-added for each of the time-resolved spectra collected during a permeation experiment, leading to a typical rate of data collection of one spectrum every 30 s. Spectral manipulation was performed using GRAMS software (Galactic Industries Corp., Salem, NH, USA). Breakthrough time (BT) was taken to be the time immediately preceding the time at which two or more consecutive

absorbances exceed three standard deviations from the background absorbance.

4.2.2.2. SEM Analysis

JEOL JSM 6060 Scanning Electron Microscope (SEM) was used to study the fibrous structure of untreated and treated chicken feather samples. The samples were dried in an oven at 95°C for 4h and fibers were placed on the metallic stubs with carbon band and then placed on the holder and introduced into the instrument at 10kV.

4.2.3. Adsorption Study with Chicken Feather

4.2.3.1. Heating Effect on Adsorption of Cu^{+2} ions on the Chicken Feather

Cu^{2+} solution with initial concentrations of 1-6 mg/L was prepared and 25ml of the solutions were added to 0.05 g of untreated chicken feathers. In order to increase the uptake capacity, chicken feather and Cu^{2+} ion complexes were heated for 20 minutes stirring on the hot plate until the temperature reached 80°C. Then the Erlenmeyer flasks were removed from the hot plate. After cooling for 15 minutes the Erlenmeyer flasks were placed to the shaker and shaken for 24 h and solutions containing heavy metal ions were filtered through Whatman filter paper (No:3). The equilibrium concentrations (C_e) of the Cu^{+2} ions in the supernatant liquid, were determined by AAS (Spectra 10A). The flame type was air-acetylene and absorption wavelengths were Cu^{+2} : (324.7 nm), Zn^{+2} : (213.9 nm), Ni^{+2} : (232 nm), Cd^{+2} : (228.8 nm).

4.2.3.2. Equilibrium Adsorption Experiments

Equilibrium adsorption experiments were performed by batch experiments. 0.05 g of untreated or treated chicken feather samples, respectively, were immersed in 25 mL aqueous solutions of various metal ion solutions with initial concentrations in the range of 1-19 mg/L for Cu^{2+} , 1-11 mg/L for Cd^{2+} , 1-31 mg/L for Ni^{2+} and 1-15 mg/L for Zn^{2+} ions. They were shaken for 24 h on a horizontal shaker at 175 rpm in 100 ml Erlenmeyer flasks at 25 °C.

Equilibrium concentrations (C_e) of the filtrated metal ion solutions were determined by AAS. For the calibration, a series of metal ion solutions with varying concentrations were prepared in the day of analysis. The filtrated solutions were also analyzed in the day of experiment.

4.2.3.3. Adsorption Kinetics Experiments

Adsorption kinetics experiments were performed in 25 ml of metal ion solutions with 0.05 g untreated and with citric acid treated chicken feathers. The initial metal ion concentrations in solutions were 20mg/L for Ni^{2+} and Zn^{2+} ions, 25 mg/L for Cu^{2+} and Cd^{2+} ions. The samples were shaken on a horizontal shaker at 175 rpm in 50 ml polypropylene tubes at 25 °C for the time intervals of 10, 20, 30, 40, 50, 60, 80, 100 minutes and 2, 3, 4, 5, 6, 7, 19, 20, 23, 24 hours. Filtrated metal ion concentrations were determined by AAS using the calibration solutions.

After each experiment, the used tubes and flasks were cleaned with dilute HNO_3 solution (respectively 0.1 N) to remove metal ions from the walls of the flasks and rinsed with deionized water.

5. RESULTS AND DISCUSSION

5.1. Heating Effect

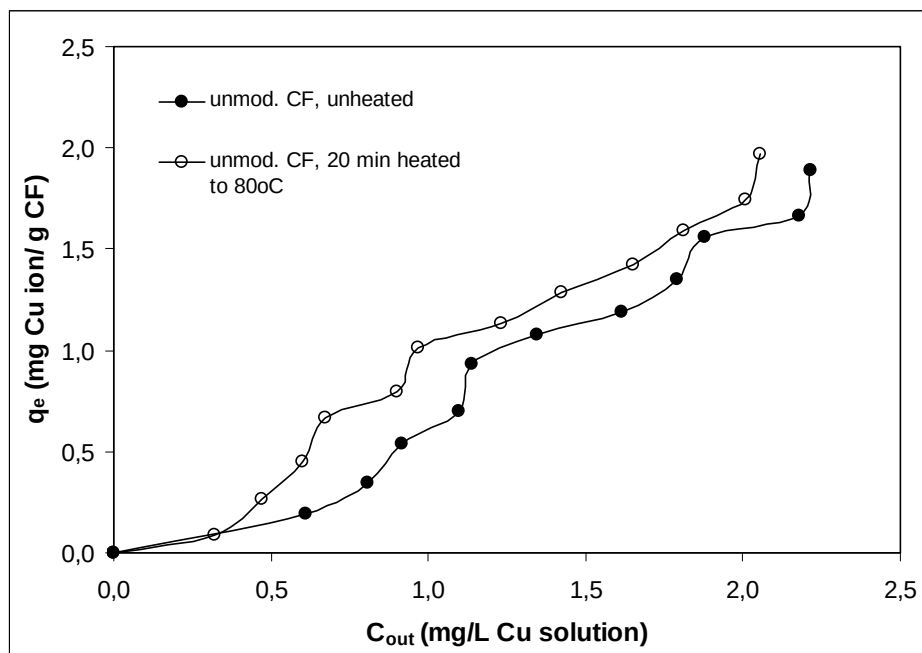


Figure 5.1: Cu^{2+} adsorption equilibrium and heating effect.

The Figure 5.1 shows that heating the solution of Cu^{2+} ions with unmodified chicken feathers affect the uptake of the metal ions slightly. Consequently, the experiments were not continued and other methods such as modification applied to increase the uptake of metal ions.

5.2. Washing Effect with NaOH

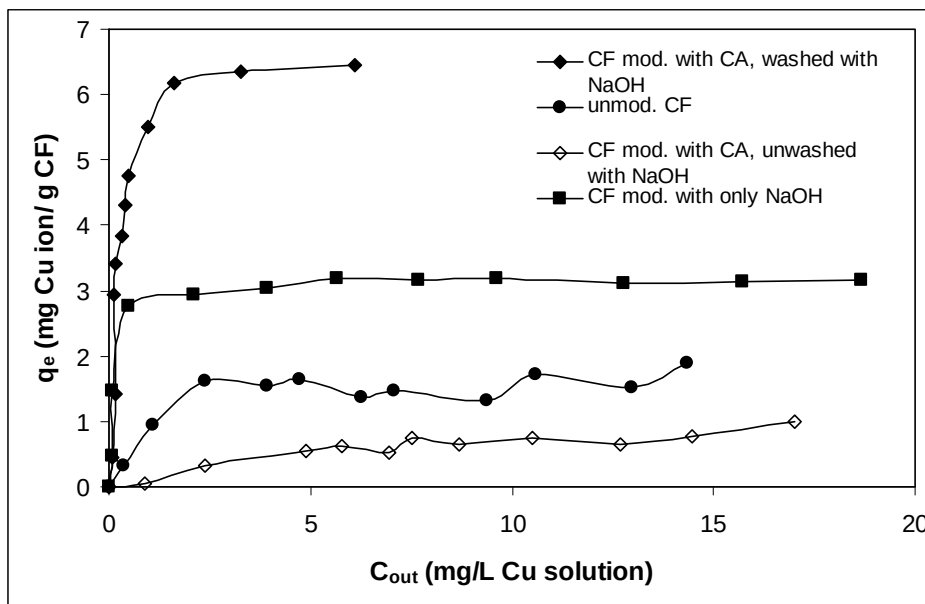


Figure 5.2: Adsorption isotherm of Cu^{2+} ions and washing effect of NaOH.

The washing effect of NaOH was studied by the modification of CF with CA by comparing the results with and without final washing with NaOH (Figure 5.2). Figure has shown that the acid treatment resulted in lower capacity of metal ions uptake if the CA modified chicken feathers were not washed with 0.05 N NaOH finally. The washing of CF with NaOH after the modification with acids however have shown not only repeatable metal ion uptake capacities, but also increased the capacity of the CF three times of its initial value. To consider the effect of NaOH, unmodified CF was also treated with 0.1 N NaOH as shown in Figure 5.2. CF treated only with NaOH showed not such high capacities, as they were obtained after acid modification with NaOH washing. These results indicate that the higher capacity of the

modified CFs is to attribute to increased -COOH (carboxyl) groups added by the acid modification of the CFs and NaOH washing causes their deprotonation (-COO^-), which are negatively charged and capable of interacting with positively charged heavy metal ions. Consequently, to increase the adsorption capacity and to provide repeatability of the experiments CFs were washed with NaOH after the treatment with CA and AA.

5.3. The Equilibrium Studies

The equilibrium studies were accomplished with CFs washed with NaOH and deionized water after the treatment with CA and AA.

5.3.1. Effect of Initial Concentration

The amount of metal ions adsorbed depends on the initial metal ion concentration. A plot of amount of heavy metal ions adsorbed versus initial Cd^{2+} , Cu^{2+} , Ni^{2+} and Zn^{2+} ion concentrations in solution were presented in Figure 5.3-5.6, respectively. As can be seen from the figures, after a linear increase of metal ions uptake as a function of initial concentration, it reaches a plateau value, after which an increase in initial concentration does not cause a further increase in metal ions uptake. The uptake amounts increased with increasing initial metal ion concentrations are as a result of the increase in the driving force (ΔC).

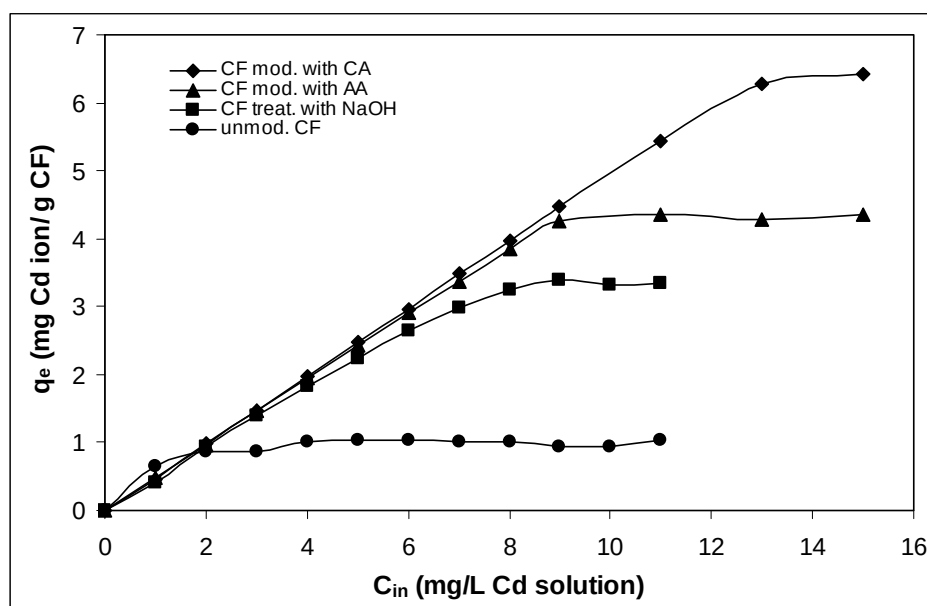


Figure 5.3: The variation of metal ion uptake with initial Cd^{2+} ion concentration.

As can be seen from figures at low concentrations the uptake of metal ions was increased with increasing initial metal ion concentration. After a certain concentration, an increase in initial metal ion concentration did not cause an increase in metal ions adsorbed, so that a saturation plateau was reached.

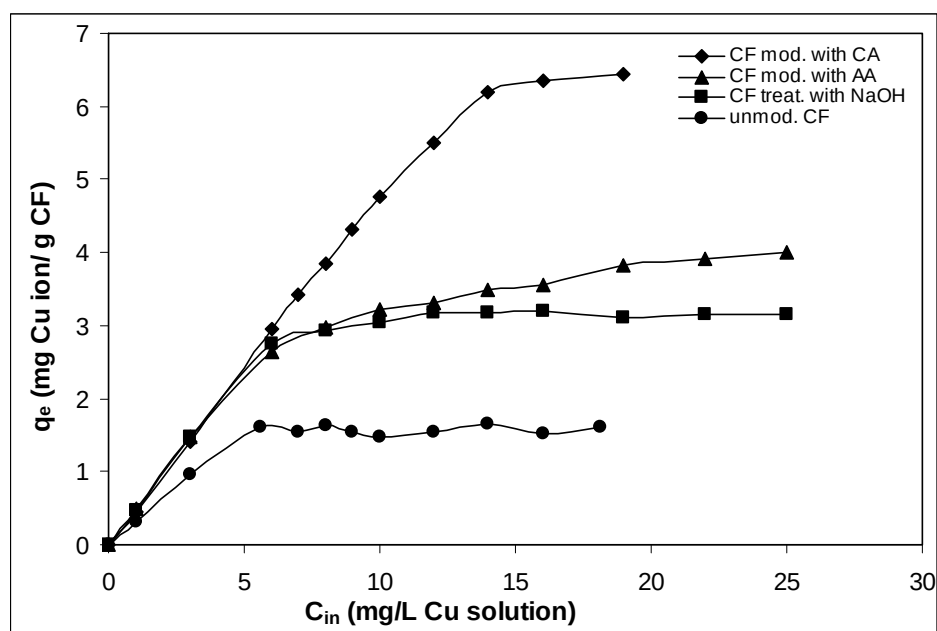


Figure 5.4: The variation of metal ion uptake with initial Cu^{2+} ion concentration.

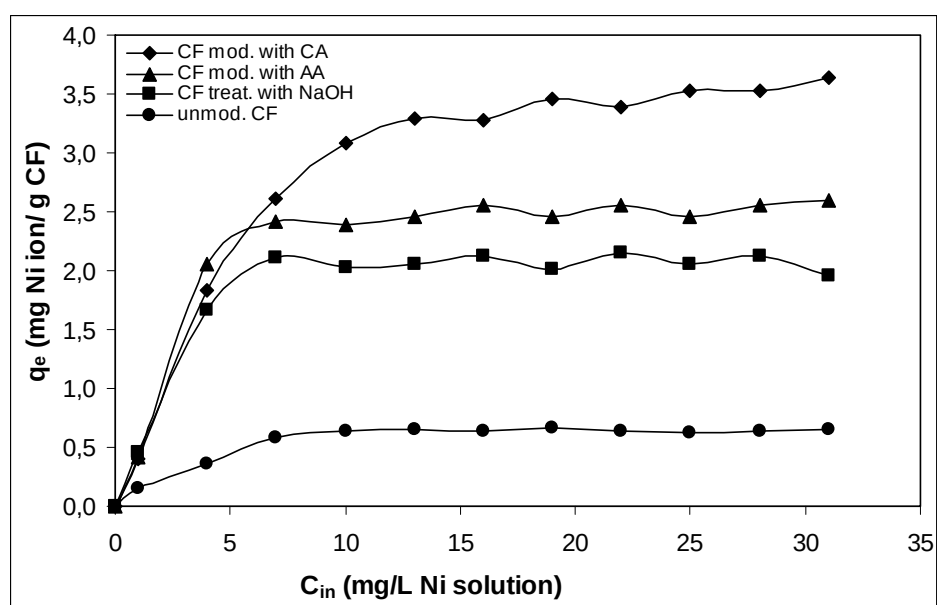


Figure 5.5: The variation of metal ion uptake with initial Ni^{2+} ion concentration.

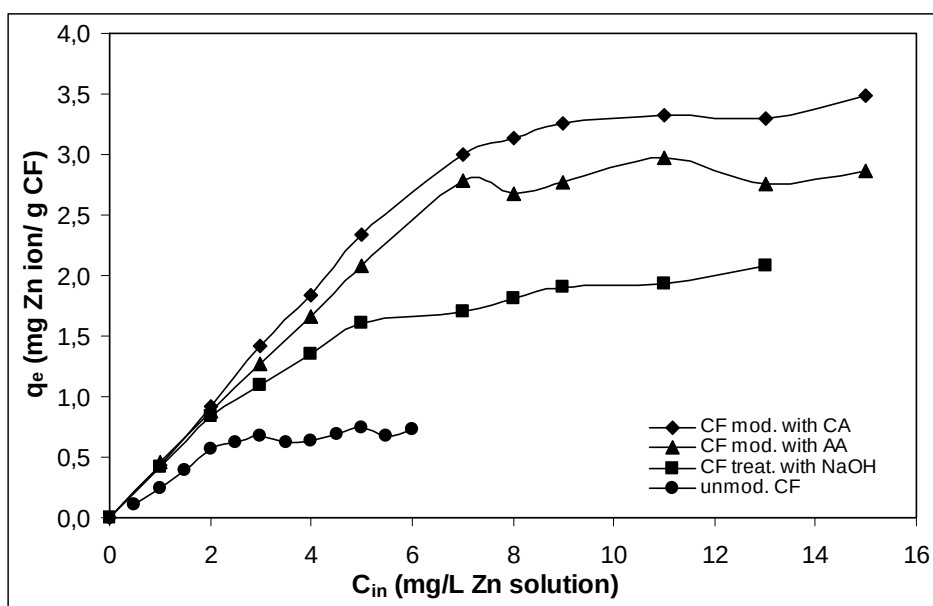


Figure 5.6: The variation of metal ion uptake with initial Zn^{2+} ion concentration.

Based on the saturation value depending on the initial concentration of heavy metal ions, different initial concentrations were chosen for different metal ions. Concentrations ranges from 1-19 mg/L for Cu^{2+} , 1-11 mg/L for Cd^{2+} , 1-31mg/L for Ni^{2+} and 1-15mg/L for Zn^{2+} metal ion solutions used in this study were sufficient to reach the equilibrium.

5.3.2. Effect of Adsorbent Type on Adsorption Equilibria

The adsorption of Cu^{2+} , Cd^{2+} , Ni^{2+} and Zn^{2+} ions onto untreated CF, treated CF with NaOH, AA and CA was studied at 25°C by varying metal ion concentrations.

The amount of metal ions adsorbed at equilibrium, q_e (mg/g), was calculated as

$$q_e = \frac{(C_o - C_e)V}{W} \quad (4.1)$$

where C_o and C_e are concentrations (mg/L) of metal ions in the initial and equilibrium solutions, respectively, V (L) is volume of the initial solution taken for equilibration, and W (g) is the weight of the chicken feather.

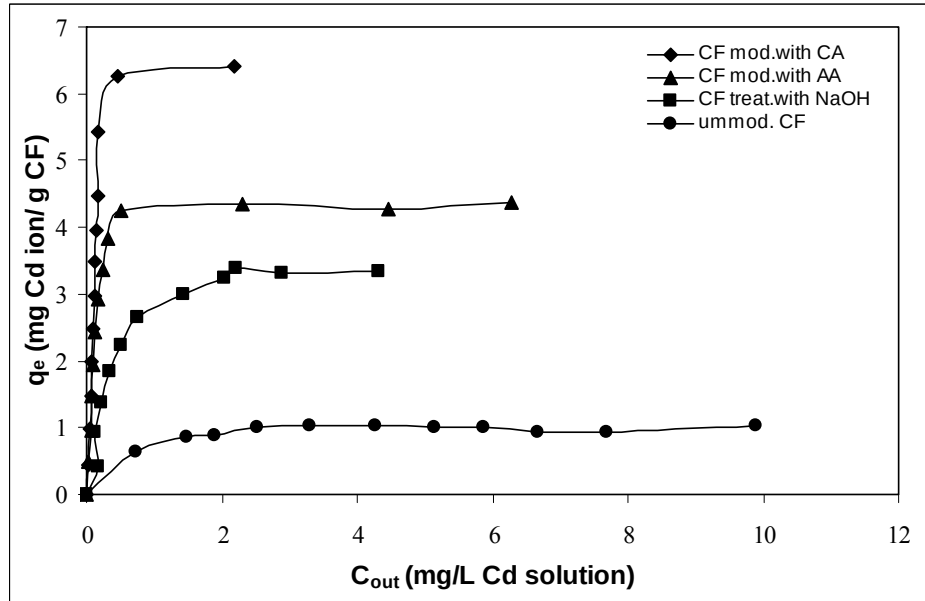


Figure 5.7: Adsorption equilibria for Cadmium adsorption onto different adsorbents.

As can be seen from Figure 5.7 uptake of metal ions increased at low concentrations and then the saturation plateau was reached a value at 6.5 mg/g for CF modified with CA, 4.5 mg/g for CF modified with AA, 3.5 mg/g for CF treated with NaOH, 1 mg/g for unmodified CF and did not change with further increase in initial Cd^{2+} concentration, suggesting that available sites on the adsorbents are the limiting factor for metal ion adsorption.

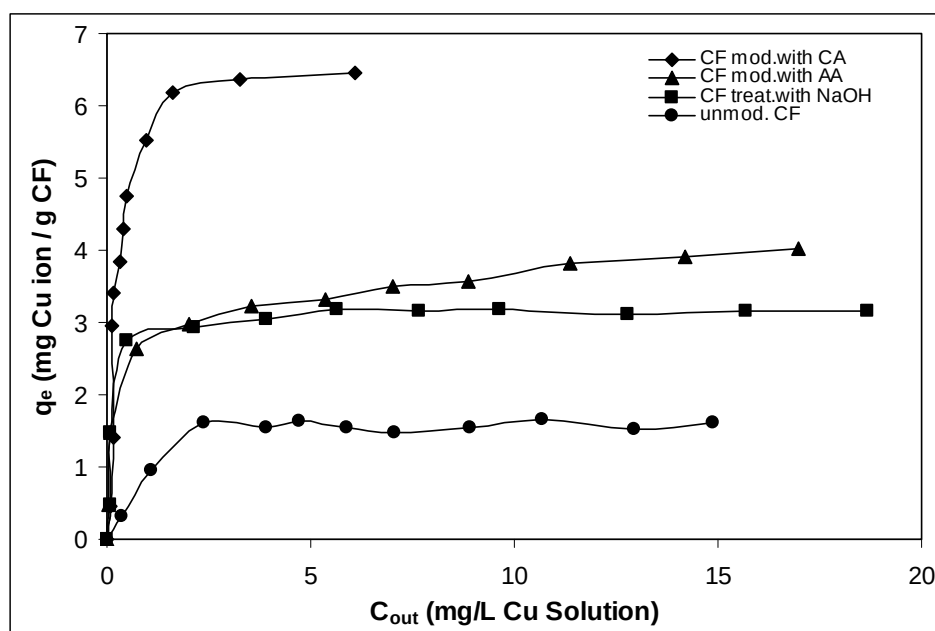


Figure 5.8: Adsorption equilibria for Copper adsorption onto different adsorbents.

Figure 5.8 shows that the maximum uptake values of Cu^{2+} ion for CF modified with CA, AA, CF treated with NaOH and unmodified CF are 6.5, 4.0, 3.2 and 1.8 mg/g, respectively.

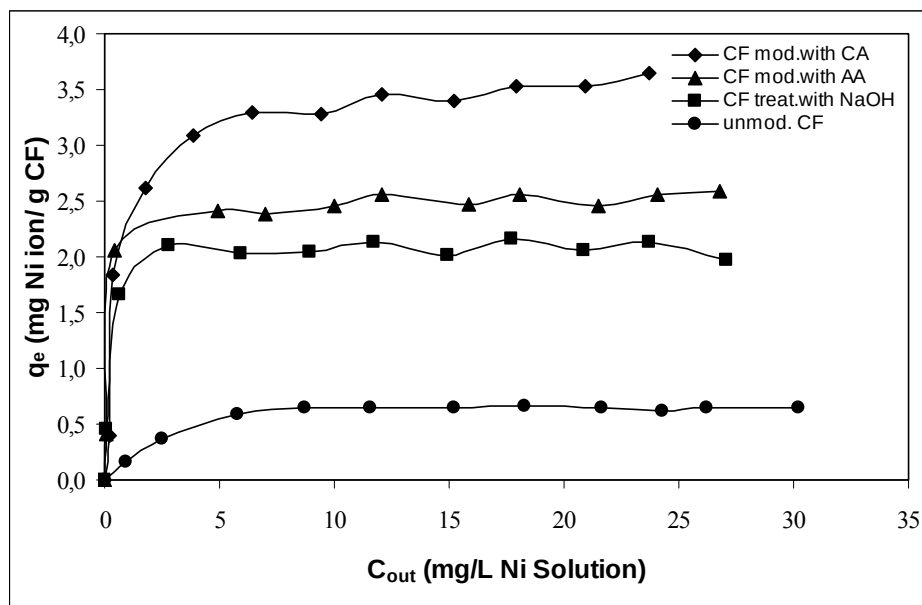


Figure 5.9: Adsorption equilibria for Nickel adsorption onto different adsorbents.

Figure 5.9 represents the uptake of Ni^{2+} ions onto the different types of adsorbents as a function of equilibrium solution concentration of Ni^{2+} ions. The uptake amounts at equilibrium are 3.6, 2.5, 2.0 and 0.7 mg/g for CF modified with CA, AA, CF treated with NaOH and unmodified CF, respectively.

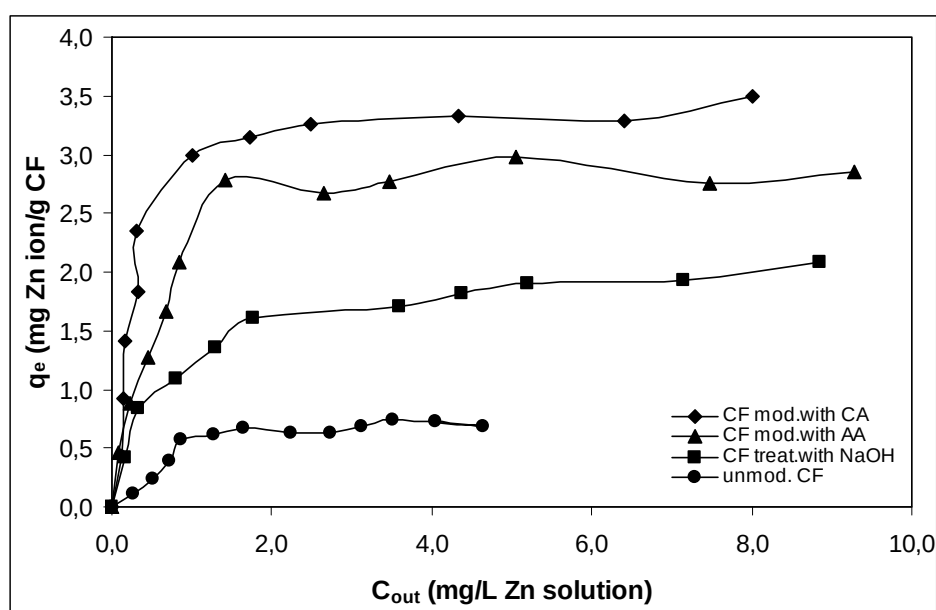


Figure 5.10: Adsorption equilibria for Zinc adsorption onto different adsorbents.

The Figure 5.10 shows that the uptake amounts at equilibrium are 3.4, 2.8, 2.0 and 0.7 mg/g for CF modified with CA, AA, CF treated with NaOH and unmodified CF, respectively.

At lower concentrations, all metal ions present in solutions could interact with the binding sites of all type of adsorbents and thus the adsorption was effective at low initial metal ion concentrations.

5.3.3. Effect of Modification of CF on Adsorption Equilibria

In order to compare the uptake capacities of CFs for different heavy metal ions, the amount of heavy metal ions adsorbed onto CF (mmol/g) versus the concentration of heavy metal ions remained in solution (mmol/L) were shown in figures below.

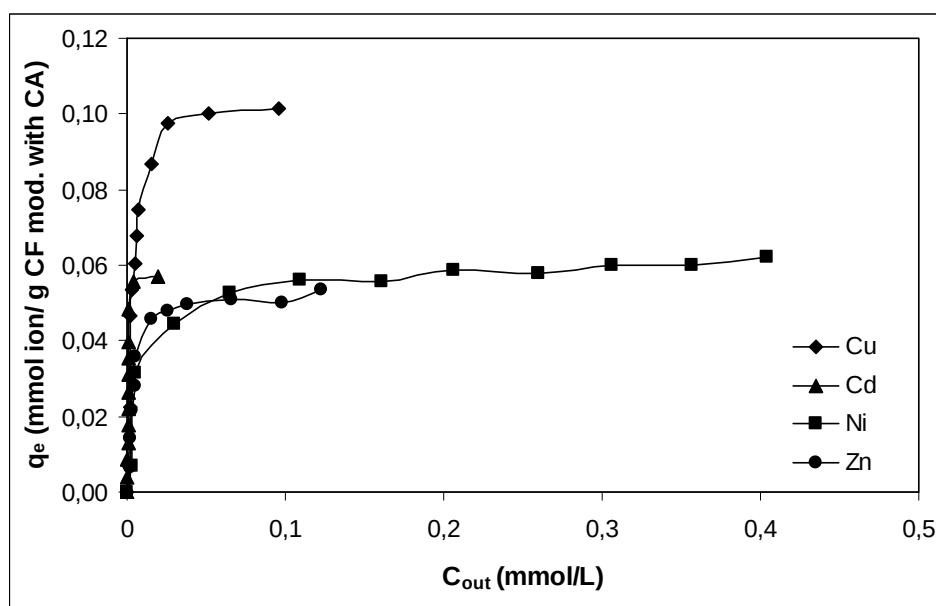


Figure 5.11: Adsorption equilibria for different metal ions adsorbed on CF modified with CA.

It can be seen from the Figures 5.11- 5.14 that adsorption capacity for different metal ions differs depending on the type of adsorbent. For CF modified with CA the metal uptake capacity decreases in the order $Cu^{2+} > Cd^{2+} \approx Ni^{2+} \approx Zn^{2+}$, as 0.1, 0.06, 0.06 and 0.058 mmol/g, respectively.

Depending on their physical and chemical properties, heavy metal ions have different affinity for oxygen-, nitrogen- and sulfur-containing ligands. The adsorption capacity of the heavy metal ions varies depending on their interactions with the active sites of the keratin molecules in CF.

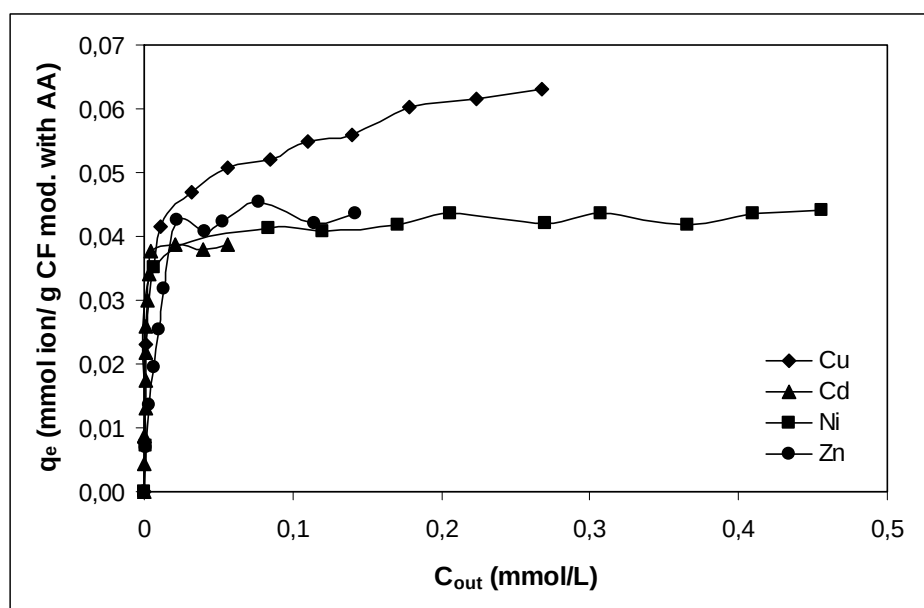


Figure 5.12: Adsorption equilibria for different metal ions adsorbed on CF modified with AA.

For CF modified with AA the metal uptake capacity decreases in the order of $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+}$, as 0.064, 0.045, 0.042 and 0.040 mmol/g, respectively.

The reactions between metal ions and active sites of the proteins can be explained with their Lewis acid and base characteristics. A Lewis acid is an electron pair acceptor and a Lewis base is an electron pair donor. The metal cations are classified into hard and soft Lewis acids.

Cd^{2+} , Cu^{2+} , Ni^{2+} , and several other ions come into an intermediate class, with Ni^{2+} shifting towards hard and Cd^{2+} , soft class. Oxygen and aliphatic nitrogen sites of the keratin molecules are classified as hard, aromatic nitrogen as intermediate, and sulfur as soft Lewis bases. It follows that Ni^{2+} would rather bind to aromatic nitrogen, and Cd^{2+} , Hg^{2+} , and Ag^+ , to SH-groups. For example, Ni^{2+} and Zn^{2+} practically do not differ in several physical and chemical properties, such as the size of the ion. However, Zn^{2+} is a constituent of numerous enzymes, whereas Ni^{2+} is found in few plant enzymes, probably because in the former case the rate of ligand exchange is higher by three orders of magnitude. Thus, diverse effects of the metals of similar properties apparently depend on the different rates of ligand exchange, and their physiological properties are immediately derived from their physical and chemical parameters.

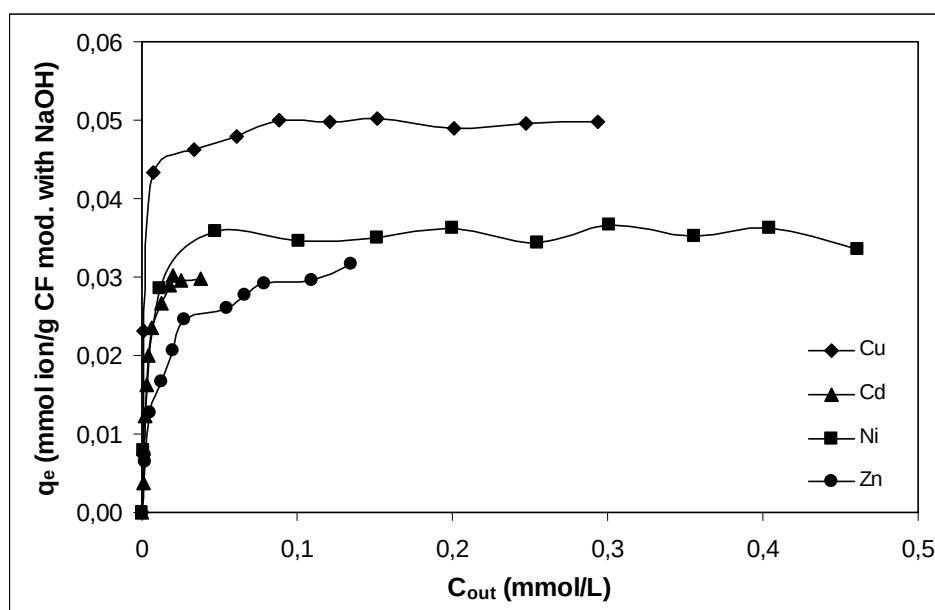


Figure 5.13: Adsorption equilibria for different metal ions adsorbed on CF treated with NaOH.

For CF treated with NaOH, the metal uptake capacity decreased in the order of $\text{Cu}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} \approx \text{Cd}^{2+}$, as 0.05, 0.035, 0.03 and 0.03 mmol/g, respectively.

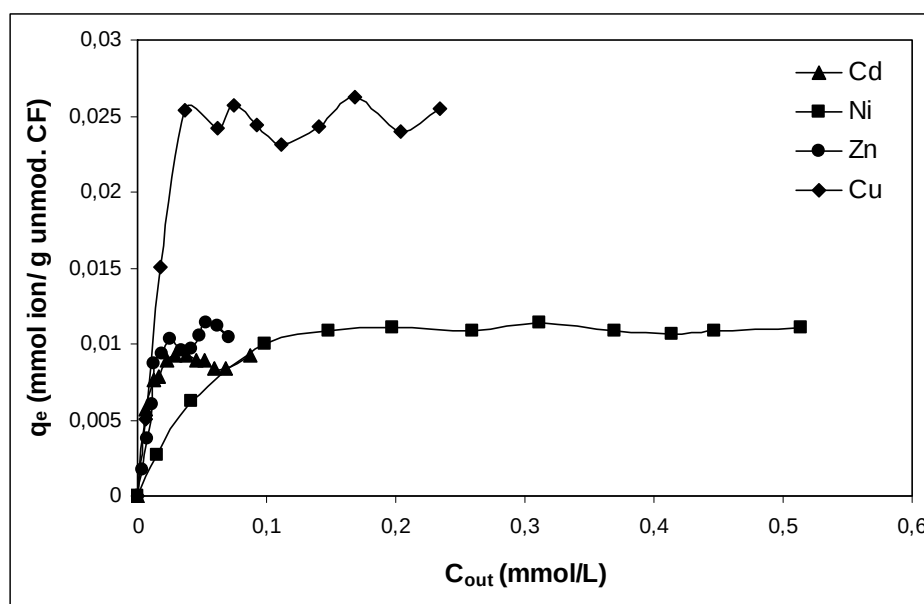


Figure 5.14: Adsorption equilibria for different metal ions adsorbed on unmodified CF.

For untreated CF, the metal uptake capacity decreased in the order of $\text{Cu}^{2+} > \text{Ni}^{2+} = \text{Zn}^{2+} > \text{Cd}^{2+}$, 0.03, 0.012, 0.12 and 0.009 mmol/g, respectively.

A comparison of the order of heavy metal uptakes depending on the adsorbent modification has shown that all the adsorbents adsorbed Cu^{2+} ions in a higher capacity than other heavy metal ions. With untreated CF and CA modified CF the capacity increase reached a value 100% higher than that of the other ions, while adsorption capacities of other heavy metal ions were almost in the same amount. With NaOH

treated CF and with AA modified CF the capacity difference remained at 70% and 40%, respectively. The higher adsorption capacity of Cu^{2+} ions onto modified and unmodified CF can be ascribed to the surface precipitation of Cu^{2+} ions at pH values of the CF solution mixtures above pH 5.

The modification of the CF increased the metal ions uptake in the order CF modified with CA > CF modified with AA > CF treated with NaOH > CF unmodified. The highest uptake values were obtained by the modification of CF with CA. Ni, Cd and Zn ions uptake at unmodified CF increased from 0.01 to 0.03 after NaOH treatment and to 0.04 with AA modification and reached to 0.06 (mmol/g) by CA modification. Cu ions uptake was obtained with unmodified CF as 0.025 and after NaOH treatment reached to 0.05 and increased with AA modification to 0.06 and obtained its highest value of 0.1 by CA modification of CF.

Douglas et al, 2006 modified soybean hulls with CA to adsorb Cu^{2+} ions. By the comparison of the modified and unmodified hulls for the adsorption they found that the modified soybean hulls showed a higher adsorption capacity for Cu^{2+} ions than the unmodified hulls.

They concluded that the addition of carboxyl groups resulted in more adsorption of Cu^{2+} ions when using modified hulls. The addition of carboxyl groups are obtained by formation of active anhydride with acid and combination with cellulose compounds. We can explain similarly the higher uptake of heavy metal ions onto AA and CA modified CF due to the increased amount of carboxyl groups added onto CF through the surface modification. Besides concentrated acids cause hydrolysis in CF molecules, that leads to an increased uptake of heavy metals.

5.3.4. Adsorption Isotherms

The results obtained by the adsorption of Cu^{+2} , Cd^{+2} , Ni^{+2} and Zn^{+2} ions onto untreated and treated chicken feathers with NaOH, acetic acid and citric acid were analyzed by the well-known models given by Langmuir, Freundlich and Dubinin–Kaganer–Radushkevich (DKR) isotherms. From the equation 3.2 the linear form of Langmuir isotherm ($1/q_e$ versus $1/C_e$) was plotted and the $q_{\max}$, K_L and R^2 values were calculated (Table 5.1).

Table 5.1: Langmuir parameters.

Heavy metal ion	Adsorbent	$q_{\max}(\text{mg/g})$	K_L	R^2
Cd^{2+}	unmod. CF	1.0964	2.0982	0.8869
	CF treat. with NaOH	5.1653	1.0602	0.6016
	CF mod. with AA	7.1225	3.0259	0.9769
	CF mod. with CA	15.2672	1.8931	0.9798
Cu^{2+}	unmod. CF	2.1829	0.4884	0.9672
	CF treat. with NaOH	3.3367	4.1224	0.6419
	CF mod. with AA	3.8880	4.9272	0.9327
	CF mod. with CA	-8,7260	-0,6297	0,8227
Ni^{2+}	unmod. CF	0.8188	0.2674	0.9816
	CF treat. with NaOH	2.1692	3.3070	0.9963
	CF mod. with AA	2.6350	3.8567	0.9941
	CF mod. with CA	4.6339	0.6067	0.8114
Zn^{2+}	unmod. CF	2.1935	0.2205	0.9238
	CF treat. with NaOH	2.1286	1.5413	0.9840
	CF mod. with AA	2.9053	2.3147	0.9836
	CF mod. with CA	6.1576	0.9233	0.7075

Langmuir parameters and correlation coefficients evaluated from the model indicate favorable conditions for adsorption of heavy metal ions onto CF. According to Hall et al., (1966), the essential features of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter K_L which is defined by the following relationship:

$$K_L = \frac{1}{1 + K_a C_o} \quad (5.1)$$

where K_L is a dimensionless separation factor, C_o the initial concentration (mg/L), K_a the Langmuir constant (L/mg). The parameter K_L indicates the shape of the isotherm and nature of the adsorption process [$K_L > 1$: unfavorable; $K_L = 1$: linear; $0 < K_L < 1$: favorable; $K_L = 0$: irreversible] (Ong et al., 2007). The values of K_L obtained were between 0 and 1 indicating the favorable adsorption of metal ions. But the values of q_{max} are not fitting the data obtained experimentally as can be seen from Figures 5.15-5.18.

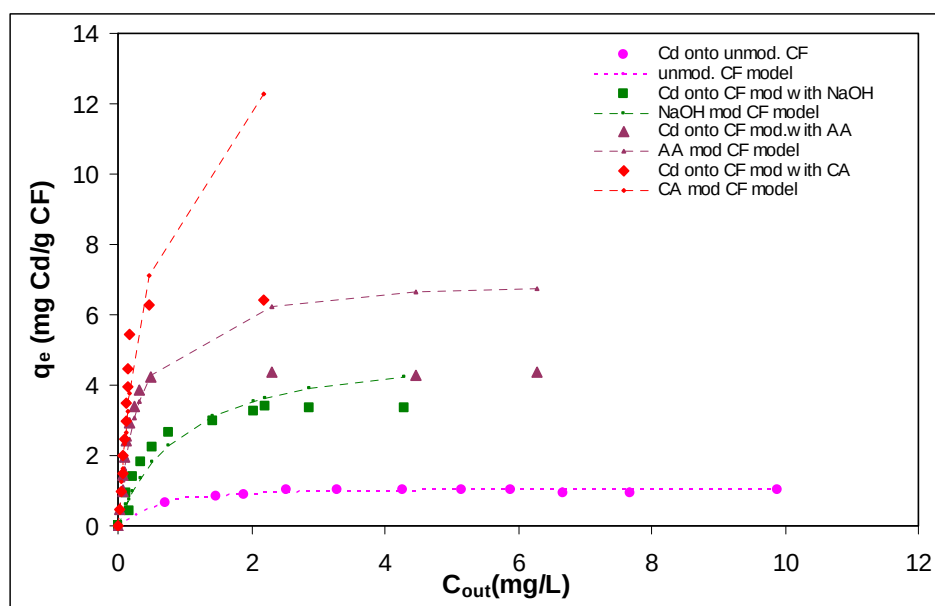


Figure 5.15: Experimental and calculated Langmuir isotherms for Cd^{2+} ions adsorbed on different adsorbents.

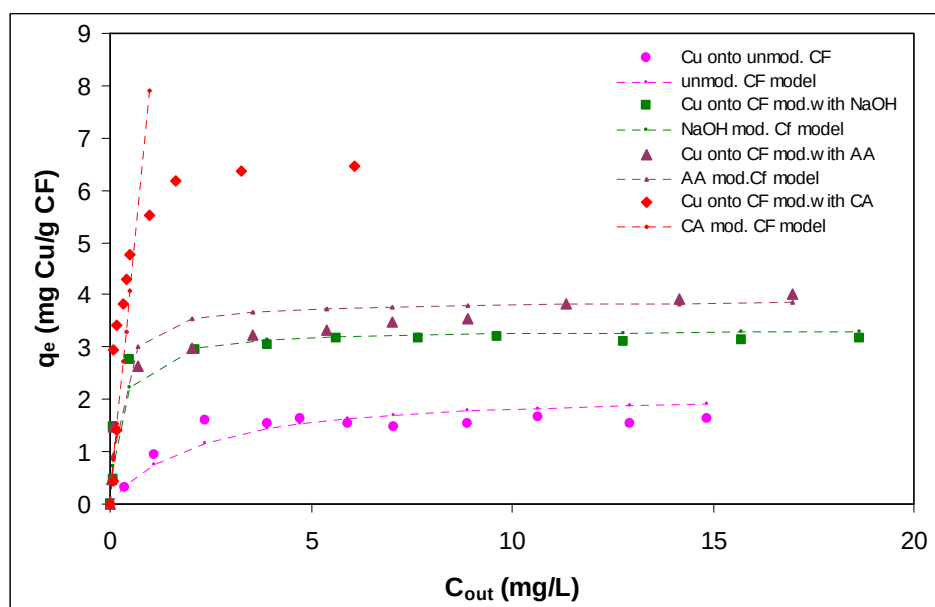


Figure 5.16: Experimental and calculated Langmuir isotherms for Cu^{2+} ions adsorbed on different adsorbents.

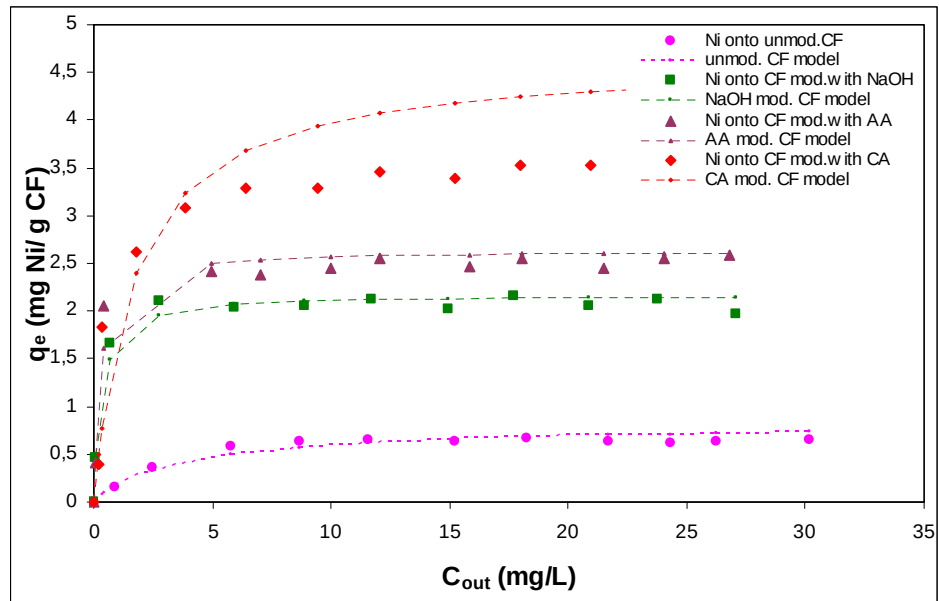


Figure 5.17: Experimental and calculated Langmuir isotherms for Ni^{2+} ions adsorbed on different adsorbents.

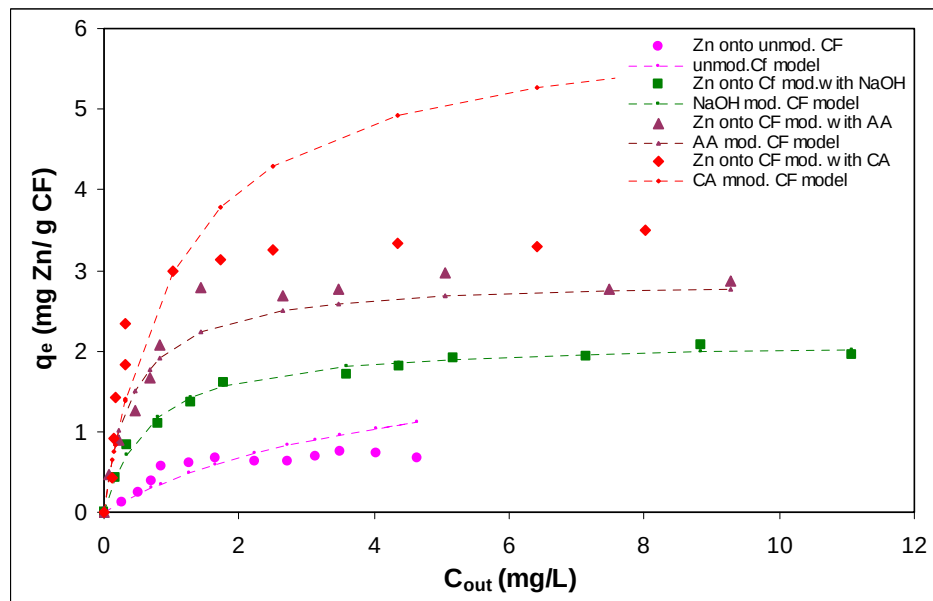


Figure 5.18: Experimental and calculated Langmuir isotherms for Zn^{2+} ions adsorbed on different adsorbents.

The adsorption data obtained also analyzed with Freundlich isotherm model from the equation 3.4. The linearized form of Freundlich isotherm was plotted according to $\log q_e$ versus $\log C_e$ and the parameters K_F , $1/n$ and correlation coefficients R^2 were listed in Table 5.2. K_F and $1/n$ are characteristic constants that can be related to the relative adsorption capacity of the adsorbent (mg/g) and the intensity of adsorption, respectively.

Table 5.2: Freundlich parameters.

Heavy metal ion	Adsorbent	K_F	$1/n$	R^2
Cd^{2+}	unmod. CF	0.7820	0.1407	0.6246
	CF treat. with NaOH	2.2919	0.4685	0.7512
	CF mod. with AA	3.5826	0.3252	0.6672
	CF mod. with CA	8.6936	0.5745	0.7474
Cu^{2+}	unmod. CF	0.7511	0.3673	0.7130
	CF treat. with NaOH	1.9530	0.2316	0.6630
	CF mod. with AA	2.1125	0.2695	0.8473
	CF mod. with CA	4.4596	0.4920	0.6215
Ni^{2+}	unmod. CF	0.2325	0.3546	0.7884
	CF treat. with NaOH	1.2081	0.2143	0.7212
	CF mod. with AA	1.3693	0.2350	0.7488
	CF mod. with CA	1.5336	0.3265	0.7036
Zn^{2+}	unmod. CF	0.3925	0.5493	0.7629
	CF treat. with NaOH	1.0710	0.3343	0.8908
	CF mod. with AA	1.6676	0.3718	0.8557
	CF mod. with CA	2.1399	0.3534	0.6835

The highest K_F value was found as 8.69 for Cd^{2+} adsorbed onto CF modified with CA. The magnitude of K_F and n values expresses affinity

for metal ions from wastewater onto CF and also indicates a favorable adsorption. If adsorption is favorable, then $1/n$ will be <1.0 or $n >1.0$ (Treybal, 1980). For four types of metal ions used in this study, the values of $1/n$ calculated from Freundlich isotherms for different adsorbents were found to be very close to each other (Figure 5.19-5.22). However, the correlation coefficients evaluated give a poor fit to the model.

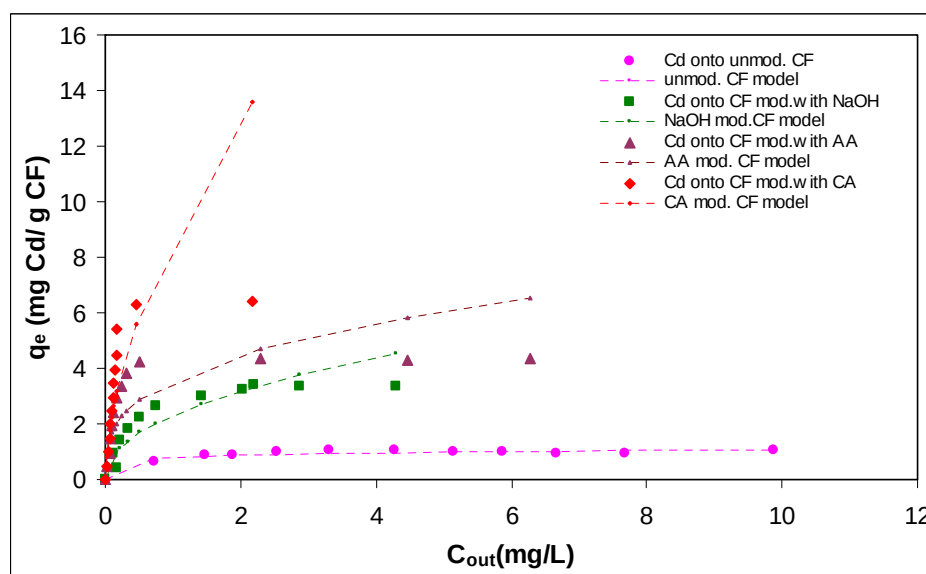


Figure 5.19: Experimental and calculated Freundlich isotherms for Cd^{2+} ions adsorbed on different adsorbents.

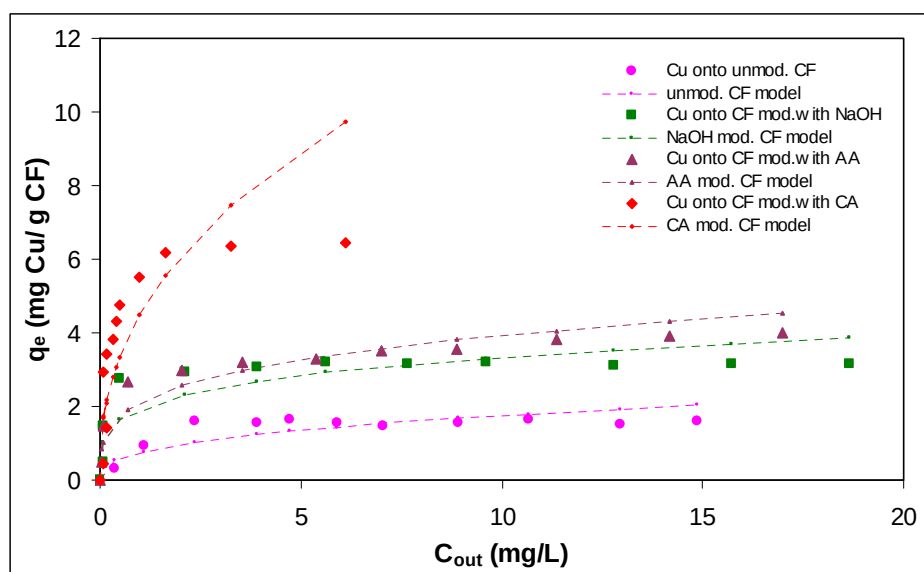


Figure 5.20: Experimental and calculated Freundlich isotherms for Cu^{2+} ions adsorbed on different adsorbents.

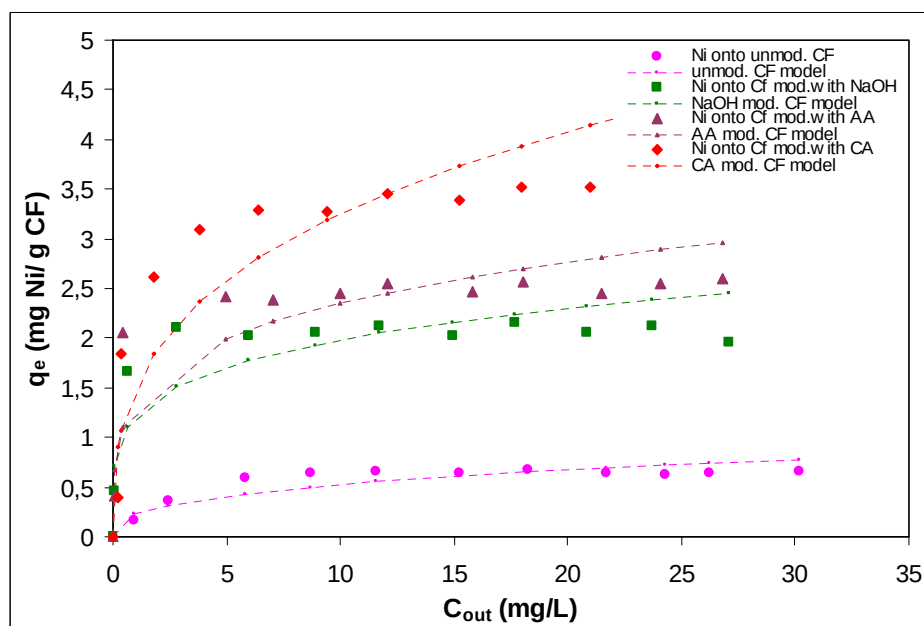


Figure 5.21: Experimental and calculated Freundlich isotherms for Ni^{2+} ions adsorbed on different adsorbents.

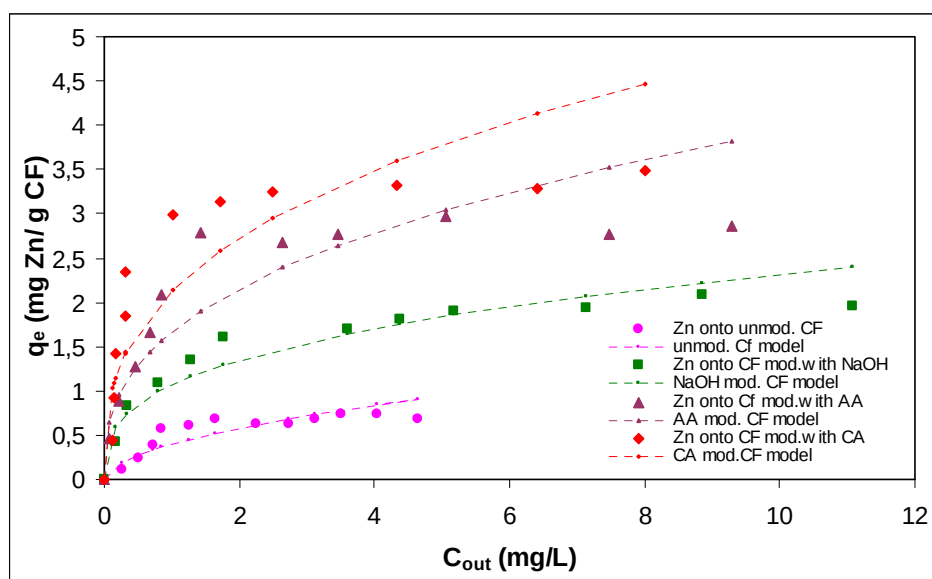


Figure 5.22: Experimental and calculated Freundlich isotherms for Zn^{2+} ions adsorbed on different adsorbents.

The Dubinin–Kaganer–Radushkevich (DKR) equation confines to the monolayer region in micro pores of adsorbent and has been widely used to explain energetic heterogeneity of solid surfaces at low coverages (Ma et al., 2007). DKR isotherm has been used to describe the sorption of metal ions on CF. From the equation $2.5 \ln q_e$ versus ε^2 was plotted to obtain the data listed in Table 5.3.

The saturation limit $q_{\max}$ may represent the adsorption capacity of the sorbent. In Table 5.3 DKR parameters and the correlation coefficients (R^2) are given. The magnitude of free energy was used for estimating the type of adsorption. The free energy E was obtained using Eqn. (3.7) (Ma et al., 2007). Since E values found in this work are less than 8 kJ/mol, the type of adsorption is physical adsorption due to weak van Der Waals forces.

Table 5.3: Dubinin–Kaganer–Radushkevich (DKR) parameters.

Heavy metal ion	Adsorbent	β (polanyi potential)	$q_{\max}$ (mg/g)	E (kJ/mol)	R^2
Cd^{2+}	unmod. CF	0.0687	1.0175	2.6978	0.9084
	CF treat. with NaOH	0.0592	3.4199	2.9062	0.8495
	CF mod. with AA	0.0322	4.8341	3.9406	0.9723
	CF mod. with CA	0.0286	7.5587	4.1812	0.9411
Cu^{2+}	unmod. CF	0.1531	1.6013	1.8072	0.982
	CF treat. with NaOH	0.0280	3.1591	4.2258	0.8099
	CF mod. with AA	0.0244	3.5187	4.5268	0.9444
	CF mod. with CA	0.0704	7.5519	2.6650	0.9553
Ni^{2+}	unmod. CF	0.3016	0.6346	1.2876	0.9628
	CF treat. with NaOH	0.0368	2.0734	3.6860	0.9957
	CF mod. with AA	0.0310	2.5181	4.0161	0.9946
	CF mod. with CA	0.0927	3.4418	2.3224	0.9129
Zn^{2+}	unmod. CF	0.0912	0.7519	2.3415	0.9691
	CF treat. with NaOH	0.0647	1.8009	2.7799	0.9467
	CF mod. with AA	0.0456	2.5726	3.3113	0.9051
	CF mod. with CA	0.0560	3.5676	2.9881	0.8943

A comparison of the $q_{\max}$ values obtained from the Figures 5.23-5.26 with that of $q_{\max}$ of the DKR model indicates that the experimental and calculated values of $q_{\max}$ by DKR model give good agreement with each other.

The data shows that the COO^- side chains are not the only binding sites for metal ions. $-\text{SH}$, $-\text{NH}_2$ and other aromatic side chains can affect the uptake of metal ions. Nevertheless for all metal ion species CF modified with CA have the highest uptake capacity indicating COO^- as an important part of binding sites.

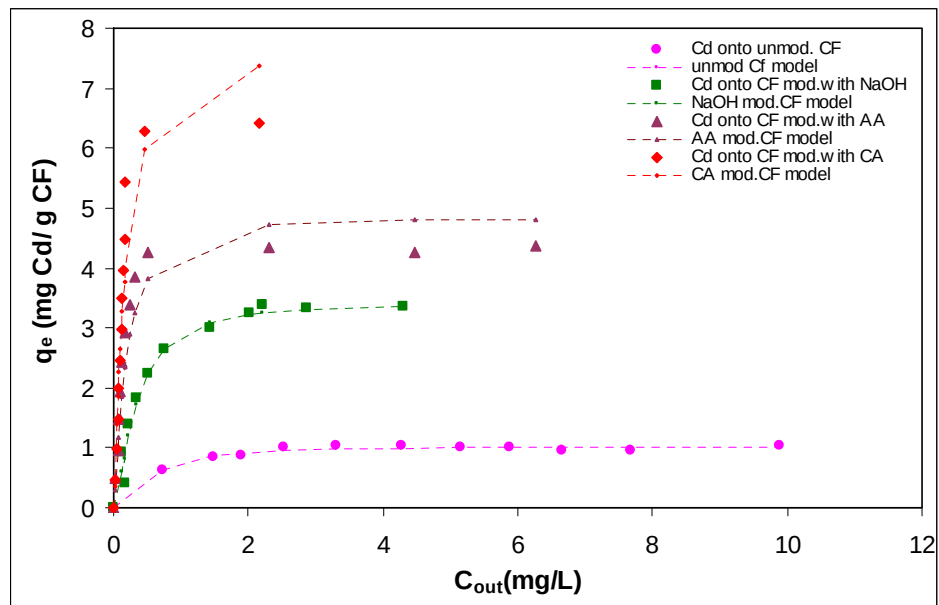


Figure 5.23: Experimental and calculated DKR isotherms for Cd^{2+} ions adsorbed on different adsorbents.

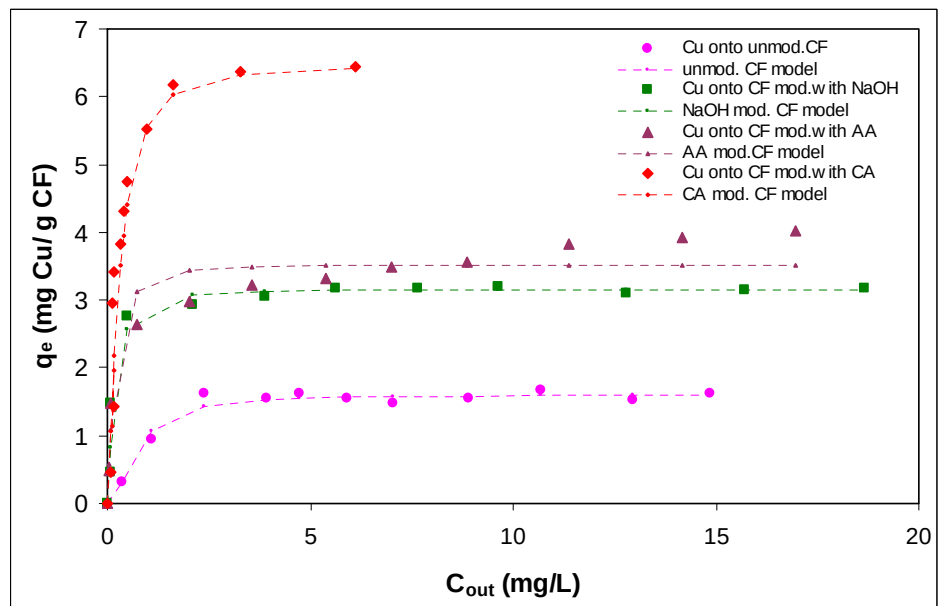


Figure 5.24: Experimental and calculated DKR isotherms for Cu^{2+} ions adsorbed on different adsorbents.

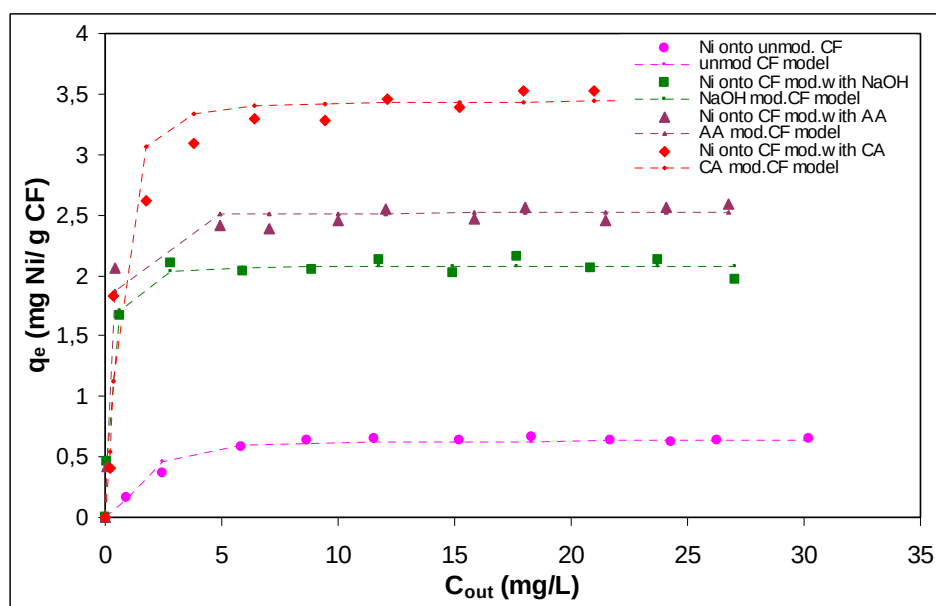


Figure 5.25: Experimental and calculated DKR isotherms for Ni^{2+} ions adsorbed on different adsorbents.

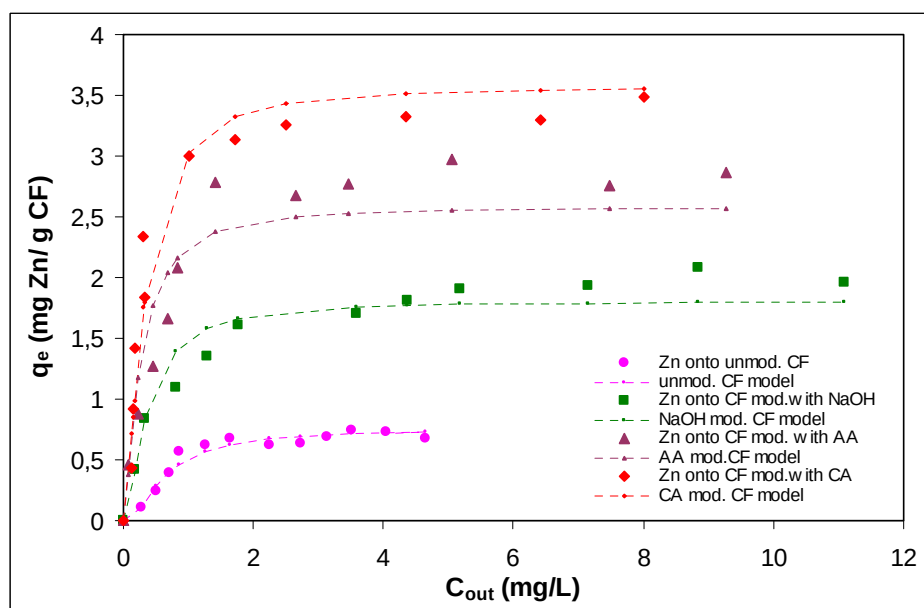


Figure 5.26: Experimental and calculated DKR isotherms for Zn^{2+} ions adsorbed on different adsorbents.

The adsorption of heavy metal ions onto keratin molecules are attributed to the interaction with the negatively charged surface functional groups and the retention by the surface complexation and surface precipitation.

Kar et al., 2004 have studied alkaline (NaOH treatment) ultrasonic processing of the chicken feathers by the cavitation effect, increasing the surface area. It was also believed that due to processing, hydroxyl ions become embedded in the surface and caused surface precipitation of metal cations, thereby improving uptake. This effect causes especially for Cu^{2+} ions to precipitate on the surface.

The speciation diagram for Cu^{2+} ions indicate that in the pH range 2–4.9, Cu^{2+} ions in aqueous solution may exist in two forms: $\text{Cu}(\text{aq})^{2+}$ and $\text{Cu}(\text{NO}_3)^+$. In the pH range 5.0–6.0 an additional two species may appear: $\text{Cu}(\text{OH})^+$ and $\text{Cu}(\text{OH})_{\text{aq}}$ (Oh et al., 2007).

The modified CFs were used to adsorb metal ions in nitrate solutions with pH 6–8. The speciation diagram for Zn^{2+} ions shows that in the pH range 2–8, Zn^{2+} ions in aqueous solution exist only in one form, $\text{Zn}(\text{aq})^{2+}$. Similar to Zn^{2+} , at pH values below 8, Cd^{2+} and Ni^{2+} ions exist in aqueous solution only as the $\text{Cd}(\text{aq})^{2+}$ and $\text{Ni}(\text{aq})^{2+}$ species (Oh et al., 2007). As we put modified CFs with CA and AA into DI water we have obtained pH values of 8.45 and 8.25, respectively, after they have been washed many times with DI water. These pH values indicate that hydroxyl ions may become embedded onto CF fibers.

The metal removal is highly dependent on pH, with adsorption increasing with decreasing acidity. This effect is much more marked for Cu^{2+} , owing to their greater capacity to form inner-sphere complexes at

the edge sites, due to their facility to hydrolyse (hydrolysis constants (pK_a) Cu = 7.9; Zn = 9.0; Ni = 9.9; Cd = 10.1). In particular, at pH 7 the order of removal capacity ($Cu^{2+} > Cd^{2+} > Zn^{2+} \approx Ni^{2+}$) is in reasonable agreement with the first hydrolysis equilibrium constants. However, at these values of pH it is possible that also metal hydroxide formation contributes to the removal process, even if the affinity series found in the pH range 6.5–8.0 does not seem to be closely related to the solubility of these hydroxides. In fact according to the values of the solubility product constants (pK_{sp}) ($Cu(OH)_2 = 19.66$; $Zn(OH)_2 = 16.91$; $Ni(OH)_2 = 14.70$; $Cd(OH)_2 = 13.60$; pK_{sp} is exceeded at pH 7.0 only for $Cu(OH)_2$ and at pH 8.0 for $Cu(OH)_2$ and $Zn(OH)_2$. The blue color of the CF after the adsorption of Cu^{2+} ions can be ascribed to surface precipitation of $Cu(OH)_2$. However, no precipitate was visible during the experiments with Zn^{2+} ions.

The adsorption capacities of modified and unmodified CF were studied in this work. It was found that the modified CF with CA showed a higher adsorption capacity of metal ions than the unmodified CF, CF treated with NaOH and CF modified with AA. The reason was that acid formed an active site and added more carboxyl group resulting in more adsorption of metal ion when using modified CF with CA (Douglas et al., 2006).

Table 5.4: Comparison of sorption capacities for different metal ions with some adsorbents.

Adsorbent	Metal Ion	Initial Metal ion Concentration	(mg heavy metal ion/ g adsorbent)	Reference
Keratin Fiber	Cu^{2+} , Ni^{2+} , Zn^{2+}	10 ppm	3.25, Very small, Very small, respectively	Kar et al., (2004)
Cassava Fiber Wastes (1.0M mercaptoacetic acid modified)	Cu^{2+}	30 mg/L	0.703	Augustine et al., (2007)
Soybean Hulls (modified with CA)	Cu^{2+}	50 ppm	48	Douglas et al., (2006)
Aspen Wood (modified with CA)	Cu^{2+}	50 mg/L	7.90	Mc Sweeny et al., (2006)
Wool (heated to 85°C)	Cu^{2+}	10 mM	9.4	Sheffield et al., (2005)
Keratin Composed Chicken Feather	Cu^{2+} , Zn^{2+}	50, 100 mg/L, respectively	6.35, 5.24, respectively	Banat et al., (2002)
Pelletized Ponderosa Pine Bark	Cu^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+}	10 mg/L	0.7, 0.4, 0.7, 0.5, respectively	Oh al, (2007)
Chicken Feather (untreated)	Cd^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+}	11,19,31,15 mg/L, respectively	1, 1.8, 0.7, 0.7 respectively	Present Study
Chicken Feather (modified with NaOH)	Cd^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+}	11,19,31,15 mg/L, respectively	3.5, 3.2, 2.0, 2.0, respectively	Present Study
Chicken Feather (modified with AA)	Cd^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+}	11,19,31,15 mg/L, respectively	4.5, 4.0, 2.5, 2.8, respectively	Present Study
Chicken Feather (modified with CA)	Cd^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+}	11,19,31,15 mg/L, respectively	6.5, 6.5, 3.6, 3.4, respectively	Present Study

5.4. Kinetics of Adsorption

The prediction of batch kinetics is necessary for the design of sorption systems. The adsorption kinetics of Ni^{2+} , Cu^{2+} , Cd^{2+} and Zn^{2+} ions onto modified and unmodified CFs was studied at constant initial metal ion concentration. Figure 5.27 illustrates the adsorbed amount of heavy metal ions onto the CA modified CFs as a function of time. The figure shows a rapid increase over the first one hour and then begins to level off. A steep increase at the initial part of the adsorption curves points to high adsorption rates and after some fluctuations the adsorbed amount reaches a plateau value in a relatively long time, with progressively slowing adsorption rates. Unmodified CFs exhibit a very similar kinetic behavior, however with lower uptake capacity (Figure 5.28).

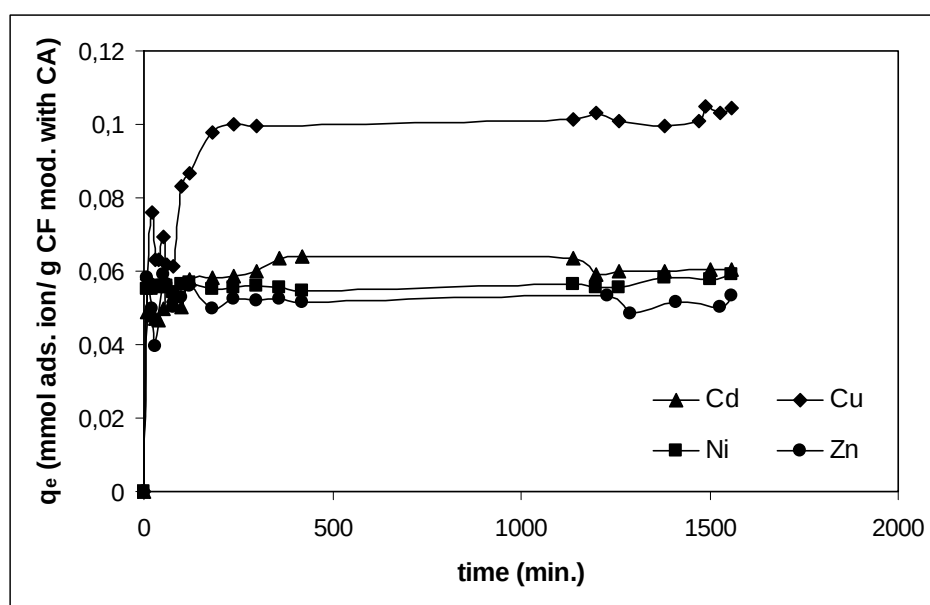


Figure 5.27: Uptake of different heavy metal ions adsorbed onto CF mod. with CA as a function of time.

The kinetics of pollutants adsorption may be controlled by a variety of processes like the transport to a solid surface, diffusion into pores, and reaction on the surface, etc. Depending on the assumed rate controlling step, a variety of kinetic equations have been proposed in the literature (Rudzinski et al., 2006).

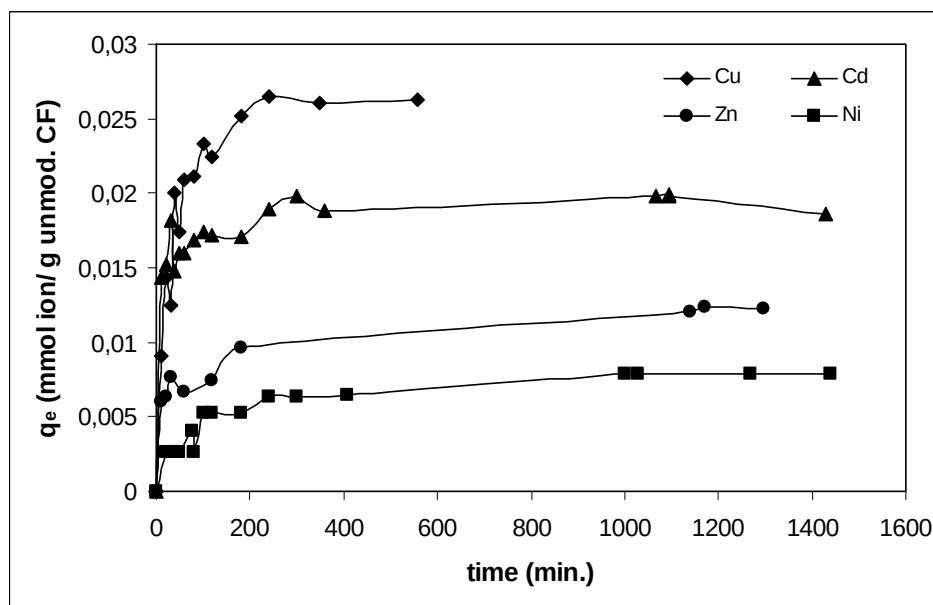


Figure 5.28: Uptake of different heavy metal ions adsorbed onto unmodified CF as a function of time.

In this investigation, pseudo-first order, pseudo-second order and Weber-Morris intra-particle diffusion model kinetics models were studied to evaluate the adsorption kinetics mechanism. Pseudo first order is to be related to the kinetic model in which reaction on the surface, transition from free to adsorbed state, is the mechanism controlling the rate of the adsorption process. Its equation indicates that a one-site occupancy adsorption takes place, adsorbing molecule reacting with one

adsorption site. By the adsorption kinetics of pseudo second order model, two-site occupancy of adsorption occurs, solute molecule reacting with two adsorption sites.

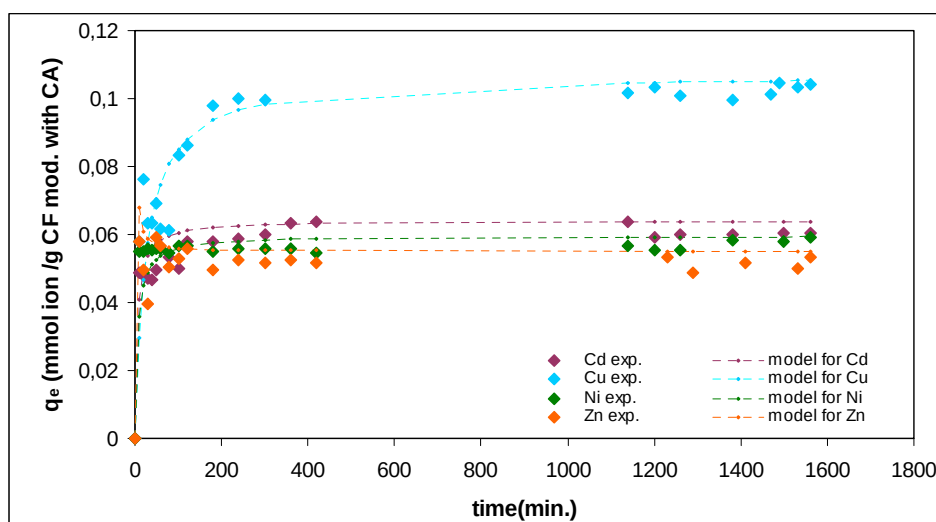


Figure 5.29: Experimental and calculated values for pseudo-second order model for different heavy metal ions adsorbed on CF mod. with CA

The equilibrium sorption capacity, q_e is obtained from the slope of the plot t/q_t versus t , and the initial sorption rate h is obtained from the intercept (Eqn. 3.15). Since q_e is known from the slope, the pseudo second order constant k_2 can be determined from the value of the initial sorption rate. The best fit values of h , q_e , and k_2 along with correlation coefficients for the pseudo first order and pseudo second order models are shown in Table 5.4.

Table 5.5: The parameters of kinetic models and the correlation coefficients for the heavy metal ions adsorbed on CF mod. with CA.

	The Pseudo-First Order Equation			The Pseudo- Second Order Equation		
	k_1	q_e calc. (mg/g)	R^2	k_2	q_e calc. (mg/g)	R^2
Cu^{2+}	0.0006	2.2116	0.7182	0.0061	6.5445	0.9979
Cd^{2+}	0.0005	1.2747	0.4891	0.0278	6.8399	0.9991
Ni^{2+}	0.0004	0.3244	0.3260	0.0462	3.3807	0.9988
Zn^{2+}	0.0006	0.4001	0.2778	-0.1695	3.3580	0.9980

The possibility of intra particle diffusion resistance affecting sorption was tested by the aid of ‘Weber and Morris’ method (Singh et al., 2007): From Eqn. 3.20 the plot of q_t versus $t^{1/2}$ is presented in Figure. 5.30. The values of I (Table 5.6) gives an idea about the thickness of the boundary layer, *i.e.* the larger the intercept the greater the boundary layer effect. Furthermore, such deviation of straight line from the origin indicates that pore diffusion may not be the only rate controlling step in the removal of sorbate.

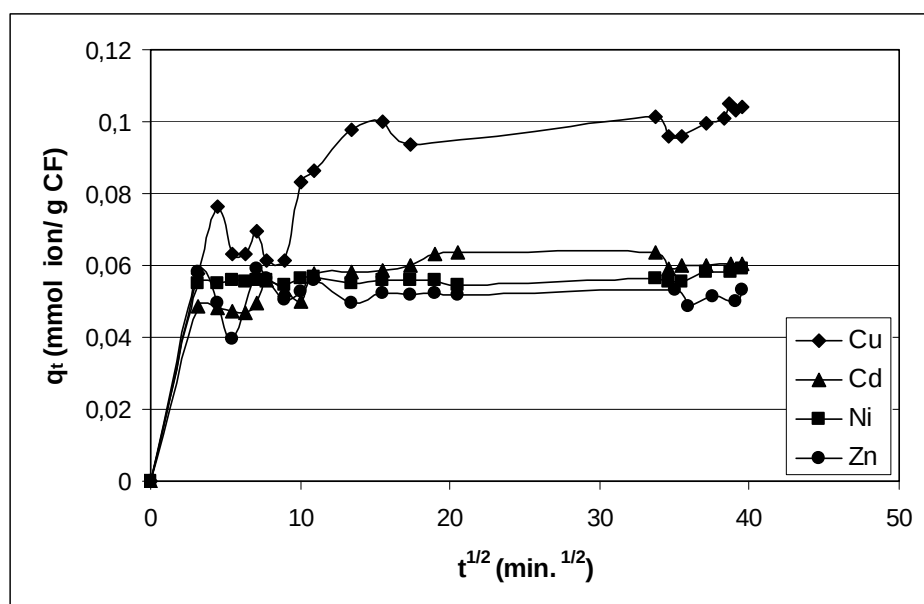


Figure 5.30: The intra-particle diffusion model

Table 5.6: The parameters of the Weber-Morris intra-particle diffusion model and the correlation coefficients for the heavy metal ions adsorbed on CF mod. with CA.

	K_{id}	I	R^2
Cu^{2+}	0.00100	0.0657	0.7037
Cd^{2+}	0.00030	0.0501	0.553
Ni^{2+}	0.00006	0.055	0.3514
Zn^{2+}	-0.00003	0.0526	0.0104

The calculated correlation coefficients are closer to unity for pseudo second order kinetics than that for the pseudo first order kinetic model and intra particle diffusion model. Therefore, the sorption can be approximated more appropriately by the pseudo second order kinetic model for the sorption of metal ions onto CF modified with CA (Figure 5.30), with reaction at the surface as its rate limiting step.

5.5. FTIR Studies

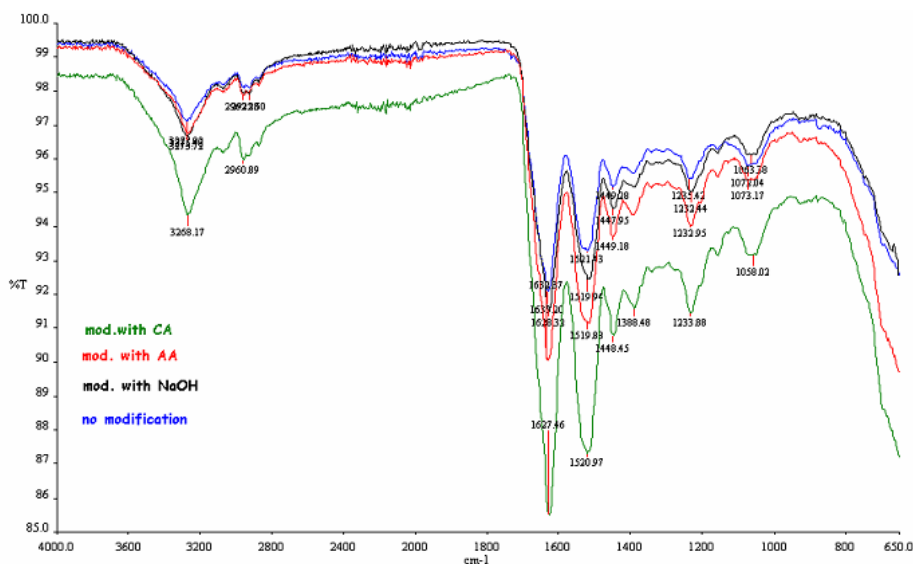


Figure 5.31: FTIR spectrum of unmodified and modified CF.

Numerous chemical groups have been proposed to be responsible for the sorption of metals by CF: these include carboxyl and amino. Their relative importance in metal sorption may depend on factors such as the quantity of sites, their accessibility, chemical state and affinity between site and metal ion. FTIR analysis of the CF was carried out using ATR-FTIR spectroscopy which provides information about the secondary structure content of proteins on the surface. Characteristic bands found in the infrared spectra of proteins and polypeptides include the Amide I and Amide II. These arise from the amide bonds that link the amino acids. The absorption associated with the Amide I band leads to stretching vibrations of the C=O bond of the amide, absorption associated with the Amide II band leads primarily to bending vibrations

of the N—H bond (Figure 5.32) (http://www.chem.uwec.edu/Chem455_S05/Pages/Manuals/FTIR_of_proteins.pdf).

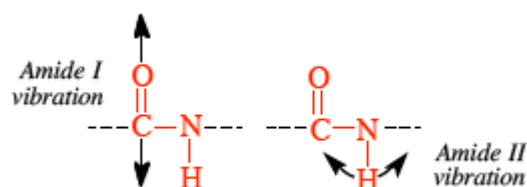


Figure 5.32: The vibrations responsible for Amide I and amide II in the infrared spectra of proteins and polypeptides.

The Amide I band is due to Carbonyl stretching vibration while Amide II is due to primarily to NH bending vibrations.

While many vibrational bands give information on protein structure, the amide I absorption ($1690\text{--}1620\text{ cm}^{-1}$) is widely used in studies of protein secondary structure. This vibrational mode originates primarily from the C=O stretching vibration. The main stretching frequencies observed by various authors and the sources of these stretches are summarized in Table 5.7.

Table 5.7: Commonly observed stretching frequencies of unmodified and modified CF (Arica et al., 2005; Murphy et al., 2007; Taddei et al., 2003; Tonin et al., 2004)

Wave number (cm ⁻¹)	Assignment
3272; 3271; 3275; 3268	N-H stretching
2962; 2962; 2962; 2961	Asymmetric stretch of aliphatic chains -CH stretching
1632.87; 1632.20; 1628.32; 1627.46	Asymmetric C=O β sheet parallel (Amide I band)
1521.43; 1519.94; 1519.88; 1520.97	(Amide II band)
1449.28; 1447.95; 1449.18; 1448.45	Symmetric C=O aliphatic residues
1388; 1388.50; 1389; 1388.48	-CO stretching in -COO ⁻
1235.42; 1232.44; 1232.95; 1233.88	N-H in-plane bend, C-N stretch Anti-parallel β -sheet (Amide III band)
1063.38; 1072.04; 1073;17; 1058.02	-CO stretching of esters and carboxylic acids

The absorption bands that comprise amide I, amide II and amide III bands are shown in Figure 5.31 and described in Table 5.7. In the CF structure the bands at 1632.87; 1632.20; 1628.32; 1627.46 for untreated CF, CF treated with NaOH, CF modified with AA and modified with CA, respectively are assigned to various types of β -turns and disorder structures. The large intensity of the band at 1627.46 cm⁻¹ is due to the greater molar absorptivity of β strands, which is obtained for CF modified with CA. Taddei et al., 2003 concluded that the gain in intensity of the 1438 cm⁻¹ band (COO⁻ symmetric stretching) can be attributed to Cu²⁺ binding of aspartate and glutamate residues occurring in the amorphous polypeptide sequences.

In this work ATR-FTIR studies were used to reveal the modifications made by NaOH, AA and CA on the CF. A close study of the FTIR spectrums have shown that Amid I peaks (C=O groups of the amid backbone vibrations) were intensified by AA and CA treatment of the CFs by derivatization of the side chains of the amino acids bound to the backbone of the protein structures. The change in the peak maxima is an important indication for the derivatization of the peptide bond, however an important change could not be observed. There is an increase in the 1388 cm^{-1} band (Figure 5.31) and this can be explained as an increase in the concentration of the surface carboxyl groups. This may be due to the hydrolysis of peptide bond under the effect of CA. As Sessa et al., 1999 concluded that CA produced an anhydride that reacted with the nucleophilic hydroxyl groups of starch and fiber as well as the hydroxyl, sulfhydryl, and amino groups of protein to produce ester or acyl derivatives. CA is known to be an excellent chelating agent. A chelate is a complex resulting from the combination of a metal ion and a multidentate ligand such that the ligand forms two or more bonds with the metal, resulting in a ring structure that includes the metal ion. CA itself possesses the coordinating functions of three carboxylic and one hydroxyl group. All these groups are known to participate in the binding of metal ions. CA as an anhydride will react with the hydroxyl functional groups of corn starch or fiber (as well as the nucleophilic NH_2 , SH , and OH groups of protein to generate ester or amide derivatives. This derivatization of CA with biobased materials results in a water insoluble chelate with two carboxylic acid and one hydroxyl group. The electron-donating species in these CA complexes are commonly referred to as ligands. The principal electron donating atoms in these ligands are oxygen, nitrogen and sulfur where oxygen, nitrogen, and sulfur contributions reside with the protein moieties and oxygen resides with the carbohydrate and CA moieties. Charged ligands form more stable

chelates than uncharged ligands, hence charged citrate ions will form more stable chelates than the carboxylic acid groups of CA.

5.6. SEM Studies

The surface morphology of chicken feather, untreated and treated as described in the experimental part, is shown in the SEM micrographs (Figure 5.33). It can be seen that all fibers have a size of 5 μm diameter.

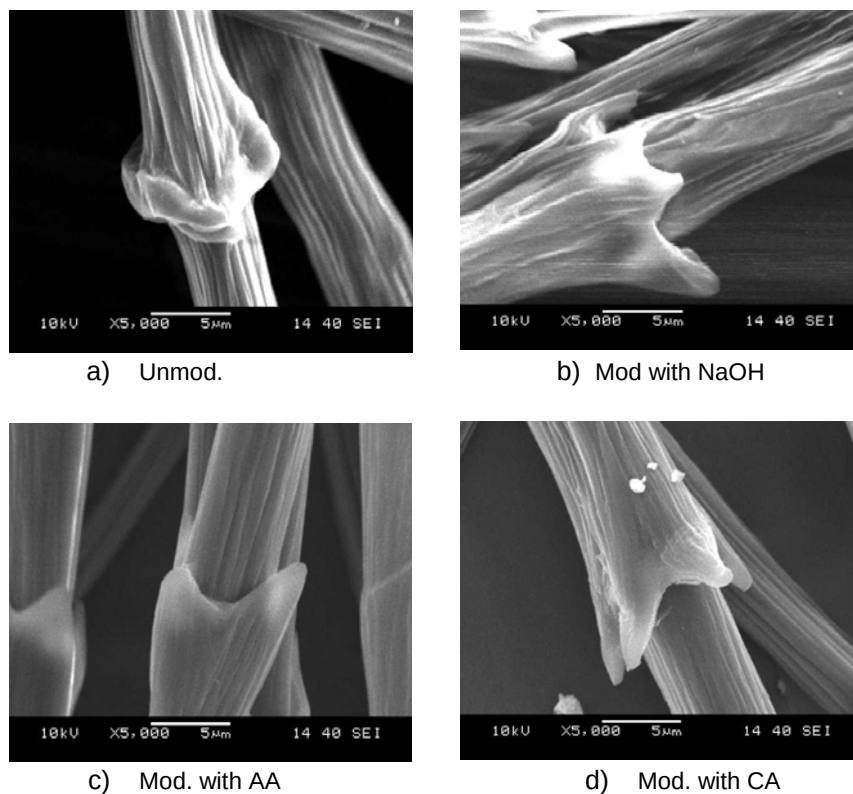


Figure 5.33: SEM picture of keratin protein material showing the fibrous structure

It is believed that when applied to feather keratin citric acid opens up the outer layer, also known as the cuticle. While the cuticle is open, it allows for a deeper penetration into the feather keratin shaft. In the

present study modification can not be seen from the SEM images, but treatment effect can be explained by the difference between the active sites of feather where it is most sharpened when modified with CA.

6. CONCLUSIONS

This study has revealed that the poultry byproduct, chicken feather, can be used to remove heavy metal ions from aqueous solutions at low concentrations. In order to avoid the possible complexation of sulfate and chloride ions with metal ions in the aqueous phase, nitrate salts were used. The sorption of heavy metal ions can be attributed to several mechanisms including electrostatic attraction, coordination (complexation), and micro-precipitation. The maximum adsorption capacity of *CF* for the removal of heavy metal ions was obtained with *CF* modified with *CA*. Experiments were performed as a function of adsorbent type and heavy metal ion concentration. The Langmuir, Freundlich and Dubinin-Kaganer-Radushkevich (DKR) isotherm models were used for the mathematical description of the adsorption of heavy metal ions onto *CFs* depending on modification type and the isotherm constants were evaluated from these isotherms. Results indicated that the adsorption equilibrium data fitted well to Langmuir and DKR isotherm models in the studied concentration range with their correlation coefficient close to unity, but considering the experimental and calculated $q_{\max}$ values, DKR model was the best model fitted. The physical adsorption due to weak van der Waals forces being important according to the values of mean free energies from DKR isotherm of adsorption less than 8 kJ/ mol. The kinetics of metal ions adsorption onto *CF* modified with *CA* was based on the assumption of the pseudo-second-order mechanism.

The analysis of ATR-FTIR spectrum confirms a slight increase in the 1388 cm^{-1} bonds in the keratin chain of chicken feather fiber treated with NaOH, AA and CA. This increase can be explained as a rise in the

amount of carboxyl groups due to CA which may cause hydrolysis of peptide bonds.

For CF, coordination was shown to play an important role in metal binding with COO^- , SH, aromatic side chains and $-\text{NH}_2$ groups. On the other hand, competitive sorption of proton and metal ions on CF may also occur.

It may be concluded from above results that *CFs* can be used for the elimination of heavy metal pollution from wastewater since it is a low-cost, abundant and locally available adsorbent.

7. REFERENCES

- Arica, M. Y., Bayramoğlu, G.,** 2005, Cr(VI) biosorption from aqueous solutions using free and immobilized biomass of *Lentinus sajor-caju*: preparation and kinetic characterization, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 253: 203–211
- Augustine, A.A., Orike, B.D. and Edidiong, A.D.,** 2007, Adsorption kinetics and modeling of Cu(II) ion sorption from aqueous solution by mercaptoacetic acid modified cassava (*manihot sculenta* cranz) wastes, *EJEAFChe*, 6 (4): 2221-2234
- Baldrian, P.,** 2003, Interactions of heavy metals with white-rot fungi, *Enzyme and Microbial Technology*, 32: 78–91
- Balköse, D. and Baltacıoğlu, H.,** 1992, Adsorption of heavy metal cations from aqueous solutions by wool fibers, *J. Chem. Tech. Biotechnol*, 54: 393-397
- Banat, F., Al-Asheh, S. and Al-Rousan, D.,** 2002, Comparison between different keratin-composed biosorbents for the removal of heavy metal ions from aqueous solutions, *Adsorption Science and Technology*, 20: 393-402
- Banat, F.A., Al-Asheh, S.,** 2000, Biosorption of phenol by chicken feathers, *Environ Eng and Policy*, 2: 85–90

Barone, J. R., Danganan, K. L., Schmidt, W. F., 2007, Protein-transition metal ion networks, *Journal of Applied Polymer Science*, 106: 1518–1525

Barrett, G. C. and Elmore, D. T., 2004, Amino Acids and Peptides, *Cambridge University Press*, United Kingdom

Buxbaum, E., 2007, Fundamentals of Protein Structure and Function, *Springer Science_Business Media Inc.*, USA

Chaiyasith, S., Cihaiyasith, P., and Septhum, C., 2006, Removal of cadmium and nickel from aqueous solution by adsorption onto treated fly ash from Thailand, *Thammasat Int. J. Sc. Tech.*, 11 (2)

Douglas, S., Pongamphai, S., Lerdtrailuck, S., Ponin, S., Polchai, S., Kaewchana, A. and Osataworanun, B., 2006, Adsorption of copper (II) ion from aqueous solution using soybean hulls, The 2nd Joint International Conference on “Sustainable Energy and Environment (SEE 2006”, E-022 (P) 21-23 November 2006, Bangkok, Thailand

Green, N., Bjerkgeng, B., Hylland, K., Ruus, A., Rygg B., Künitzer A., 2003, Hazardous substances in the European marine environment: trends in metals and persistent organic pollutants, *Topic Report 2/2003*, *European Environment Agency*, Copenhagen

Güler, Ç., Çobanoğlu, Z., 1997, Su kalitesi, birinci baskı, *Çevre Sağlığı Temel Kaynak Dizisi No: 43*, Ankara

Gutherie, R.E. and Laurie, S.H., 1968, The binding of copper(II) to mohair keratin, *Aust. J. Chem.*, 21: 2437-43

Hartwig, A., Asmuss, M., Ehleben, I., Herzer, U., Kostelac, D., Pelzer, A., Schwerdtle, T. and Bürkle, A., 2002, Interference by toxic metal ions with DNA repair processes and cell cycle control: molecular mechanisms, *Environmental Health Perspectives*, 110

Hou, W., Chen, X., Song, G., Wang, Q., Chang, C. C., 2007, Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (*Lemna minor*), *Plant Physiology and Biochemistry*, 45: 62-69

Inglezakis, V., Pouloupoulos, S., 2006, Adsorption, ion exchange and catalysis: design of operations and environmental applications, *Elsevier Science*

Jenne, E. A., 1998, Adsorption of metals by geomedia: data analysis, modeling, controlling factors, and related issues, *Adsorption of Metals by Geomedia*, Academic Press.

Jiang, L. F., Yao, T. M., Zhu, Z. L., Wang, C., Ji, L. N., 2007, Impacts of Cd(II) on the conformation and self-aggregation of Alzheimer's tau fragment corresponding to the third repeat of microtubule-binding domain, *Biochimica et Biophysica Acta* 1774: 1414–1421

Jonak, C., Nakagami, H., and Hirt, H., 2004, Heavy metal stress. activation of distinct mitogen-activated protein kinase pathways by copper and cadmium, *Plant Physiology*, 136: 3276–3283

Joshi, N., 2003, Biosorption of heavy metals, Masters of Science in Biotechnology Thesis, *Thapar Institute of Engineering and Technology*, Patiala.

Kar, P. and Misra, M., 2004, Use of keratin fiber for separation of heavy metals from water, *J Chem Technol Biotechnol* 79:1313–1319

Kraemer, R.A., Choudhury, K., Kampa, E., 2001, Protecting water resources: pollution prevention, Thematic Background Paper, *International Conference on Freshwater*, Bonn, (December 2001)

Ma, W., Ya, F. Q., Han, M., Ren, W., 2007, Characteristics of equilibrium, kinetics studies for adsorption of fluoride on magnetic-chitosan particle, *Journal of Hazardous Materials*, 143 : 296–302

Malandrino, M., Abollino, O., Giacomino, A., Aceto, M., Mentastrì, E., 2006, Adsorption of heavy metals on vermiculite: influence of pH and organic ligands, *Journal of Colloid and Interface Science*, 299: 537-546

Markovska, L. T., Meshko, V. D. and Marinkovski, M. S., 2006, Modeling of the adsorption kinetics of zinc onto granular activated carbon and natural zeolite, *J. Serb. Chem. Soc.* 71 (8–9): 957–967

Mc Sweeney, J. D., Rowell, R. M., Min, S.-H., 2006, Effect of citric acid modification of aspen wood on sorption of copper ion, *Journal of Natural Fibers*, 3(1)

Miura, J., Yamada, M. and Nakamura, Y., 1985, Selective preconcentration of copper from aqueous solution by using keratine gel, *Fresenius Z. Anal. Chem.*, 321: 575-577

Murphy, V., Hughes, H., McLoughlin, P., 2007, Cu(II) binding by dried biomass of red, green and brown macroalgae, *Water Research*, 41: 731– 740

Oh, M. and Tshabalala, M. A., 2007, Pelletized ponderosa pine bark for adsorption of toxic heavy metals from water, *BioResources*, 2(1): 66-81

Ong, S. A., Seng, C. E., Lim, P. E., 2007, Kinetics of adsorption of Cu(II) and Cd(II) from aqueous solution on rice husk and modified rice husk, *EJEAFChe*, 6 (2): 1764-1774

Perego, P. and Howell, S. B., 1997, Molecular mechanisms controlling sensitivity to toxic metal ions in yeast, *Toxicology and Applied Pharmacology*, 147: 312–318

Pingli and SenGupta, A. K., 2004, Ion exchange and solvent extraction: a series of advances, Volume 16, Marcel Dekker, Ink. New York

Plácido Moore, G. R., Martelli, S. M., Gandolfo, C., Amaral Sobral, P. J., Laurindo, L. B., 2006, Influence of the glycerol concentration on some physical properties of feather keratin films, *Food Hydrocolloids*, 20:975–982

Rao, V. A., Satapathy, A., Mishra, S. C., 2007, Polymer composites reinforced with short fibers obtained from poultry feathers, *Proceedings of International and INCCOM-6 Conference Future Trends in Composite Materials and Processing December 12-14*

Roundhill, D. M., 2004, Novel strategies for the removal of toxic metals from soils and waters, *Journal of Chemical Education*, 81

Rudzinski, W., Plazinski, W., 2006, Kinetics of solute adsorption at solid/solution interfaces: A theoretical development of the empirical pseudo-first and pseudo-second order kinetic rate equations, based on applying the statistical rate theory of interfacial transport , *Phys. Chem. B*, 110 (33): 16514 -16525

Sangwichien, C., Aranovich, G.L., Donohue, M.D., 2002, Density functional theory predictions of adsorption isotherms with hysteresis loops, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 206: 313-320

Seregin, I. V. and Kozhevnikova, A. D., 2006, Physiological role of nickel and its toxic effects on higher plants, *Russian Journal of Plant Physiology*, 53(2): 257–277.

Sessa, D.J., Wing, R. E., 1999, Metal chelation of corn protein products: citric acid derivatives generated via reactive extrusion, *Industrial Crops and Products*, 10: 55–63

Sheffield, A., and Doyle, M.J., 2005, Uptake of copper (II) by wool, *Textile Res. J.* 75(3): 203–207

Singh, R., Shah, A. V., Shah, B. A., 2007, Sorption behavior of Cu(II) from acidic solution using weathered basalt andesite products, *E-Journal of Chemistry*, 4 (4): 587-605

Smalinskienė, A., Gailevičiūtė, R., Lesauskaitė, V., Sadauskienė, I., Abdrakhmanov, O., Ivanov, L., 2005, Effects of cadmium and zinc ions on mitotic activity and protein synthesis in mouse liver, *Medicina (Kaunas)*, 41(6)

Taddei, P., Monti, P., Freddi, G., Arai, T., Tsukada, M., 2003, Binding of Co^{2+} and Cu^{2+} cations to chemically modified wool fibres: an IR investigation, *Journal of Molecular Structure* 650: 105–113

Takahashi, K., Yamamoto, H., Yokote, Y. and Hattori, M., 2004, Thermal behavior of fowl feather keratin, *Biosci. Biotechnol. Biochem.*, 68(9): 1875-1881

Thawley, S., Morrisa, S., Vosloo, A., 2004, Zn and Cd accumulation in *Potamonautes warreni* from sites in the North–West Province of South Africa, *International Congress Series* 1275: 180–188

Thomas, W. J., Crittenden, B., 1998, Adsorption technology and design, *Elsevier Science & Technology Books*

Tonin, C., Aluigi, A., Bianchetto Songia, M., D'Arrigo, C., Mormino M. and Vineis, C., 2004, Thermoanalytical characterization of modified keratin fibres, *Journal of Thermal Analysis and Calorimetry*, 77: 987–996

Ünyayar, S., Çelik, A., Çekiç, F. Ö. and Gözel, A., 2006, Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*, *Mutagenesis*, 21: 77–81

Valstar A., 2000, Protein surfactant interactions, *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology*, 563.

Whitford, D., 2005, Proteins structure and function, John Wiley & Sons Ltd, England

Winandy, J. E., Muehl, J. H., Glaeser, J. A., Schmidt, W., 2007,
Chicken feather fiber as an additive in MDF composites, *Journal of Natural Fibers*, 4(1)

<http://en.wikipedia.org/wiki/Cysteine>

<http://www.advance-health.com/zinc.html#Zinc%20Toxicity>

<http://www.advance-health.com/zinc.html#Zinc%20Toxicity>

[http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/Pollution
_air?OpenDocument](http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/Pollution_air?OpenDocument)

http://www.cadmium.org/env_exp.html

http://www.nidi.org/index.cfm/ci_id/101/la_id/1.htm#Nickel%20Use

[http://www.worldscibooks.com/chemistry/etextbook/p297/p297_chap1_1
.pdf](http://www.worldscibooks.com/chemistry/etextbook/p297/p297_chap1_1.pdf)