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Fluoride Removal from Aqueous Solution Using Chitosan Adsorbent

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PROCESS ENGINEERING STREAM

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Abstract

In this study the extraction of chitosan from fish scale was studied and the performance towards fluoride adsorption from aqueous solution was evaluated by batch experiments. The methods used for extraction of chitosan were demineralization, and deproteinization followed by deacetylation. Proximate analysis, FTIR and XRD analysis were carried out on the obtained chitosan. Physicochemical properties of obtained chitosan was determined by solubility, water uptake and oil uptake capacity analysis. The water binding capacity and fat binding capacity and solubility were found to be 576%, 219.5% and 83.8%, respectively. Degree of Deacetylation was found to be 84%. The effects of initial fluoride concentration (10, 15 & 20mg/l), adsorbent dosage (1, 2 & 3gm) and pH of the solution (5, 7 & 9) were analyzed by Design-Expert version 7.0.0 of full factorial versus quadratic model response surface methodology and the optimal conditions that gave maximum/efficient removal efficiency were selected. The adsorption capacity of fluoride by chitosan from aqueous solution was found to be 3.248mg/g; and 3gm of adsorbent dosage, pH value of 7 and 10mg/l initial concentration with 98.47% removal efficiency were selected as the optimal conditions. The kinetic studies revealed that the data best fits for pseudo second order regression model and the adsorption process followed Freundlich isotherm model. The adsorbent after fluoride removal regenerated using 0.1N NaCl aqueous solution. The results indicate that chitosan adsorbent extracted from fish scale has a good capability in the removal of fluoride ions from aqueous solution.

Keywords: Chitosan; Batch experiments; Demineralization; Deproteinization; Deacetylation; Kinetics; Isotherm

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Acronym

WHO	World Health Organization
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-Ray Diffraction
DDA	Degree of Deacetylation
DA	Degree of Acetylation
HF	Hydrogen Fluoride
ANOVA	Analysis of Variance
CS	Chitosan
WBC	Water Binding Capacity
FBC	Fat Binding Capacity
CP	Crude Protein
TISAB	Total Ionic Strength Adjustment Buffer
AOAC	Association of Analytical Chemists
C.V	Coefficient of Variation
Std.Dev	Standard Deviation

Chapter One

1. Introduction

1.1. Background

Water is a universal solvent and it is an indispensable natural resource on this earth, which makes all life survive. About 97% of earth's surface is covered by water and most of the animals and plants have 60% to 65% water content in their body. Water is used for potable, irrigation, and transportation, washing and waste disposal for industries and is used as a coolant in thermal power plants. Water shapes the earth's surface and regulates the climatic conditions(Gupta et al, n.d.). Water on the earth's surface obtained from two principal natural sources: lakes, rivers, streams and fresh water are categorized under Surface water and those of borehole water and well water are considered to be ground water. Ground water is one of the major sources of water on the earth. In many countries all over the world, groundwater is extracted for potable purposes. About 2 billion people around the world depend on groundwater as their main drinking water source *(An, For, Investigation, & Annaduzzaman, 2015)*.

Groundwater containing dissolved ions beyond the permissible limit is harmful and not suitable for domestic use (Ibrahim, Asimrasheed, & Prabhakar, 2011). As a natural element, fluoride is universally present in varied water bodies, and which is beneficial up to 0.7 mg/L but damages if it exceeds 1.5 mg/L, which is the limit recommended by the World Health Organization (2004). Fluoride pollution has been a global environmental concern for decades and has caused great concerns due to its widespread nature and threat to human health *(Li et al., 2018).* Weathering of rocks and leaching of fluoride bearing minerals are the major reasons which contribute to elevated concentration of fluoride in groundwater. The other important natural phenomenon that contributes to high fluoride is evaporation (Ibrahim et al., 2011).

Fluoride pollution has been observed not only in various minerals processing but also in some natural water systems over large areas in Asia, Africa, America, and Europe where the fluoride concentration can range from 0.01 to 3 mg/L in fresh water and 1-35 mg/L in ground water.
Occurrence of fluoride in groundwater has drawn worldwide attention due to its considerable impact on human physiology. The assimilation of fluoride into the human body from potable

water at the level of 1.0 mg/L enhances bone development and prevents dental carriers *(Sivarajasekar, Paramasivan, Muthusaravanan, Muthukumaran, & Sivamani, 2017).* Fluoride has long been a recognized water-related health concern in Ethiopia, as in other parts of the East African Rift. Concentrations of fluoride greater than the WHO guideline value of 1.5 mg/l have been found in ground waters from several parts of Ethiopia but are recognized to be highest in the Rift zone *(Valley, 1996).* Concentrations often greater than 10 mg/l are found in waters from the Rift valley. A recent analysis of the water samples from this region (along the roads from Addis to Hawassa and from Addis to Nazareth) by the Chemical Engineering Department of Addis Ababa University (AAU) shows that the fluoride concentration ranges on average from 5 to 25 mg/liter *("Berhanu Assefa," 2006).*

1.2. Fluoride

Fluoride is the ionic form of fluorine, a halogen and the most electronegative of the elements of the periodic table. It is ubiquitous in nature. Fluoride combines reversibly with hydrogen to form the acid, hydrogen fluoride (HF). Much of the physiological behavior of fluoride (for example, its absorption from the stomach, distribution between extra- and intracellular fluid compartments and renal clearance) is due to the diffusion of HF. Owing to its high affinity for calcium, fluoride is mainly associated with calcified tissues. Its ability to inhibit, and even reverse, the initiation and progression of dental caries is well known. It also has the unique ability to stimulate new bone formation, and as such, it has been used as an experimental drug for the treatment of osteoporosis*(Information, 1997)* .

1.3. Fluoride Environmental Occurrence

Fluorine is the most electro-negative reactive element known, as result not found free in the environment. Fluoride is found at significant levels in a wide variety of minerals, including fluorospar CaF2, cryolite Na3AlF6, and fluoroapatite Ca10 (PO4)6F2 (Murray, 1986). The average crustal abundance is 300 mg kg-1. Fluoride is 1 commonly associated with volcanic activity and fumarolic gases. Thermal waters, especially those of high pH, are also rich in fluoride. Fluoride can also be released to the environment from anthropogenic sources through discharge of agricultural and industrial products such as glass, electronics, steel, aluminum, pesticide and fertilizer manufacture's *("Agegnehu Alemu," n.d.).*

1.4. Health effects of fluoride and fluorosis

Fluoride is well recognized as an element of public health concern. Fluoride is present universally in almost every water (higher concentrations are found in groundwater), earth crust, many minerals, rocks etc. It is also present in most of everyday needs, viz. tooth pastes, drugs, cosmetics, chewing gums, mouthwashes and so on (Ibrahim et al., 2011). Fluoride ions in aqueous solutions converted into HF in acidic environments such as those of the exists in stomach, and up to about 40% of ingested fluoride is absorbed in the stomach as HF. Prolonged and excessive intake of fluoride may result in a serious public health problem called fluorosis, which is characterized by dental mottling and skeletal manifestations such as crippling deformities, osteoporosis, and osteosclerosis. Endemic fluorosis is now known to be global occurrence and affecting many millions of people in different continent *(Sivarajasekar et al., 2017).* Fluorosis is dreaded disease caused by consumption of excess fluoride. This is caused chiefly through consumption of water having excess fluoride content (Ibrahim et al., 2011). Fluorosis does not only affect people's health; it also has serious economic and social consequences. For instance, appearance-related and psychological problems are caused by the repulsive effect of dental fluorosis, particularly among the youth. The prevalence of fluorosis and the related widespread health problems stigmatize entire villages *(Datturi, Steenbergen, Beusekom, Kebede, & Ababa, 2015).* Concentrations of fluoride above 1.5 mg/l in drinking water cause a lot of health problems through drinking water, such as brain damage, dental and skeletal fluorosis. These clinical conditions are commonly encountered in the Rift Valley. Over 14 million people are at risk. The teeth have brown discoloration. Pitting and chipping of the teeth cause functional problems. Dental fluorosis is a lifelong handicap. Skeletal fluorosis is caused by high concentrations of fluoride in drinking water consumed over many years. Symptoms include joint pains, progressive stiffness and limitation of mobility leading to severe invalidity (crippling skeletal fluorosis). Disabling neurological complications (paralysis of limbs) occur in about ten percent of the skeletal fluorosis cases. In the present of under nutrition and dietary calcium deficiency children in high fluoride areas develop deformity of the lower limbs, knock-knee (genu valgum) *("Fluoride Problems in Ethiopian Drinking Water," n.d.)*. Children who live in a fluorosis area have five times higher odds of developing low IQ than those who live in a slight fluorosis area. Owing to the toxicity of fluoride for children it is important to develop efficient and cheap defluoridation treatment of the water of fluorosis areas.

Table 1-1 Effects due to Fluoride contamination

Fluoride concentration	Health effects
>Permissible limit	Effects neurodevelopment in children
1-4 mg/l	Fractures and skeletal fluorosis
>4 mg/l (or 12 mg/day)	Kidney injury leading to polyuria and dehydration
>1 mg/l (range: 4-21 mg/l)	Dental fluorosis
50 mg/l	Suppresses endocrine glands like thyroid
0.5 mg/l	Effects aquatic organisms of soft water

Source *(Thakuria, 2016)*

Thus, water and wastewater containing high fluoride concentration (> 1.5 mg/l) must be treated before used or discharged. Nowadays, several techniques have been developed for treating fluoride-polluted water. The commonly used methods for fluoride removal include coagulation, separation, precipitation, adsorption, ion-exchange, electrolysis, electro dialysis, electrochemical methods and reverse osmosis. In comparison to the above mentioned techniques for fluoride removal from drinking water, adsorption process is widely considered the most appropriate defluoridation technique. This is due to its flexibility and simplicity of design, relative ease of operation, cost effectiveness and it produces high quality of water *(Amalraj & Pius, 2017).* In this study the use of chitosan as adsorbent from the raw waste fish shell was used for the removal of fluoride from synthetic aqueous solution.

1.5. Statement of the problem

Marine environment represents as source for many aquatic animals or crustaceans like fish which are used as food consumption all over the world including Ethiopia. The scales of crustaceans were composed polysaccharides as chitin and its derivative chitosan. However, the scales generated during fish processing disposed as waste to oceans and lakes which creates environmental pollution. The scales represent approximately 1% of total residues and do not have any nutritional value *(Fonseca, Gustavo Graciano, 2016).* This waste material has either to be discarded or converted to value added products, and this has led to the production of several useful biochemical and nutrients, such as chitin and its derivative form of chitosan. Even though Fish processing plants are not yet discovered there are a lot of different retail shops and institutes including Arbaminch Fish Corporation and Abawengele fish processing unit of Bahirdar.

According to report 2012 Abawengele fish processing unit does have a very small mill which can accommodate 100 Kg of waste fish per day *(Report, 2012).*

During fish processing mostly the meat or flesh is taken and the shell and head portions are disposed as waste or offal results in accumulation of waste for lack of knowledge in processing under good hygienic conditions. There is also a trend in Lake Hawassa that inedible part of fish removed during filleting is thrown to the lake or damped in landfilling as unusable unless otherwise it is roamed by other pelicans and predators. The available methods for the preparation of valuable component of chitin and its derivative chitosan from disposed waste parts (shell and head) are chemical methods which involve demineralization, Deprotination and deacetylation are usually used compared to the biological methods which uses enzymes and accounts for high cost relatively. On the other hand potable ground water pollution by fluoride beyond the permissible limit set by world health organization especially in the rift valley zones of Ethiopia has drawn wide attention because of the consequences it had on dental fluorosis, skeletal fluorosis and brain damage for this study. Adsorption technique is one among the available technologies for defluoridation process. The use of chitosan as a suitable adsorbent for defluoridation technique not much introduced here in Ethiopia which arise of great interest.

Therefore alternative use this waste converting into the more valuable component of chitin and its derivative chitosan using chemical method arise a promising interest due to the reduction in the amount of waste generated and simultaneously defulorized excess amount fluoride containing ground water with a positive economic impact.

1.6. Objective

1.6.1. General objective

The general objective of this study was fluoride removal from aqueous solution using Chitosan adsorbent extracted from fish scale.

1.6.2. Specific objectives

- To extract chitosan from fish scale using chemical method.
- To study the effects of various Physico-chemical parameters on the bio-adsorption performance and removal efficiency of chitosan.
- To study the proximate analysis of chitosan and analyze using FTIR and XRD

- To study the test and optimal condition for removal of fluoride from synthetic solution by batch experiment.
- To study the bio-adsorption isotherms that best fits for via developed models of Langmuir and Freudilick.
- To study the adsorption kinetics using chitosan adsorbent for fluoride removal.
- To study the regeneration of chitosan adsorbent using NaCl solution.

1.7. Significance of the study

Extraction of chitosan from fish shell waste component of chitin as a renewable and biodegradable resource has many advantages:-

1. Protect environmental pollution related with waste from fish shell and the conversion of this waste into valuable component of chitin.
2. Adsorb fluoride ion in waste water which brings health related problems such as dental fluorosis.
3. Substitute the expensive commercial adsorbents with an affordable cost.

Chapter Two

2. Literature Review

2.1. Recent studies on Defluoridation

Sahli *et al.* (2007) have examined the defluoridation of salty water by adsorption on the natural chitosan and by electro-dialysis. The coupling of two techniques decreased the fluoride obviously. The adsorption by chitosan was extremely fast and come to a maximum at 5 minutes. The defluoridation was increased with increase in pH from 2 to 6 and after that diminished up to pH 10. The chitosan cut down the 3.25 mg/l of fluoride in brackish water to 0.9 mg/l by a progressive batch reaction which was lower than the WHO standard of 1.5 mg/l *(Waghmare & Arfin, 2015a).*

A new adsorbent namely Bentonite/chitosan beads have been synthesized and studied for its defluoridation efficiency. Bentonite was activated and the beads were prepared using the inverse suspension polymerization method. The effect of temperature, contact time and initial fluoride concentration on the adsorption capacity of the adsorbent has also been investigated (Zhang et *al., 2013).* Vijaya and Krishnaiah (2009) have investigated fluoride removal potential of chitosan coated silica (CCS) from water. The FTIR result revealed that hydroxyl and amine groups were accountable for defluoridation of water. The monolayer adsorption capacity of Langmuir model was 44.4 mg/g at pH of 4.0. The experimental data fitted well with Langmuir and Freundlich isotherm models and in addition the pseudo-second-order kinetic model. Desorption of 91% was done by 60ml volume of NaOH solution in 60 minutes *(Waghmare & Arfin, 2015a).*

The effectiveness of chitosan in the adsorption of F from water was evaluated at different concentrations of F; the significance of contact duration, pH, and adsorbent dose was examined in laboratory conditions. To understand the mode of adsorption and its kinetics, the Langmuir, Freundlich, and Timken isotherms and the pseudo-first-order, the pseudo second order, and the intra particle diffusion model equations for the equilibrium adsorption data were analyzed. The results for the adsorption of F onto chitosan were best described by the Langmuir isotherm and the pseudo-second order models with the correlation values for both being 0.997. A contact time of 180 min, an adsorbent dosage of 5 g/L, and a pH of 7 were considered to be the

optimal operational conditions and gave a fluoride removal efficiency of 87%. It was concluded that; fluoride adsorption by chitosan can effectively remove fluoride from water*(Akbari, Jorfi, Mahvi, Yousefi, & Balarak, 2018).*

Chitosan-Fe3+ complex with high chemical stability material were synthesized and the performance towards Fluoride adsorption was evaluated by batch experiments. The adsorption process reached equilibrium at 1 hour. The maximum adsorption capacity reached 2.34 mg/g of F- at an initial concentration of 50 mg/L of F- and adsorbent dosage of 10 g/L. Moreover, no significant change in the fluoride removal efficiency was observed in the pH range of 3.0-10.0. The adverse influence of sulphate on fluoride removal was the most significant, followed by bicarbonate and nitrate, whereas chloride had slightly adverse effect. Adsorption process followed the pseudo-second-order kinetic model, and the experimental equilibrium data were fitted well with the Langmuir-Freundlich and D-R isotherm models. Thermodynamic parameters revealed that fluoride adsorption was a spontaneous and exothermic process. The chitosan-Fe3+ complex could be effectively regenerated by NaCl solution *(Patnaik, Pc, Rn, & Ak, 2016).*

In this study the use of Ethiopian Tilapia fish scale waste which is composed of its useful constituents ultimately converted in to chitosan end product is investigated on the removal of fluoride from waste water as indicated in the previous literature using chemical method. In the previous researches the removal efficiency of each treatment not considered taking into account this issue and adding the factors affecting adsorption was investigated.

2.2. Defluoridation in Ethiopia

In Ethiopia, defluoridation started as early as 1962 in Wonji sugar cane plantation by using activated alumina (AA) community filters. However, the community filters were not in continuous operation due to technical problems and limited supply of AA. In 2007, the sugar factory installed a piping system to provide low fluoride water from Adama/Nazreth and abandoned the use of the filters. The other defluoridation method implemented in Ethiopia are aluminum sulphate based household and community filters, known as the Nalgonda Technique, implemented by the Water Resource Office of Oromiya financed by UNICEF and the Catholic Relief Services (CRS) respectively. Currently the filters are not in use due to various technical,

financial and social challenges *(Esayas, Mattle, & Feyisa, 2009).* A research was conducted and aimed at designing and developing a household defluoridation unit that is simple, inexpensive and that uses locally manufactured Aluminum Sulfate that will reduce the fluoride concentration to the recommended range. The defluoridation unit developed by the researchers is simple, just a bucket with a tap. The bucket can be produced from plastic, clay or metal. A sample defluoridation unit has been developed in the Chemical Engineering laboratory with a plastic bucket and it has been checked to be effective. For the sample produced in the laboratory the cost is around 25 Birr, just the cost of the bucket and the tap. The cost of locally produced Aluminum Sulfate and lime is 2. 5 Birr/Kg and 1 Birr/ Kg respectively, hence the total cost of chemicals for treating a 20 liter bucket of water is less than 10 cents *("household defluoridation unit belay woldeyes , nurelegne tefera and lemma dendena department of chemical engineering," 2007)*.

2.5. Methods for Defluoridation of Water

Defluoridation was the conventional and widely tested method for supplying safe water to the fluorosis affected communities. Defluoridation is defined as, "The downward adjustment of level of fluoride in drinking water to the optimal level *("Identification and Comparison of Flaws in Conventional Treatment Techniques of Fluoride," 2015)*. Defluoridation methods broadly classified into four categories; coagulation and precipitation, membrane process, ion exchange process and adsorption process.

2.5.1. Coagulation and Precipitation

Lime and alum are the most usually utilized coagulants for Nalgonda technique for defluoridation of water. Expansion of lime prompts precipitation of fluoride as insoluble calcium fluoride and raises the pH value up to 11– 12. As the lime leaves a leftover of 8.0 mg F-/l, it is constantly connected with alum treatment to guarantee the best possible fluoride removal. As a first step, precipitation happens by lime dosing which is trailed by a second step in which alum is added to bring about coagulation. At the point when alum is added to water, basically two reactions happen. In the first reaction, alum reacts with an alkalinity's portion to deliver insoluble aluminum hydroxide [Al (OH) 3]. In the second reaction, alum reacts with fluoride ions in the water. Best fluoride removal is proficient at pH range of 5.5 – 7.5 *(Razbe, Kumar, & Kumar, 2013).*

2.5.2. Membrane process

The membrane separation process is more well-known from industrial viewpoints for defluoridation of groundwater, wastewater treatment and sea water desalination. In a membrane separation process, particles are isolated on the premise of their molecular size and shape with the utilization of extraordinarily composed semi-permeable membrane. The semi-permeable membrane is frequently a thin, nonporous or porous polymeric film, ceramic, or metal material or even a liquid or gas. The membrane must not dissolve, disintegrate or break (Waghmare & Arfin, 2015b). Reverse osmosis and electro dialysis are two membrane filtration processes which can be used for the removal of fluoride. Similarly, use of RO membranes for fluoride removal from contaminated water sources has also been reported.

Reverse osmosis

In reverse osmosis the hydraulic pressure is exerted on one side of the semi permeable membrane which forces the water across the membrane leaving the salts behind. The relative size of the pollutants left behind depends on the pressure exerted on the membrane. Recent work by Fox KR, (1981) and Huxstep MR, (1981) has shown RO to be effective in reducing traced concentration of these contaminants. The improvements in design and materials of the membranes have made the water treatment process economically competitive and highly reliable.

Electro dialysis

In electro dialysis, the membranes allow the ions to pass but not the water. The driving force is an electric current which carries the ions through the membranes. The removal of fluoride in the reverse osmosis process had been reported to vary from 45 to 90 % as the pH of the water was raised from 5.5 to 7 *(Kinyua, 2016).*

2.5.3. Ion-Exchange process

Fluoride can be removed from water supplies with a strongly basic anion-exchange resin containing quaternary ammonium functional groups. The removal takes place according to the following reaction: Matrix-NR_3 + Cl^- + F^- → Matrix-NR_3+F^- +Cl^-.

The fluoride ions replace the chloride ions of the resin. This process continues until all the sites on the resin are occupied. The resin is then backwashed with water that is supersaturated with

dissolved sodium chloride salt. New chloride ions then replace the fluoride ions leading to recharge of the resin and starting the process again. The driving force for the replacement of chloride ions from the resin is the stronger electronegativity of the fluoride ions *(Razbe et al., 2013).*

Bone Char:

Bone charcoal is the ground animal bones, which are carbonized at high temperatures (1000-16000c) to remove all the organics. Bone charcoal consists of a skeleton of calcium phosphate and carbonates, cellular in structure with a very great number of minute tubes and channels. The skeleton is coated or lined with carbon in a state of very fine subdivisions and high activity. The adsorptive power of bone charcoal resides in its activated carbon content form. If the carbon was burnt off, the calcium phosphate skeletal has title or no decolorizing power, although it will adsorb dissolved salts. The suggested removal mechanism was again the replacement of carbonate radical with fluoride. Bone charcoal in sizes between 28 to 48 meshes had been used with success in many full-scale installations for the defluoridation of drinking water. The regeneration of exhausted bed is done with caustic soda. Bone charcoal was found to be soluble in acid and fluoride removal increases with decreasing pH. Due to this problem of solubility and for reason of water consumption and distribution a pH of greater than 7 is considered as desirable.

2.5.4. Adsorption

Adsorption is a physiochemical waste water treatment in which dissolved molecules are attached to the surface of an adsorbent by physical/chemical forces. Depending on the nature of the interactions, ionic species and molecular species carrying different functional groups may be held to the surface through electrostatic attraction to sites of opposite charge at the surface or physiosorbed due to action of van der Waals forces or chemisorbed involving strong solute adsorbent bonding. This technique is quite popular due to its simplicity as well as the availability of a wide range of adsorbents and it proved to be an effective and attractive process for the removal of non-biodegradable pollutants from waste water (Sivarajasekar et al., 2017).

Adsorption of fluoride on to solid absorbent usually occurs through three phases *(Flanagan & Road, 2014):* (1) diffusion or transport of fluoride ions to the external surface of the adsorbent

from bulk solution across the boundary layer surrounding the adsorbent particle, called external mass transfer; (2) adsorption of fluoride ions on to particle surfaces; (3) the adsorbed fluoride ions probably exchange with the structural elements inside adsorbent particles depending on the chemistry of solids, or the adsorbed fluoride ions are transferred to the internal surfaces for porous materials (intra particle diffusion).

2.5.4.1. Factors affecting adsorption process

Fluoride removal efficiency always depends on raw water quality profile, i.e., initial fluoride concentration, solution pH, environment temperature, contact time and adsorbent dose. The adsorption of fluoride ion by adsorbent depends on the interactions between the solution and the surface of adsorbent. Adsorption process can be assumed to be complete when equilibrium was achieved between the fluoride ions and the adsorbent. However, specific time was needed to maintain the equilibrium interactions to ensure that the adsorption process is complete *(Sivarajasekar et al., 2017).*

Adsorption of fluoride ion from potable water is mainly influenced by the pH of the solution. pH can influence the surface charge of the adsorbent and the degree of ionization of the fluoride ions. In a particular pH range, most fluoride ion adsorption was enhanced with pH, increasing to a certain value followed by a reduction when further pH increases. The dependence of the fluoride ion uptake on pH can be associated with both the surface functional groups on the adsorbent and also the chemistry of the solution *(Panchore, Sharma, Sharma, & Verma, 2016).*

Initial concentration of fluoride ion can alter the fluoride removal efficiency through a combination of factors such as the availability of specific surface functional groups and the ability of surface functional groups to bind fluoride ions. Initial concentration of solution can provide an important driving force to overcome the mass transfer resistance of fluoride ion between the aqueous and solid phases *(Sivarajasekar et al., 2017).* Temperature plays a double role in the fluoride adsorption process. Temperature can impact the physical binding processes of fluoride to adsorbent. Most adsorption studies were conducted at room temperature in laboratory settings. As temperature increased, sorption was shown to be less favored most likely due to increased Deprotination or hydroxylation of the surface causing more negatively charges adsorbent surface *(Panchore et al., 2016).* The adsorbent dosage is an important parameter because this determines the capacity of an adsorbent. The removal of fluoride ions increases with

an increase in the adsorbent dosage. The effect of adsorbent dosage on adsorption was studied by varying the amount of adsorbents and keeping the other parameters constant *(Ushakumary.E.R, 2013).*

The amount adsorbed on to the adsorbent is in a state of dynamic equilibrium with the amount desorbed from the adsorbent. The time required to attain this state of equilibrium is termed as the equilibrium time. The amount adsorbed at the equilibrium time reflects the maximum adsorption capacity of the adsorbent under the operating conditions *(Ushakumary.E.R, 2013).*

2.6. The strength and weakness of defluoridation methods

To conquer the hazardous wellbeing impact of fluorosis, different approaches for defluoridation are exists like coagulation – precipitation, membrane separation processes, ion exchange, adsorption techniques and others (electro-dialysis and electrochemical). Each approach has their strengths and weakness and worked productively under ideal condition to remove fluoride to more noteworthy range. The strength and weakness of the four defluoridation methods: precipitation and coagulation, membrane process, ion exchange process and adsorption process are given in the table.

Table 2-1 the strength and weakness of defluoridation methods

Defluoridation methods	Strength	Weakness
Coagulation and Precipitation method	• Generally utilized technique • which is more practical when contrasted with other defluoridation technique and • Is easy to understand.	• Required chemical dosages are high (Al (OH) 3 up to 700 – 1200 mg/l). • Release of aluminum in treated water which may bring about Alzheimer's syndrome. • The utilization of aluminum sulfate as coagulant expands the sulfate ion concentration greatly which prompting cathartic impacts in human.
Membrane process	• The process permits the treatment and disinfection of water in one step. • It ensures constant water quality. • No chemicals are required and very little maintenance is needed.	• The process is expensive in comparison to other options. • The water becomes acidic and needs pH correction. • Lot of water gets wasted as brine. • Disposal of brine is a problem.

Ion Exchange	• Removes fluoride up to 90 -95%; retains the taste and color of water intact	• Efficiency is reduced in presence of other ions like sulfate, carbonate, phosphate and alkalinity. • Regeneration of resin is a problem because it leads to fluoride rich waste, which has to be treated separately before final disposal. • The technique is expensive because of the cost of resin, pretreatment required to maintain the pH, regeneration and waste disposal..
Adsorption process	• The process can remove fluoride up to 90%. • Treatment is cost-effective.	• The process is highly dependent on pH and works best only in a narrow pH range (5–6). • High concentration of total dissolved salts (TDS) can result in fouling of the alumina bed.

2.7. Adsorbents for Fluoride removal

There are various types of adsorbents used for defluoridation techniques. These includes:-

- Activated alumina
- Natural materials/Geo materials
- Carbonized /activated biomass
- Carbon Nano-sorbents

2.7.1. Bio-adsorbents

Bio-adsorbents are living and dead biomass as well as cellular products such as polysaccharides that can be used for binding metal ions. Bio-adsorbents unlike other conventional water treatment materials contain several binding organic groups which can be modified to increase their adsorptive capacity for fluoride removal. They are of low cost, high efficiency, minimum chemical and physical sludge no additional nutrient requirement, they can be regenerated and they have high possibility of fluoride recovery. Biological materials, such as chitin, chitosan, peat, yeasts, fungi or bacterial biomass, are used as effective and good sorbents to remove fluorides from solutions. Dead biomass can be used as an effective bio-adsorbent material for the removal of fluoride ions from aqueous solutions. It has advantages over other methods such as low operating cost, minimization of the volume of chemical and high effectiveness in detoxifying much diluted wastes *(Kasimu, 2018).*

Bio-sorption is an emerging technique for water treatment utilizing abundantly available biomaterials. Various bio sorbents have been developed for fluoride removal. Among Various bio-sorbents, chitin and chitosan derivatives have gained wide attention effective bio-sorbents due to their low cost and high contents of amino and hydroxyl functional groups which show significant adsorption potential for the removal of various aquatic pollutants *(Flanagan & Road, 2014).*

2.7.2. Chitin derivatives (Chitosan) as bio adsorbent

Chitosan is a biomaterial, primarily produced from the alkaline deacetylation (40–50% NaOH) of chitin where this N-deacetylation is almost never complete. The chitosan is considered as a partially N-deacetylated derivative of chitin. It is an abundant natural biopolymer obtained from the exoskeletons of crustaceans and arthropods which is a non-toxic copolymer consisting of b-

(1,4)-2-acetamido-2-deoxy-Dglucose and b-(1,4)-2-anaino-2-deoxy-D-glucose units. Each glucosamine unit contains a free amino group, and these groups can take on a positive charge which gives amazing properties of chitosan. These biopolymers offer a wide range of unique applications including bioconversion for the production of value-added food products, preservation of foods from microbial deterioration, formation of biodegradable films, recovery of waste material from food processing discards, purification of water and clarification and de-acidification of fruit juices *(Al-manhel, Al-hilphy, & Niamah, 2016).*

Chitin is formed a linear chain of acetyl-glucosamine groups while chitosan is recovered by removing enough acetyl groups (CH3-CO) from chitin therefore the chitin molecule and the resultant product is found to be soluble in most diluted acids. The actual variation between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful of chitin *(S, Kalyanasundaram, & Ravi, 2017).* Despite chitin being abundant and having exceptional functional features such as biocompatibility, bioactivity, biodegradability or high mechanical strength, it has limited utility due to its poor solubility. This makes chitin not very serviceable and shifts attention towards chitosan (CS), which is the main derivative of chitin. Chitin can be converted into Chitosan through enzymatic or chemical processes; however, the chemical conversion is preferred due to its lower cost and its suitability for mass production (Muxika, Etxabide, Uranga, Guerrero, & Caba, 2017).

Chitin and Chitosan are linear polysaccharides, comprised of two monomeric units namely N-acetyl-2-amino-2-deoxyd-glucose (N-acetylated groups) and 2-amino-2-deoxy-d-glucose residues (N-deacetylated groups, amino groups). Chitin samples contain low amount of 2-amino-2-deoxy-dglucose and hence it is less soluble in acidic solvents, whereas Chitosan samples contain lesser number of N-acetyl- 2-amino-2-deoxy-d-glucose and hence it is soluble in acidic solvents *(Anitha et al., 2014).*

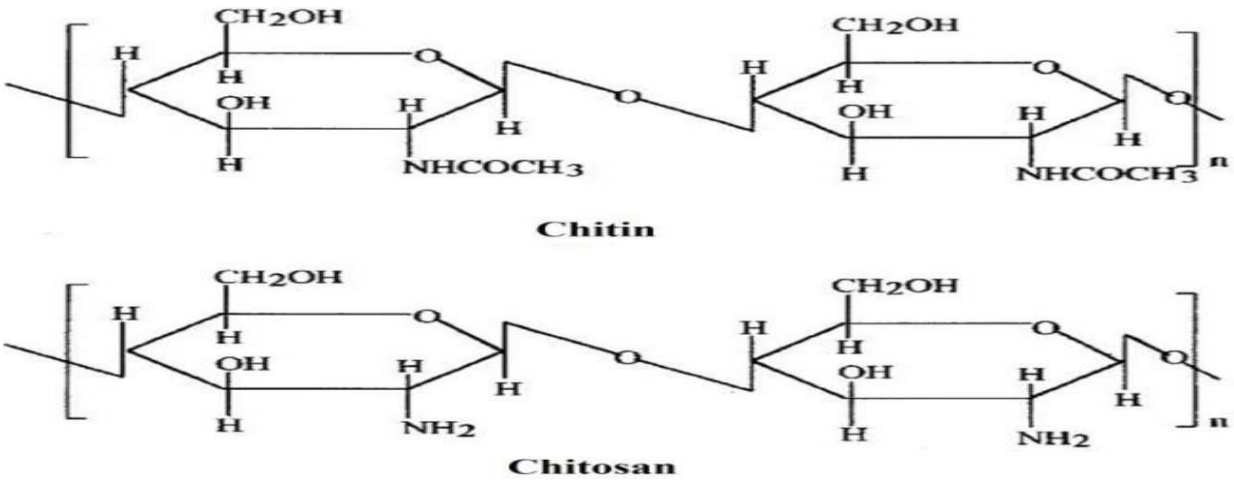

Figure 2-1 Chemical structures of chitin and chitosan

2.8. Sources of Chitin and Chitosan

Marine environment represents about a half of the whole world's biodiversity, and nearly the 70% of Earth's surface is covered by oceans and seas, which means approximately 90% of the biosphere. As it is a chiefly non-explored terrain, marine organisms represent a great source of novel compounds that include both small molecules and macromolecules *(Ruocco, Costantini, Guariniello, & Costantini, n.d.).*

Main marine origin materials can be distributed in three main groups, polysaccharides, proteins and lipids. Among many polysaccharides that can be obtained from marine environment, chitin is the one that stands out due to its availability, as it is the second most abundant natural polymer after cellulose (*Cahú et al., 2012)*. Polysaccharide is a natural molecular structure that distributes broadly in nature. Polysaccharide is a carbohydrate polymer which consists of ten to thousands monosaccharide units, which is normally glucose. The monosaccharide is the building unit of the biopolymer which is usually a six membered heterocyclic structure with oxygen and carbon atom. They have widely present in marine life and easily extracted from fish scale.

Chapter Three

3. Extraction of Chitosan from Fish scale

3.1. Materials

Hydrochloric acid and sodium hydroxide were mainly used in the treatment steps. Sodium hypochlorite as a reagent used for decolorization of the intermediate chitin. Distilled water was used for washing the scales after each treatment to maintain neutrality. Overhead Magnetic stirrer was used to perform the reaction between fish scales and prepared alkaline and acidic solutions. The scales after each treatment were separated using muslin cloth. Oven dryer was used for sample drying. pH meter was used to measure the pH of the scales after each treatment. Mortar and pestle were used for grinding of the samples.

3.2. Methods

Fish scales procured from Lake Ziway washed and sun dried for three days to undergo the pretreatment step. Extraction of Chitosan from fish scale done according to the standard methods: demineralization, deproteinization followed by deacetylation.

3.2.1. Chemical Demineralization

Demineralization of fish scales was performed at constant stirring using 1 M of HCl aqueous solution (1g/15ml) for 4 hr. in order to remove the minerals such as calcium carbonate in the scale into water-soluble calcium salts with release of carbon dioxide at room temperature. The residue after demineralization washed by distilled water till neutrality and oven dried overnight at 50^{o}c.

3.2.2. Chemical Deproteinization

Deproteinization breaks the covalent bonds between chitin and protein linkages. The residue left from demineralization was deprotenized by 1M of aqueous sodium hydroxide solution (1g/15ml) at room temperature in order to remove the crude protein for 24 hr. A 1M aqueous solution of NaOH is the common solution for the deproteinization of chitin *(Noh, 2005).*

3.2.3. Chemical deacetylation

The residue left after deproteinization was decolorized using sodium hypochlorite solution and the intermediate product obtained is called chitin. Further treatment was performed in order to achieve the chitosan. Chitosan was obtained by removal of the acetyl group using concentrated aqueous sodium hydroxide solution (40% NaOH) at 50^{o}c for 2 hr.

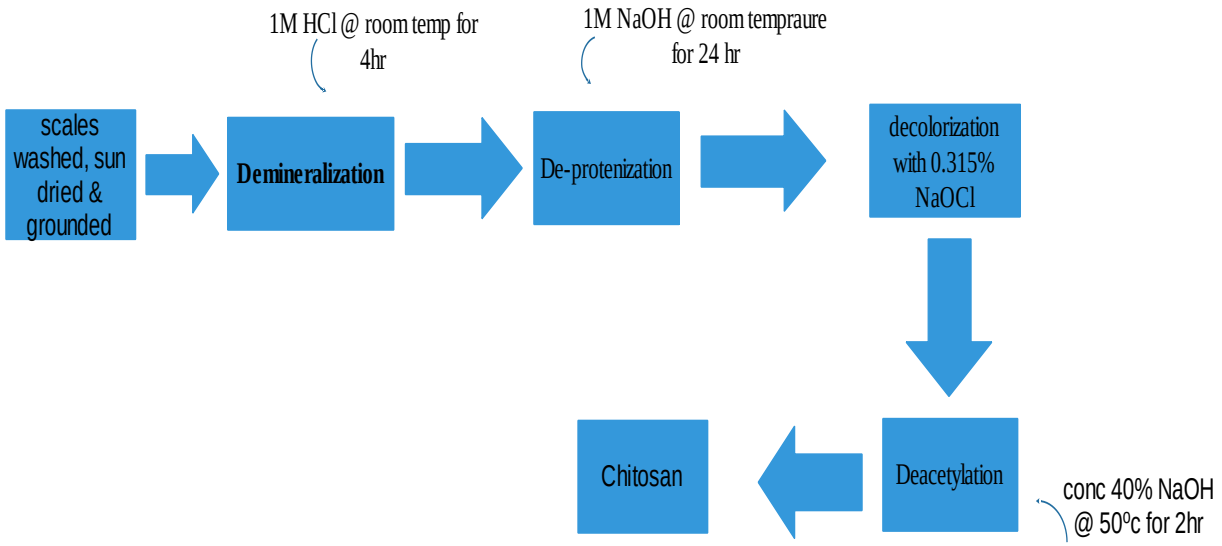

Figure 3-1 extraction procedure of chitosan

3.3. Characterization of chitosan

Chitosan was characterized for various parameters such as acid solubility, water and fat binding capacity of chitosan, degree of deacetylation and chitosan yield.

3.3.1. Materials

Acetic acid was used to dissolve chitosan, distilled water was used to wash the sample and to maintain neutrality, Water and Niger oil were used to test the capacities, Methyl orange added as indicator during titration for deacetylation test, HCl and NaOH solution was used to perform titration, Centrifuge was used for separation of the chitosan sample from its supernatant liquid. Vortex mixers thoroughly mixed and dispersed the contents of chitosan in water as well as Niger oil. Digital reading pipettes were used to take and add the samples in small amounts. Burette was used to add and mix specified amount of titrant to chitosan solution during titration.

3.3.2. Methods

Solubility in acid solution *(Kumari, Rath, & A, 2016)*

1.5gm of chitosan sample taken in triplicate measured; put in to a known weight of centrifuge tube. 15ml of 1% acetic acid poured and dissolved the sample for 30 min using incubator shaker at 250 rpm and room temperature. Then the mixture in the tube was centrifuged at 5000 rpm for 15 min and filtered. The residue left was washed using distilled water till neutrality and oven dried at 50^{o}c overnight. The mass of the particles was weighed and the percentage solubility was calculated.

Water and Fat Binding Capacity (WBC &FBC): according to the modified method of Wang and Kinsella (1976).

Chitosan sample of (0.5 gm.) was measured and put into a known weight of centrifuge tube. 10 ml of Water or Niger oil was added to the sample and the mixture dispersed for 2 min using vortex mixer. The dispersed sample and then left at room temperature for 30 min was shaken for 5 sec every 10 minutes and centrifuged at 3200 rpm for 25 minutes. Finally the supernatant decanted, the tube was weighed and the capacities calculated respectively *(Kumari et al., 2016).*

Degree of Deacetylation (DD): by the method of acid-base titration.

Chitosan sample (0.5gm) was measured and dissolved in 30 ml of HCl (0.1M) solution. Methylene orange (6 drops) pipetted and the mixture stirred vigorously with the addition of 0.1 M NaOH solution till the red chitosan solution changed to yellow color. The amount of NaOH consumed was labeled and the DD calculated accordingly (A.Y.Allam, 2016).

Chitosan yield

The percentage yield of chitosan was calculated on dry basis. The percentage yield was obtained as the ratio of dried extracted chitosan after deacetylation process relative percentage of starting dry raw material.

3.4. Proximate analysis

The proximate analysis conducted for chitosan sample were moisture content, ash content, mineral content and crude protein content.

3.4.1. Materials

Crucible, spatula, furnace, desiccator and those materials used by JIJE private limited company for protein and mineral content determination.

3.4.2. Methods

Moisture content according to the method of *(AOAC, 1990)*.

The moisture content of produced chitosan calculated on dry basis. The sample taken in triplicate (1.5gm of chitosan) was kept in an oven at 105^{o}c overnight and put in to the desiccator till constant weight. It is calculated as the relative difference between the weight of initial dry sample and oven dried samples. The moisture content also determined using moisture analyzer instrument in the AAiT thermal laboratory.

Protein content determination

The protein content of untreated raw and treated or deprotenized fish scale was determined in JIJE Labo glass limited Share Company according to the method of Kjelhdahl.

Mineral content determination

The mineral content of untreated raw and treated or demineralized fish scale also determined in JIJE Labo glass limited Share Company according to Ash digestion-EDTA Titration method.

Ash content determination

The Ash content of treated and untreated raw fish scale was determined in JIJE Labo glass Limited Share Company according to the method of AOAC official method 923.03- Direct method.

3.5. Standardization of process efficiencies

Demineralization efficiency determination

The demineralization efficiency was calculated from the ratio of relative difference of the total ash content of the raw fish scale and residue left from demineralization in the proximate analysis to that of raw fish scale.

Demineralization efficiency =

$$\frac{(\text{g of sample X Ash content (\%) of sample}) - (\text{g of residue X Ash content (\% of residue)}}{\text{g of sample X Ash content (\%) of sample}} \text{ X 100..3.1}$$

Deproteinization efficiency determination

The deproteinization efficiency was calculated from the ratio of relative difference of the crude protein content of the raw fish scale and residue left from deproteinization in the proximate analysis to that of raw fish scale.

Deproteinization efficiency =

$$\frac{(\text{g of sample X CP content (\%) of sample}) - (\text{g of residue X CP content (\% of residue)}}{\text{g of sample X CP content (\%) of sample}} \text{ X 100 ... 3.2}$$

3.6. Analysis of chitosan using FTIR and XRD

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR analysis was used to identify the organic and inorganic functional groups of chitosan adsorbent. The instrument operated using conventional potassium bromide pellets (KBr) in the wavelength range of 500-4000cm^{-1} of model number 65 FT-IR spectrum (PerkinElmer). The functional groups expected for this analysis were aldehyde (carbonyl group), amino acids (1^0 and 2^0 amines) and hydroxyl (OH). The FTIR analysis was performed in Addis Ababa University College of natural and computational science chemistry department.

X-Ray diffraction spectroscopy (XRD)

The wide angle X-ray diffraction analysis used to test and compare the amorphous and crystalline structures between the raw fish scales and an end chitosan product. The instrument recorded the patterns with D8 Avance XRD of Bruckner powder diffract meter in the scale and chitosan with Cu radiation (40KV and 15mA) at a scan rate of 10^0/min with diffract gram for 2theta angels ranged from 5^0 to 50^0. The XRD analysis was performed in Addis Ababa University College of natural and computational science chemistry department.

3.7. Batch adsorption experiments

Preparation of stock solution

In order to know the efficiency of chitosan adsorbate fluoride stock solution (1000mg/l) was prepared by dissolving anhydrous sodium fluoride powder (2.21gm) in one liter of distilled water. One hundred ml working solution of (10, 15 and 20 mg/l) of fluoride concentration was prepared by using the law of serial dilution. Batch adsorption experiment was carried in an Erlenmeyer conical flask with (1, 2 and 3gm) adsorbent dosage at various pH (5, 7 and 9). The effect of pH on adsorption was studied by adjusting the pH of working solution using 0.1M HCl or 0.1M NaOH solution. The contents are thoroughly mixed by magnetic shaker at a speed of 250 rpm at room temperature (25±2) for 3 hr. equilibrium time according to the preliminary tests done. At the end of the experiment, the supernatant in the flask was decanted and filtered by Whatman No.1 filter paper. The fluoride level in the supernatant was measured by the help of fluoride ion specific electrode analyzer by diluting with total ionic strength adjustment buffer (TISAB) in Addis Ababa city government, Environmental Protection Authority (EPA).

Total ionic strength adjustment buffer (TISAB)

In order to regulate the effects of other ions those interfere on fluoride ion total ionic strength adjustment was prepared. It was prepared by adding 57 ml of glacial acetic acid, 7 g of sodium citrate, 58 g of sodium chloride (NaCl) and 2 g of EDTA to 500 ml of distilled water. The mixture was dissolved and its pH was adjusted to 5.3 with 6 molar NaOH. Finally the solution in the volumetric flask filled with double distilled water up to one thousand ml *(Temsgen, 2017).*

3.8. Effects of adsorption conditions on performance of chitosan adsorbent

3.8.1. Effect of adsorbent dosage

Test solution of 100 ml was taken from the stock solution prepared in section 3.9.1 above and the effect was checked by adding adsorbent amount of 1gm to it and the mixture was stirred for 180 min at room temperature (25±2). The mixture was allowed to settle and the solution was filtered to retrieve the clear solution after total ionic strength ionic buffer was added to the mixture and shaken thoroughly. Fluoride concentration analysis was performed in Addis Ababa city government, environmental protection authority. The procedure was repeated for chitosan dosage of 2gm and 3gm respectively. The mass of chitosan that gave the least residual concentration of fluoride ion in the solution identified as optimum dosage of chitosan.

3.8.2. Effect of fluoride ion concentration

Solutions of different fluoride concentration 10mg/l, 15mg/l and 20mg/l was prepared from the stock solution in section 3.9.1 above and the effect of adsorbate concentration on removal efficiency was studied for equilibrium time of 180 minutes (3hr), mixture agitated at a speed of 250 rpm and room temperature (25±2) for all the solutions at fixed adsorbent dosage. The initial concentrations were taken from the range obtained in average 5-25mg/l in the rift valley from studies done by Addis Ababa University.

3.8.3. Effect of pH

pH was also another important parameter on the removal efficiency of fluoride from aqueous solution. Effect of solution pH was studied by varying its value in the range 5-9 for various concentrations of adsorbate (10mg/l, 15mg/l and 20mg/l) at 200 rpm on a magnetic shaker for 180 min of contact time fixed for all batch experiments for which the equilibrium was attained at room temperature (25±2) on fixed amount of sorbent dosage. The pH at which maximum removal efficiency of fluoride achieved was termed as optimum pH. Fluoride concentration analysis was performed in Addis Ababa city government, Environmental Protection Authority.

3.8.4. Determination of contact time

Using optimum dosage of chitosan that was the conditions for which maximum fluoride removal efficiency was achieved at room temperature (25±2), contact time was studied for the time intervals of 30 min starting from 30 min to determine the optimum time for adsorption of fluoride. The time experiment was terminated at t = 180 min for all concentrations when the adsorption process attained equilibrium using fluoride ion specific electrode at different concentrations of fluoride solution at fixed amount, 3gm of adsorbent dosage and pH value equal to 7 from Appendix B.

3.9. Experimental Design and Statistical Analysis

To analyze the experimental data a statistical software Design Expert Software version 7.0.0 was used. Design of experiment mostly used the statistical techniques to design and analyze the experimental data. The use of such techniques helps to draw accurate and valid conclusions for a set of experiments. In this study three factors were selected initial fluoride concentration, adsorbent dosage and pH for batch adsorption experiment. Full Factorial design three levels of each factor, one factor at a time was studied. Analysis of variance (ANOVA) was used to

determine the necessary conditions on the fluoride removal and the regression model used to test the interaction effect between the factors. Analysis of variance (ANOVA) justified the adequacy and significance of the model. The Model variance and residual (error) variance compared by F-test value that is based on 95% confidence interval. The input selected variables likely have a significant effect on the output variable (removal efficiency) for p-value of less than 0.05. It is calculated by dividing the model mean square by residual mean square. The results were tabulated as shown below in the table.

Table 3-1 Experimental factors and levels for testing fluoride removal efficiency

Factor	**Levels**		
	Low actual	Medium	High actual
Initial fluoride concentration	10	15	20
Adsorbent dosage	1	2	3
pH	5	7	9

The selections of these factors were based on the effect they had on removal efficiency of fluoride compared with other factors such as contact time, temperature and agitation speed relatively. It was crucial to test the effect of pH on batch adsorption experiment due to the fact that the variation occurred in the removal efficiency both in acidic and basic conditions and hence the most efficient pH was needed. Adsorption process terminated when the equilibrium was achieved between adsorbent and fluoride solution therefore specific time was needed for the batch experiment to ensure that the equilibrium time was achieved according to preliminary test done for selected experimental conditions. Initial concentration of fluoride was selected as one crucial parameter because it alters the removal efficiency by providing mass transfer resistance between the solution and adsorbent. Most adsorption studies conducted at room temperature in the laboratories due to the Deprotination of the adsorbent surface which causes more negative charges on the adsorbent surface and had less likely effect on the adsorption process. Adsorbent dosage is also another important parameter because it determines the capacity of the adsorbent and hence selected as one parameter for this study.

3.10. Modeling analysis

The equilibrium adsorption capacity, removal efficiency and adsorption capacity at time t were determined by using:

$$qe = \frac{(co - ce)}{mac} Xv \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

$$qt = \frac{(co - ct)}{mac} Xv \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .3.2$$

$$E(\%) = \frac{(co-ce)}{co} X100 \ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

Where qe (mg/gm) is the equilibrium adsorption capacity, co (mg/L) is the initial concentration of fluoride ions in the solution, ce (mg/L) is the equilibrium concentration of the fluoride ions in the solution, ct is the concentration of fluoride ions in the solution at time t, v (L) is the volume of the fluoride solution, mac (gm) is weight of chitosan. E (%) is removal efficiency.

3.11. Isotherm modeling of fluoride adsorption

Adsorption isotherms are viable for describing the relationship between solute molecules and adsorbent surface, highlighting the distribution of solute molecules between the liquid and solid phases when an adsorption process reaches equilibrium state. The analysis of isotherm data by fitting different isotherm models is a significant step to determine the suitable model which can be used for design purposes. Hence, the correlation of equilibrium data using either a theoretical or empirical equation is essential for interpretation and prediction of the extent of adsorption, in deciding the maximum adsorption capacity of adsorbate for a given adsorbent *(Sivarajasekar et al., 2017).* To evaluate the adsorption capacity of chitosan for the removal of fluoride, two isotherms namely Freundlich and Langmuir are used. The isotherms of a given data were drawn by varying the concentration of the fluoride solution from 10mg/l to 20mg/l at fixed adsorbent dosage, contact time and pH value. The isotherm models compared from the data fit in terms of their correlation coefficients (R^2). The Freundlich model is an empirical equation based on adsorption on a heterogeneous surface. The isotherm constants $\frac{1}{n}$ and k_F were calculated from the slope and the intercept of the plot of log_{qe} vs.log_{Ce} . These constants related to the measure of adsorption intensity or surface heterogeneity and adsorption capacity, respectively. Values of

$\frac{1}{n}$ lying between 0 and 1 and the n-values lying in the range of 1–10 confirm the favorable conditions for adsorption.

The general form of Freundlich and log scale is given by

$$q_{e} = k_F \, Ce^{\frac{1}{n}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

$$log_{qe} = log_{kF} + log_{Ce^{\frac{1}{n}}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

Linear form of Langmuir Isotherms is given by

$$q_e = {(Q^0 bCe)}/{(1 + bCe)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

$${Ce}/{qe} = \left({1}/{Q^0 b}\right) + {Ce}/{Q^0} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

The linear plot of ${Ce}/{qe}$ vs. Ce indicates the applicability of Langmuir isotherm. The Langmuir constants Q^0 and b are related to adsorption capacity and adsorption energy, respectively. Maximum adsorption capacity (Q^0) represents monolayer coverage of sorbent with sorbate and b represents the energy of adsorption and varies with temperature. To find out the probability of isotherm, the important characteristics of Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L.

$$R_L = {1}/{(1 + bC_o)}; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

Where b is the Langmuir isotherm constant and C_0 is the initial concentration of fluoride (mg L^{-1}). The R_L-values between 0 and 1 indicate favorable adsorption *(Amalraj & Pius, 2017)*.

3.12. Adsorption kinetics

Adsorption kinetic parameters are useful for the prediction of adsorption rate that gives important information for modeling the process and designing adsorption-based water treatment systems. Two adsorption kinetic models are pseudo-first-order and pseudo-second-order models.

A simple pseudo-first order kinetic model is given as

$$\mathrm{Log}(q_e - q_t) = logq_e - k_1 t/2.303 \; ; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.6$$

Where q_t is the amount of fluoride on the surface of the chitosan at time t (mg g^{-1}) and k_1 is the equilibrium rate constant of the pseudo-first-order adsorption (min^{-1}). The linear plots of $\mathrm{Log}(q_e - q_t)$ vs t give straight lines indicating the applicability of Pseudo-first-order model.

The Pseudo-second-order model is also widely used and the most popular linear form of pseudo second-order model is

$$t/q_t = {}^{1}/_{h} + t/q_e \; ; \; q_t = \frac{{q_e}^2 k_2 t}{1+{q_e}^2 k_2 t} ; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

Where q_t the amount of fluoride on the surface of chitosan at any time, t (mg g-1), k_2 is the pseudo-second-order rate constant (g mg-1 min-1), q_e is the amount of fluoride ion adsorbed at equilibrium (mg g-1) and the initial adsorption rate, $h = {q_e}^2 k_2$ (mg g-1 min-1). The value of q_e (1/slope), k_2 (slope2/intercept) and h (1/intercept) of the pseudo-second order equation can be found out experimentally by plotting t/q_t against t *(Amalraj & Pius, 2016).*

3.13. Regeneration study

High adsorption capacity and good reusability were of utmost importance for any adsorbent, which would significantly promote economic value of adsorption method. It was evident that the NaCl desorption reagent could efficiently regenerate the chitosan based on ion exchange reaction. Therefore, NaCl was a promising desorption reagent (Patnaik et al., 2016). Regeneration of chitosan adsorbent was conducted using sodium chloride solution. The purpose of regeneration was to remove the F^- on the surface of the adsorbent. The exhausted chitosan powder was retrieved in 0.1N NaCl (1gm/20ml) aqueous solution for 12 hr. and then washed

with deionized distilled water followed by drying in the oven at 50^0C. The chitosan adsorbent with dosage of 3gm where maximum removal efficiency achieved was reused in the next two runs of adsorption experiments.

Chapter Four

4. Results and Discussion

4.1. Physico-chemical parameters of chitosan

Solubility test in acid solution (acetic acid)

1.5 gm. of chitosan sample taken in triplicate and measured weight of centrifuge tubes were depicted as shown in the table below. The acid solubility was calculated by using the equation:-

Table 4-1 weight of chitosan sample in acid solution

Weight of empty tube	Initial weight of tube + chitosan	Final weight of tube + chitosan
6.7106	8.2106	6.881
6.0070	7.507	5.432
6.8001	8.3001	6.643
Average = 83.8±0.07		

The results are triplicate determination mean±standard deviation

$$solubility(\%) = \frac{(initial\ weight\ of\ (tube+chitosan))-(final\ weight\ of\ (tube+chitosan))}{(initial\ weight\ of\ (tube+chitosan))-(weight\ of\ empty\ tube)} X100 \ldots\ldots\ldots\ldots\ldots .4.1$$

Acetic acid solubility of chitosan was calculated taking the average value for triplicate experiment by using the equation above and the value was found to be 83.8% at solution pH of 4. During deacetylation reaction there would be conversion of acetamide groups in chitin (-$NHCOCH_3$) into free amino groups (-NH_2) along chitosan macromolecule and because of the free proton able amine groups in the D-glucosamine units attracts ionic compounds and enables chitosan solubility in dilute aqueous acidic solvents. The solubility of chitosan in acetic acid indicated the purity of chitosan. The presence of carboxyl group in acetic acid would facilitate the dissolution of chitosan due to the hydrogen interaction between the carboxyl group and the amine group of chitosan. The solubility value also suggests the removal of protein.

Degree of Deacetylation

Degree of deacetylation of chitosan is an important property because it was used to determine the quality of chitosan produced in which it affects physical, biological and chemical properties of chitosan such as adsorption process in water treatment and covalent linking. It is defined as the number of D-glucosamine units and is an indicator for free amino groups (-NH2) in chitosan sample.

$$(-\mathrm{NH2})\% = \frac{0.016(C1V1-C2V2)}{W}100; \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.4.2$$

$$DD\% = \frac{203(-NH2\%)}{16+42(-NH2\%)}100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4.3$$

Where C1, V1, C2, and V2 are the concentrations and volumes for the HCl standard solution and NaOH standard solution, respectively, and W is the weight of the sample.

C1 = 0.1M HCl solution, C2 = 0.1M NaOH solution, V1 = 30ml = 0.03 liter of HCl solution used and the amount of NaOH solution consumed during the reaction for color change from methyl orange to red obtained using the relation C1V1=C2V2 and found to be 27ml =0.27 liter substituting the values to the above equation:-

$$(-\mathrm{NH2})\% = 0.080$$

$$DD\% = \frac{203(0.080)}{16+42(0.080)}100 = 84\%$$

The degree of deacetylation of value of chitosan prepared from fish scale was obtained to be 84%. The degree of deacetylation of typical commercial chitosan is usually ranged between 66 and 95%. Therefore the value obtained is in a good agreement as discussed in the literature part and categorized in medium degree of deacetylation according to the classification. The higher degree of deacetylation value of chitosan indicates the higher removal of inorganic components.

Water and Fat Binding Capacity of Chitosan

The fat and water binding capacity of chitosan sample taken in triplicate was measured and given in the table below and calculated using the equations accordingly to the method of modified Wang and Kinsella (1976).

Table 4-2 Water and Fat bound mass of the samples

Initial sample mass (g)	Fat bound (g)	Initial sample mass(g)	Water bound (g)
7.2106	15.532	7.7106	43.284
6.507	14.721	7.0070	42.465
7.3001	15.8333	7.8001	43.9095
Average = 219.5±0.05		Average = 576±0.21	

The results are triplicate determination mean±standard deviation

$$\text{WBC}(\%) = \frac{(\text{water bound})\text{gm}}{(\text{initial sample})\text{gm}} \text{ X } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4.4$$

$$\text{FBC}(\%) = \frac{(\text{fat bound})\text{gm}}{(\text{initial sample})\text{gm}} \text{ X } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4.5$$

The obtained values for Physico-chemical properties of fish chitosan; fat binding capacity and water binding capacity obtained was 219.5 and 576 respectively and in a good agreement with the available ranges written in the literature. Relatively fat binding capacity of chitosan is lowest compared with water binding capacity. The higher water binding capacity of chitosan was related with its degree of deacetylation value which increases the amount of free amino group (-NH_2) along the structure of the biomolecule that enhances its ability to bind with water. Amine group contains hydrogen ions which makes chitosan can easily interact with water through hydrogen bonding.

Yield of chitosan

The amount/weight of dry fish scale used = 400gm and about 150gm of dried chitosan extracted;

$$chitosan\ extraction\ yield(\%) = \frac{dried\ extracted\ chitosan\ weight(g)}{fish\ scale\ waste(g)}\ X\ 100\% \ldots\ldots\ldots\ldots 4.6$$

$$yield(\%) = \frac{150gm}{400gm}\ X\ 100\% = 37.5\%$$

Using the above equation conversion of waste fish scale into valuable component of chitosan the yield is found to be 37.5% which is quite high. The existence of loss of the chitosan particles during washing after each treatment of extraction and weigh or mass loss as a result of removal of acetyl group during deacetylation decreases the yield to some extent.

4.2. Proximate analysis

Moisture content

Sample of fish scale chitosan taken in triplicate (1.5gm) on dry basis for the given data as shown below in the table and analyzed for moisture content using both the equation and moisture analyzer *(AOAC, 1990).*

Table 4-3 Weight of the samples before and after drying

Mass of sample(W1,gm)	Mass of crucible(W2,gm)	Mass of sample and crucible(W3,gm)
1.5	23.6	24.49
1.5	21.5	22.41
1.5	22.48	23.26
Average = 42.6±0.038		

The results are triplicate determination mean±standard deviation

$$moisture\ content\ (\%) = \frac{W1-(W3-W2)}{W1}\ X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots 4.7$$

Where $W1$, $W2$ and $W3$ were mass of sample, mass of crucible and mass of a sample and crucible respectively. The moisture content was calculated using the equation above and found to be 42.6%. The presence of higher moisture content implies that due to the hygroscopic nature of chitosan; it has the capability to absorb moisture and its binding capacity with water molecule due to availability of amine group of chitosan enhance its absorption capacity and the result obtained agreed and nearly similar with the value obtained using moisture analyzer in the lab which is equal to 39.8%.

Ash content determination

The ash content of chitosan measured in JIJE labo glass private limited company for triplicate of the sample using AOAC method for both untreated and treated sample found to be 45.40 and 0.60 respectively. The decreased value of ash content indicates the efficiency of demineralization step for removal of salt as well as high quality grade of chitosan. Ash value must not increase on 1% by *No and Meyers, 1995*.

Protein content determination

Protein content analysis was done in JIJE labo glass private limited company according to the method of Kjelhdahl method. The analysis was done for both raw untreated sample and treated sample and the results obtained to be 33% and 6.7% respectively. This shows that initially the sample was rich in nitrogen amount relatively to the treated sample. The decreased amount of nitrogen was due to the process of deproteinization by sodium hydroxide solution.

Mineral content determination

The mineral content of raw fish scale and that of chitosan was analyzed in terms of $CaCo_3$ which mainly comes from Ca in JIJE Labo glass limited Share Company according to Ash digestion-EDTA Titration method. It was observed that the reduction from 13.36% to 0.14% definitely assures the removal of mineral from the raw fish scale which supports the demineralization step.

Table 4-4 Summary of the results of proximate analysis

Proximate analysis (%)	Ash content	Ca	Crude Protein	Moisture content
Untreated sample of fish scale	45.40±0.56	13.36±0.06	33.0 ±0.64	-
Treated sample (chitosan)	0.60±0.13	0.14±0.02	6.7±0.22	42.6%±0.038

The results are triplicate determination mean±standard deviation

4.3. Standardization of process efficiencies

Determination of demineralization efficiency

From the proximate analysis given above for the ash content obtained for treated and untreated sample and 0.75gm of raw sample and demineralized residue the demineralization efficiency was calculated. The value of ash content decreased from 45.40 to 0.60 shows that the removal of inorganic matter such as calcium carbonates and phosphates from the scale due to demineralization process which increases percentage removal efficiency of these minerals and calculated as:

Demineralization efficiency =

$$\frac{(g\ of\ sample\ X\ Ash\ content\ (\%)\ of\ sample) - (g\ of\ residue\ X\ Ash\ content\ \ (\%\ of\ residue)}{g\ of\ sample\ X\ Ash\ content\ (\%)\ of\ sample}\ X\ 100$$

$$= \frac{(0.75X\ 45.40) - (0.75\ X\ 0.60)}{0.75\ X\ 45.60}\ X\ 100 = 98.2\%$$

Therefore from the above result it was concluded that the demineralization process is efficient in removing almost all the inorganic components of the scale and the conditions used for this purpose were suitable and enhance the demineralization process in a good manner.

Determination of deproteinization efficiency

From the proximate analysis given above for the crude protein content obtained for treated and untreated sample and 0.75gm of raw sample and deprotenized residue the deproteinization efficiency was calculated. The value of protein content decreased from 33% to 6.7% shows that the removal of crude protein from the scale due to deproteinization process which in turn increases the removal efficiency of deproteinization and given below *(Kumar & Ravi, 2017)*.

Deproteinization efficiency =

$$\frac{(g\ of\ sample\ X\ CP\ content\ (\%)\ of\ sample) - (g\ of\ residue\ X\ CP\ content\ \ (\%\ of\ residue)}{g\ of\ sample\ X\ CP\ content\ (\%)\ of\ sample}\ X\ 100$$

$$= \frac{(\ 0.75X\ 33) - (0.75\ X\ 6.7)}{0.75X33}\ X\ 100 = 79.69\%$$

Almost more than half of crude protein is removed from the scale during deproteinization process from the result obtained. The Force of covalent bond due to protein connectivity between chitin and chitosan results in a difficulty of the total removal of crude protein from sample; and also in deproteinization efficiency. Deproteinization efficiency below 80% indicates higher concentration of protein which requires harsh treatment unfortunately leads to undesirable deacetylation and alter depolymerized of chitin.

4.4. XRD and FTIR analysis

X-ray powder diffraction (XRD)

XRD mostly used to determine the crystal planes and provide strong evidence for polymorphic structure of the compound for which different x-ray diffraction patterns was obtained and tells the crystal structures. It was observed that chitosan samples extracted from fish scales exhibited strong reflection at 2theta values of 25.81 and 32.14^0 as shown below in the figure. The intense peak at 32.09 for fish scale chitosan sample indicates the presence of hydroxyapatite mineral content ($Ca_5(PO_4)_3(OH)$); is the hydroxyl end member of the complex apatite group which denotes the crystal unit cells comprises two entities. The peak values obtained for this work is more or less similar to previous studies. The XRD result suggests fishery wastes are most remarkable and good sources of chitosan and apatite content on this peak shows that chitosan had a good potential for defluoridation and the result further confirms the analysis of FTIR. The XRD analysis also indicated that when compared to chitosan the scale had high peak and amorphous in structure however chitosan shown crystalline structures. Generally the sharpness of the bands is higher in the scales samples than in their chitosan analogues with a slight decrease in the crystalline percent. XRD patterns indicated chitosan sample exhibited comparable degree of crystallinity and had consistent peak in between the range $30\text{-}40^0$.

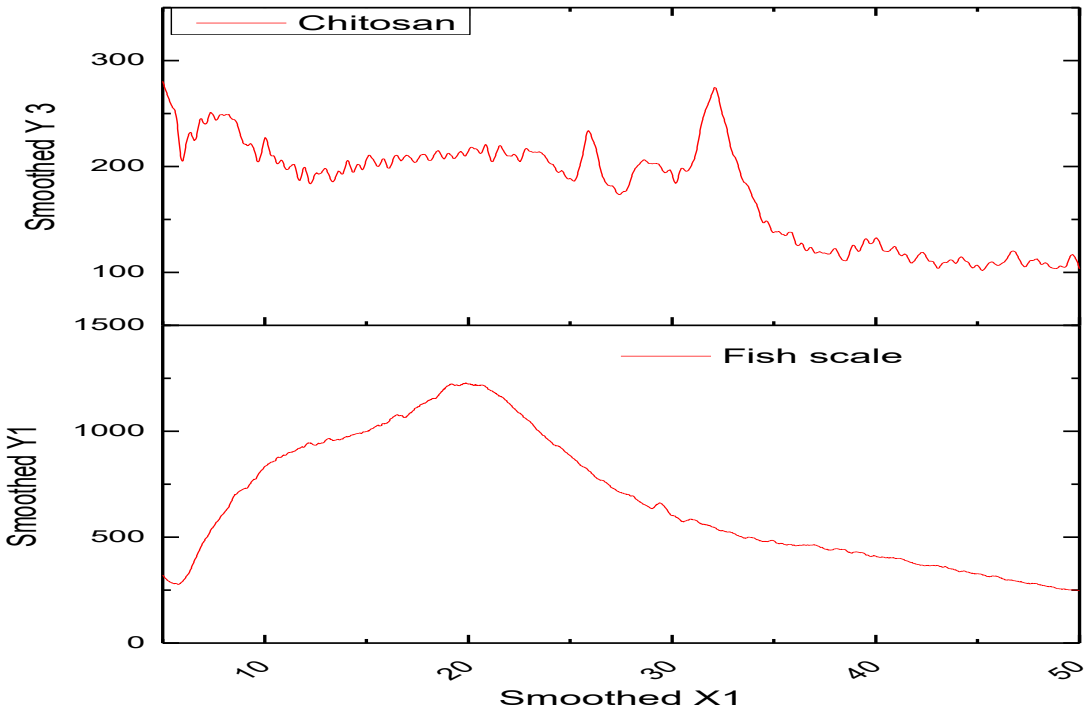

Figure 4-1 X-ray diffraction pattern of fish scale and chitosan

Fourier transforms infrared spectroscopy (FTIR)

Chitosan is a polysaccharide and known for its acetyl glucosamine groups. The presence of amine, carboxyl and hydroxyl functional groups represents unique properties of chitosan sample.

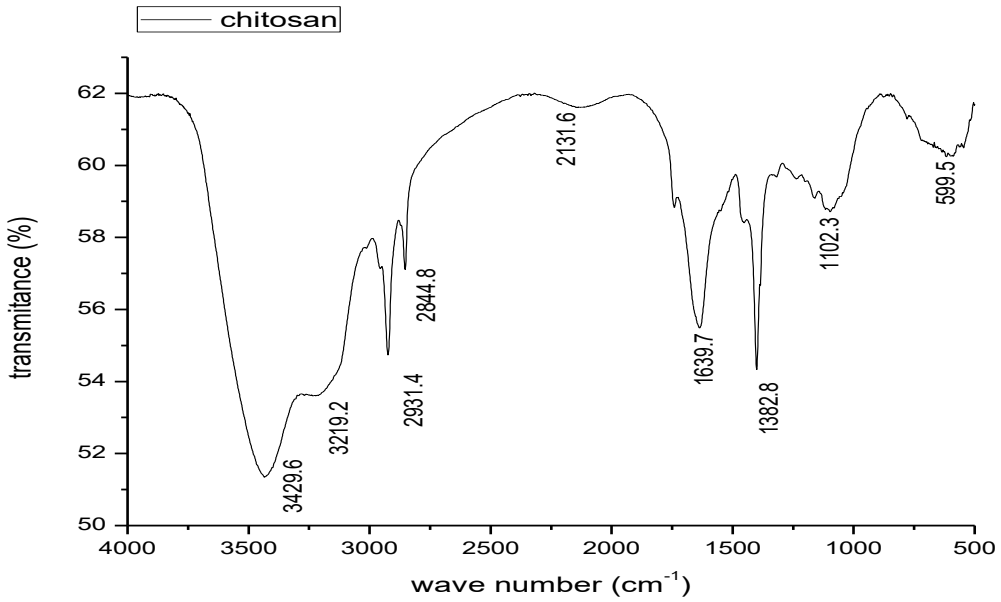

Figure 4-2 FTIR spectrum of Chitosan

A broader spectrum observed around 3429 and 3219 indicates the formation of hydrogen bond due to the axial deformation of O-H group which overlaps with the bond of axial deformation of amine NH_2 of free amino groups. The most significant spectrums observed in chitosan were 1639cm^{-1} and 1382cm^{-1} which are assigned to the carboxyl (C=O) stretching from acetamide ($-nHCOCH_3$) and C–H bending and symmetric CH3 deformation. The band observed at 1102 corresponds to the stretching vibration of C-O. The peaks observed at 2931 and 2844 were assigned to symmetric and asymmetric stretching of methyl group, CH_3 in $NHCOCH_3$ and methylene group, CH_2 in CH_2OH shows the extent of conversion of chitin to chitosan. Finally the band at 599 corresponds to the NH-out of plane bending. Bands displayed as a result of FTIR analysis that corresponds to stretching and vibration of O-H, N-H and CO bonds confirmed the formation of chitin/chitosan polysaccharide. The band corresponds to 1639 formed Schiff's base as a result of the reaction between carbonyl group of glutara aldehyde and amine group of chitosan chains. Schiff's base is group specific reaction of aldehydes with amines under basic conditions. The commercial chitosan used for comparison is tabulated in the table and more or less similar with the current study.

Table 4-5 comparison of current study with commercial chitosan

	Frequency(cm^{-1})		
Functional group	commercial chitosan	current study	reference
Amine, NH2 stretch and hydroxyl, O-H stretch	3429	3429	*(Kitin, Ahyat, Mohamad, Ahmad, & Azmi, 2017)*
Aliphatic compound, -CH2 stretch	2921	2844	
Secondary amide, C=O stretch	1643	1639	
Aliphatic compound, -CH2 bend	1430	1384	
C-O-C glycosidic linkage	1154	1102	

4.5. Effects of adsorption conditions on removal efficiency

4.5.1. Effect of pH

The effect of solution pH for defluoridation at different values of pH 5, 7 and 9 at various concentration and room temperature depicted in the figure below. The removal efficiency of chitosan increased through with pH and then started to decline after it was reached the equilibrium pH where maximum removal efficiency of fluoride was obtained. This was because the acidic pH provide with more adsorption sites to chitosan adsorbent for fluoride removal than the alkaline pH. Also the reason behind for maximum removal at the acidic pH is negatively charged fluoride ions exhibit electrostatic attractions towards the positively charged amine groups of fish scale chitosan. On the other hand free amine group of chitosan could not be protonated and the electrostatic repulsions between carboxyl ($-COO^-$) and phosphate groups of chitosan with fluoride ion minimizes the efficiency at alkaline conditions.

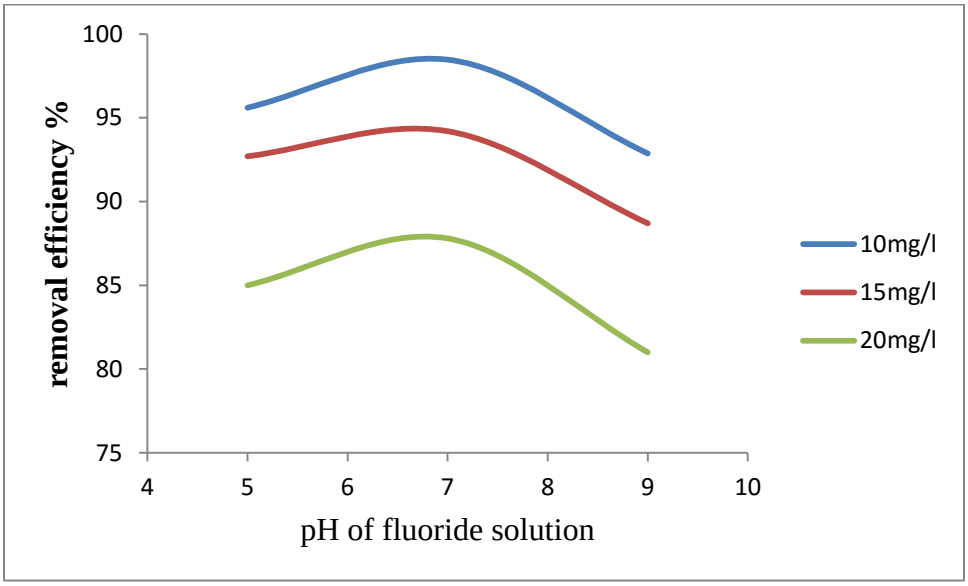

Figure 4-3 Effect of pH on removal efficiency

The value of pH for which maximum removal efficiency of fluoride for different fluoride ion concentration obtained was 7. Hence it denotes the equilibrium pH.

4.5.2. Effect of contact time

From figure4.4 sorption increased with contact time. Sorption rate increased during initial period of contact time between the adsorbate and adsorbent this was because of at the beginning of adsorption the availability of more sites on the adsorbent surface and the high concentration gradient between the solution and solid phase (increment of unimolecular layer). The removal efficiency increases at a slower rate gradually with contact time till the adsorption equilibrium was reached saturation point and remains constant after 180 minutes onwards. The adsorption process was mainly due to the hydroxyl and amine functional groups of chitosan adsorbent. The effect of contact time on removal efficiency at various initial concentration of fluoride was drawn using the data points from Appendix B at 3gm of adsorbent dosage and pH of 7.

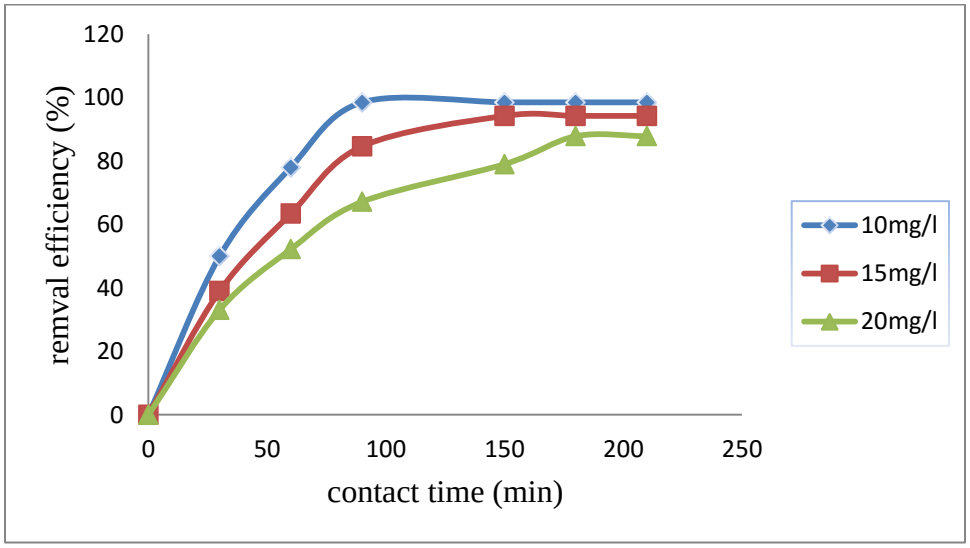

Figure 4-4 Effect of contact time on removal efficiency for different fluoride concentrations

4.5.3. Effect of fluoride ion concentration

The higher uptake of fluoride at low concentration was due to the availability of more isolated fluoride ions. At higher fluoride ion concentration, sites for adsorption of the adsorbent became limited and saturate which results in lower removal efficiency at fixed adsorbent dosage and contact time. However the adsorption capacity unlikely increases due to the concentration gradient between the aqueous and solid phase increased and overcome the mass transfer resistance as a result of increasing driving force. The removal efficiency decreased from 98.47 to 87.8 as the initial concentration increased from 10 to 20mg/l at fixed adsorbent dosage and pH from Appendix A.

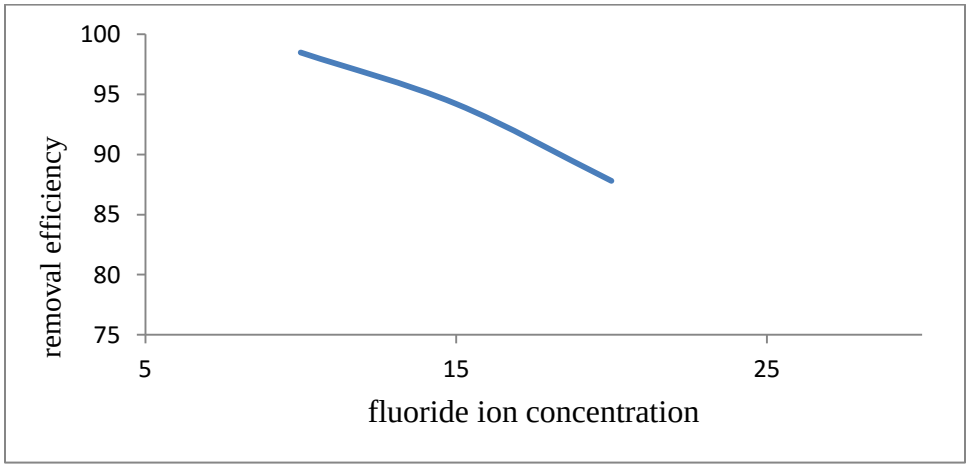

Figure 4-5 Removal efficiency as a function of fluoride ion concentration

4.5.4. Effect of adsorbent dosage

The removal efficiency of fluoride ion increased with increase in adsorbent dose due to the availability of large amount of active adsorption sites of the chitosan adsorbent. However the amount of adsorbed Fluoride per gram of adsorbent decreases because the active sites for the removal of fluoride are not saturated. When the mass of adsorbent was increased, the capacity of all the active sites on the surface of the adsorbent was not used fully and thus leading to a decrease in adsorption per unit of adsorbent dosage. As shown in the figure below the percent removal efficiency of fluoride increased from 96.5% to 98.47%; with increasing the adsorbent dosage from 1g to 3g at fixed fluoride concentration and pH of the solution from appendix A.

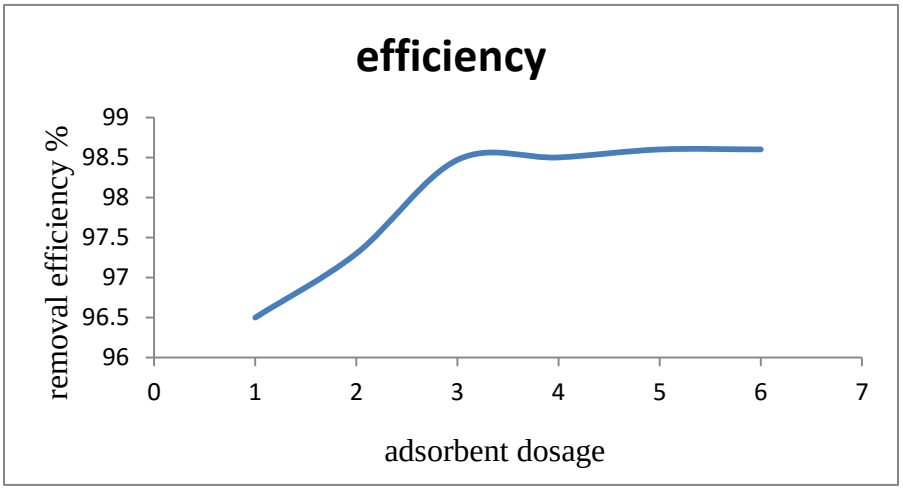

Figure 4-6 Removal efficiency as a function of adsorbent dosage

The removal efficiency of fluoride ion increased with adsorbent dosage up to a certain limit and then it remains almost constant this is due to the increase in the adsorbent surface. 3g of adsorbent dosage is selected as the saturation level where further increase in the adsorbent dosage beyond this value does not show any appreciable improvement in the removal of fluoride.

4.6. Analysis of regression model equation for batch adsorption study

Design of experts is an important tool to study the different conditions of batch adsorption experiments. On this regard the optimal removal efficiency of fluoride was studied by full factorial design analysis. Full factorial analysis allows you to have factors that each has a different number of levels. Quadratic versus two factor interaction (2FI) model of full factorial

design was used for the analysis of optimal removal efficiency and was suggested by design expert version 7.0.0 version for the analysis of optimal removal efficiency.

Table 4-6 ANOVA for response surface quadratic model of chitosan adsorbent

Source	Sum of Squares	Df	Mean Square	F Value	p-value prob >F	
Model	914.75	9	101.64	171.48	< 0.0001	
A-conc	719.09	1	719.09	1213.23	< 0.0001	
B-pH	48.51	1	48.51	81.85	< 0.0001	
C-dosage	41.83	1	41.83	70.58	< 0.0001	
AB	2.20	1	2.20	3.71	0.0708	
AC	11.04	1	11.04	18.63	<0.0005	Significant
BC	1.53	1	1.53	2.58	0.1269	
A^2	20.65	1	20.65	34.83	< 0.0001	
B^2	69.02	1	69.02	116.45	< 0.0001	
C^2	0.88	1	0.88	1.49	0.2393	
Residual	10.08	17	0.59			
Cor Total	924.82	26				

The Model F-value of 171.48 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AC A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Pred R-Squared" of 0.9724 is in reasonable agreement with the "Adj R-Squared" of 0.9833. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 44.383 indicates an adequate signal.

Table 4-7 Model adequacy parameter

Std.Dev	0.77	R-Squared	0.9891
Mean	89.31	Adj R-Squared	0.9833
C.V%	0.86	Pre R-Squared	0.9724
Press	25.5	Adeq Precision	44.383

This model can be used to navigate the design space. The coefficient of determination (R^2) of the model indicated a good fit between predicted values and the experimental data points. It also indicates the extent of variation in the removal efficiency. Predicted R^2 is a measure of how good the model predicts response value. The coefficient of variation and standard deviation indicate the degree of precision. The low values of C.V (0.86) and Std.Dev (0.77) show the adequacy with which the experiment is conducted. C.V is the standard deviation expressed in the form of percentage of the mean. Estimated by dividing the standard deviation by the Mean and multiplying by hundred. Adjusted- R^2 a measure of the amount of variation around the mean defined for the number of terms in the model.

From the above results obtained pH of fluoride solution, dosage of the adsorbent, initial fluoride concentration and the interaction effect between pH and adsorbent dosage have significant effect on the removal efficiency of fluoride.

Predicted versus actual graph is a plot of the actual response values versus the forecast response values. It helps one detect a value, or group of values, that are difficult to predicted by the model. The graph of predicted versus actual shows that the model equaton is best fitted between the predicted and actual values of experimental data points in which the scatter points are closer each other and fits to the straight line equation. The closer of the scatter points towards the straight line indicates the good selection of process variables in the removal of fluoride from aqueous solution.

The normal probability plot indicates whether the residuals keep on a normal distribution, in which case the points will follow a straight line. Expect some scatter even with common (normal) data. normal probablity plot implies the experimental data points fits the straight line which assures the quadratic versus 2FI (two factor interacction) model choosen satisfies the assumption of ANOVA in which the distribution error is approximately normal.

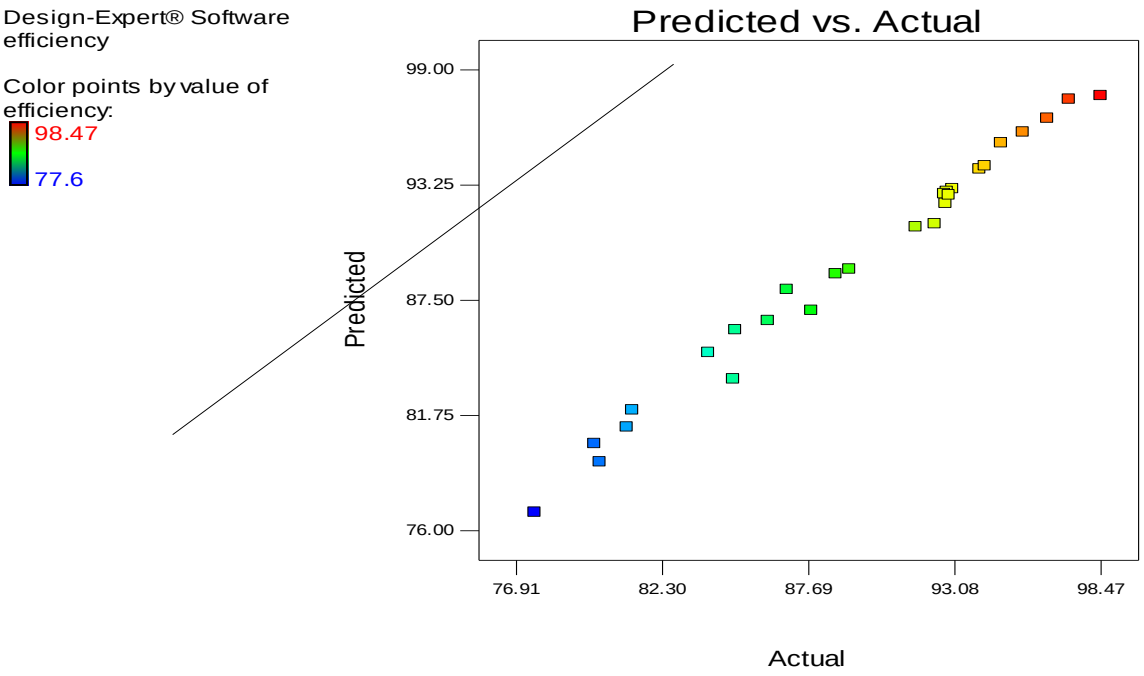

Figure 4-7 predicted versus actual graph

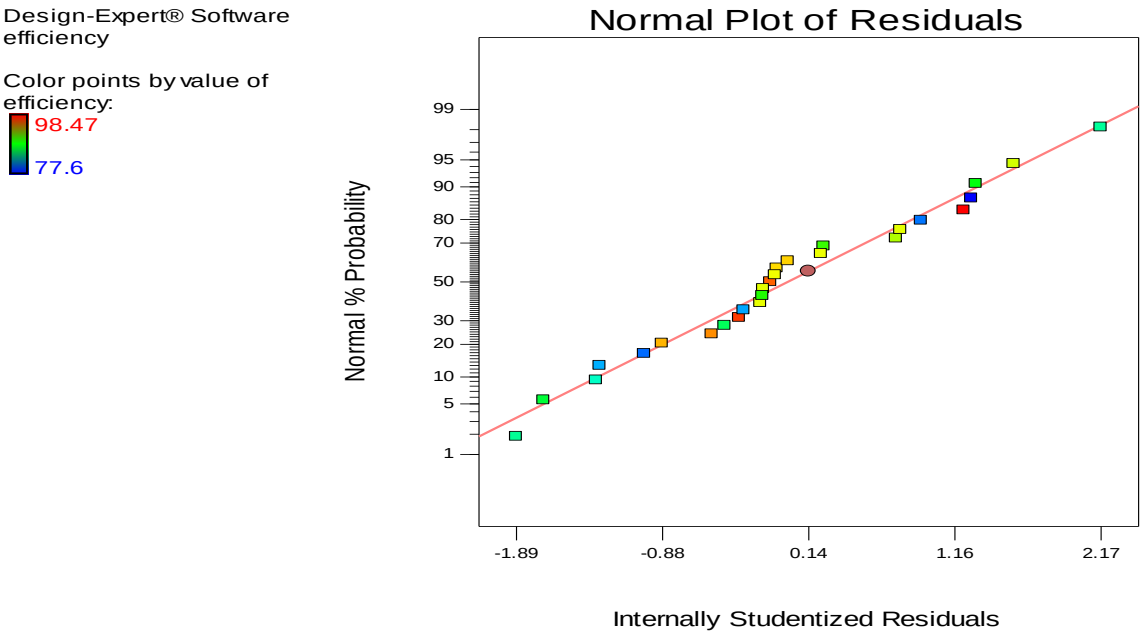

Figure 4-8 normal versus residual graph

4.7. Interaction effects of testing conditions on the removal efficiency

The interaction effect between concentration and adsorbent dosage is significant whereas the other interaction effects were not significant. The surface methodology of 3 level full factorial interaction effects was depicted in the figure below by contour plot and 3-D. The contour plot is a two dimensional representation of the response for selected factors. In the 3-D plot the model can be displayed in three dimensions which may provide with a clearer view of the surface. From the figure below the interaction effect between fluoride concentration and adsorbent dosage with maximum removal efficiency was observed towards the edges of the design points in red color. The removal efficiency is maximum for adsorbent dosage of 3g and fluoride concentration of 10mg/l at actual factor of pH = 7 as shown below in the two dimensional contour plot. The same is true for 3D surface plot interaction effect which provide with maximum removal efficiency at the end point of adsorbent dosage = 3gm and fluoride concentration of 10mg/l using actual pH factor = 7. The plots are displayed as shown in the graph.

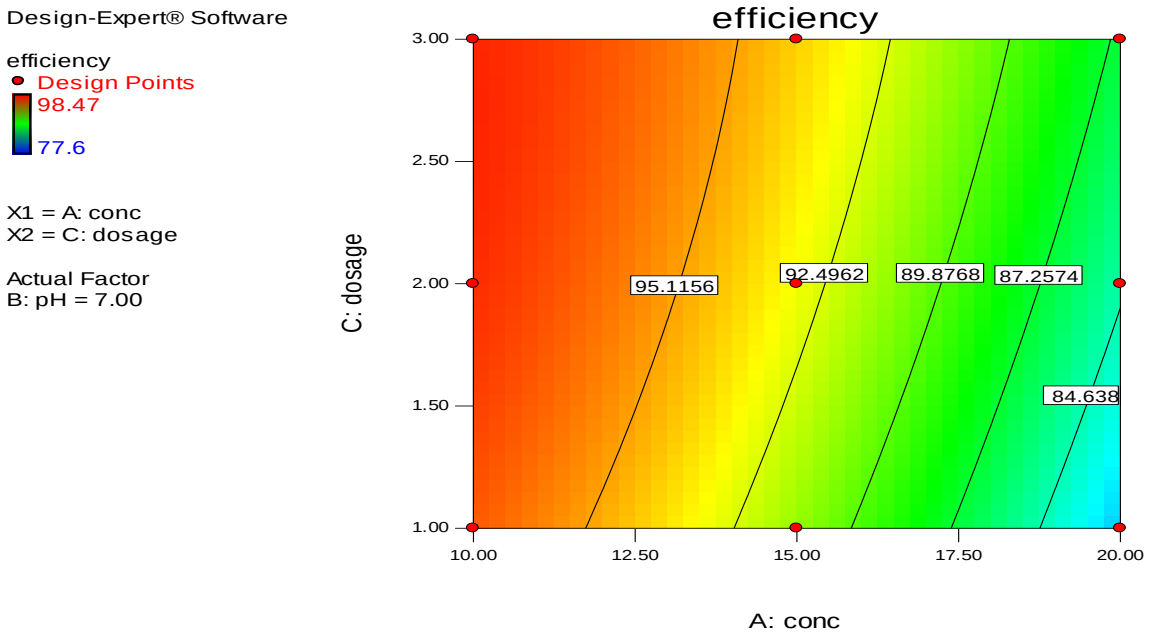

Figure 4-9 Contour plot of between concentration of fluoride and adsorbent dosage

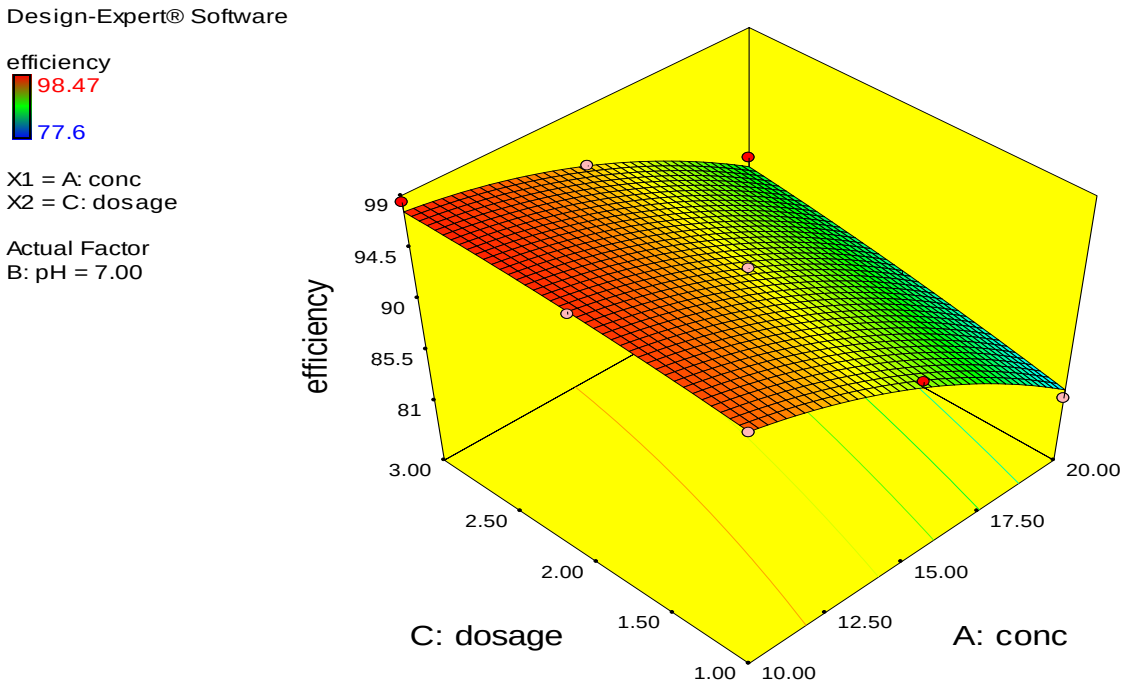

Figure 4-103D-surface plot interaction effect between concentration of fluoride and adsorbent dosage

4.8. Development of regression model equation

The model equation that correlates the response percentage removal efficiency to optimal defluoridation conditions in terms of actual factors removing the insignificant terms from the regression model is shown below. The regression coefficient with negative sign decreases the optimal parameter while that of positive sign increases. The final equation of predicted model in terms of actual factors is given by:

efficiency = +53.70431 + 0.87806 * conc + 12.04917 * pH + 1.42861 * dosage + 0.19183 * conc * dosage - 0.074200 * $conc^2$ - 0.84792 * pH^2 - 0.38333 * $dosage^2$

4.9. Optmization of Process Variables

Inorder to obtain the optimium conditions in which maximium removal efficiency of fluoride it was necessary to perform the numerical optimization for a combination of other parameters in a range that gaves as maximum removal efficiency. In this conditions the combination of parameters obtained which gaves maximium removal efficiency were fluoride concentration of

10mg/l, adsorbent dosage of 3gm and pH of 6.56 with desirabiltiy of 0.972 selected. For the selected conditions the maximium removal efficiency obtained was 97.8901% from figure4.11

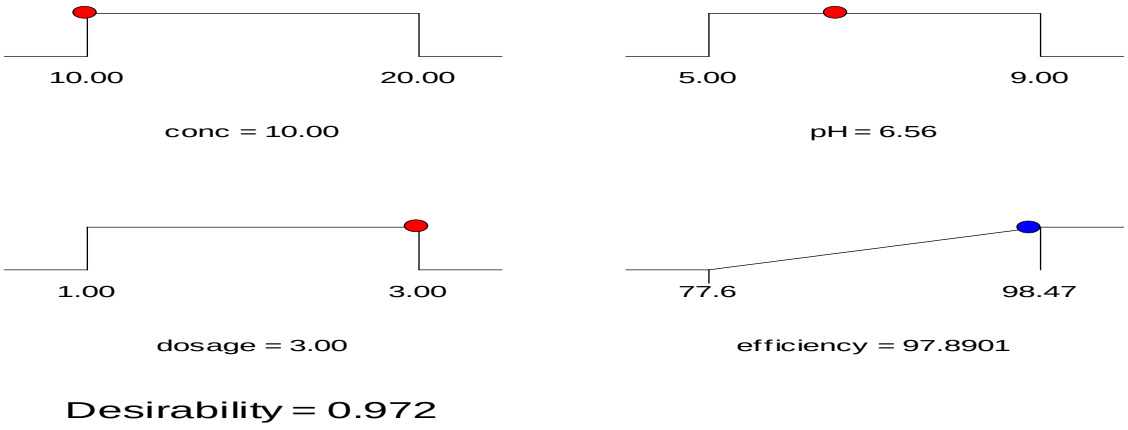

Figure 4-11 optimum conditions of desirability function

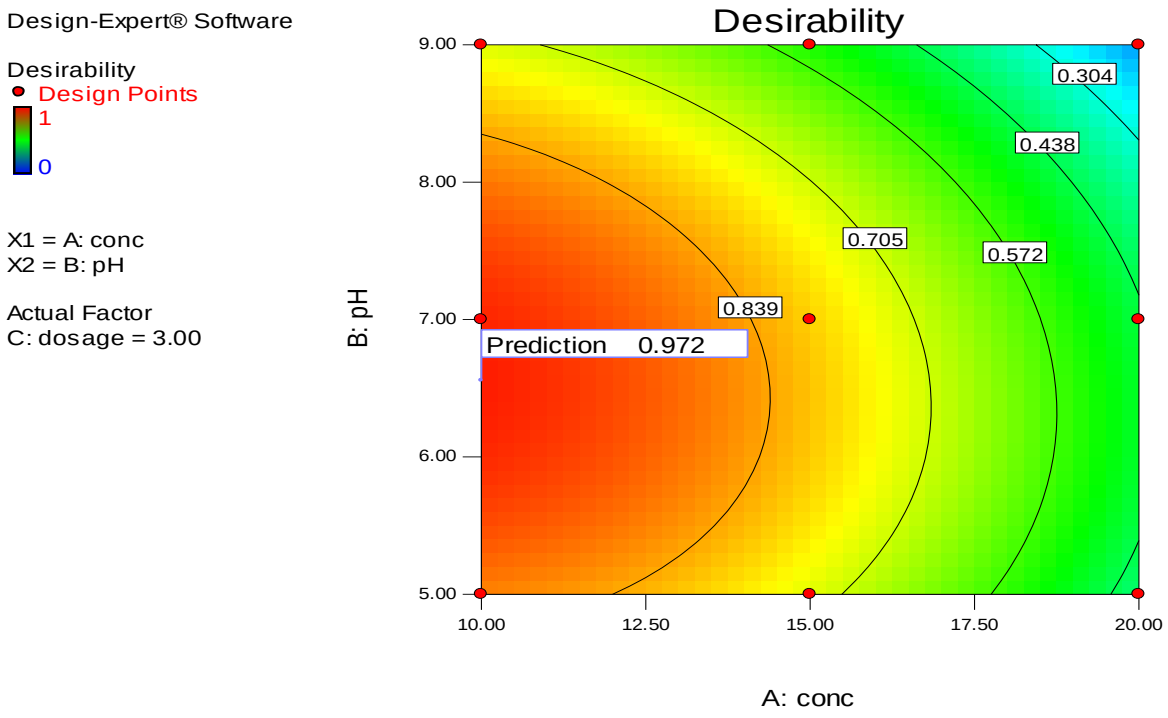

Figure 4-12 graphical solution of optimum conditions with maximum removal efficiency

The desirability functions of triplicate experiments were performed to validate the optimum defluoridation conditions and the mean value was taken. The results for triplicate experiments under optimum defluoridation conditions were 97.3, 98.25 and 99.74. The mean value of 98.43 obtained was closely related with the data obtained from optimization analysis using desirability ramp function. The use of chitosan adsorbent at the optimal conditions of the predicted model equation therefore enhances the removal of fluoride ion from aqueous solution and validates optimal conditions.

4.10. Adsorption isotherms

Langmuir Isotherm

As shown in the figure below the linear plot of Ce/qe vs. Ce gave a straight line with a slope of 0.79806 and intercept = 0.15911. The values of the parameters for Langmuir isotherm determined from the slope and intercept. The results obtained were Qm = 1.253mg/g, b = 5.0157L/mg and correlation coefficient (R^2) = 0.99118. The linear plot was drawn using the data points listed in table4.8.

Table 4-8 Langmuir isotherm data

Ce	Qe	$1/qe$	Ce/qe
0.153	0.65646	1.52332	0.23307
0.87	0.942	1.06157	0.92357
2.44	1.1706	0.85426	2.0844

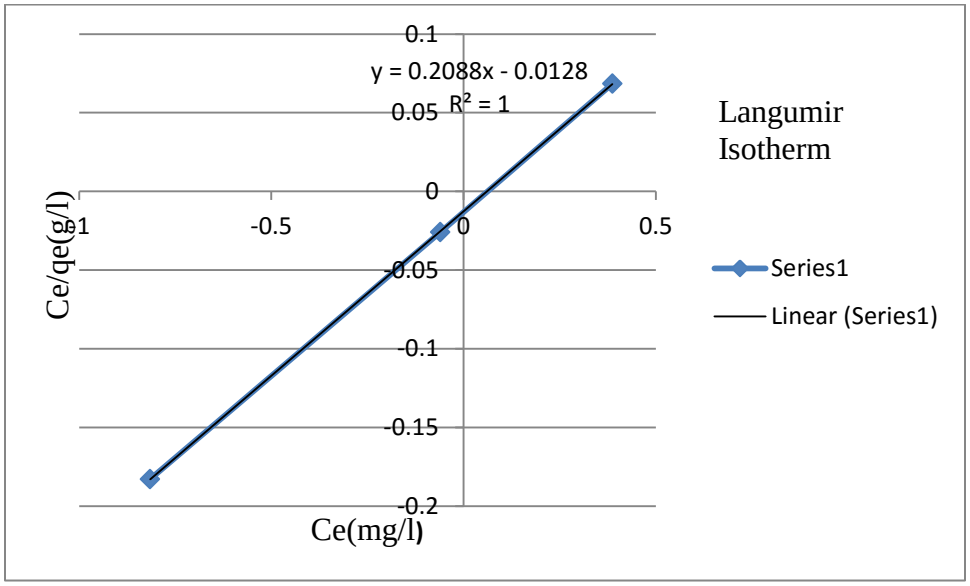

Figure 4-13 Langmuir isotherm for adsorption for 3g adsorbent dosage and pH of 7

Separation equilibrium or equilibrium parameter for Langmuir isotherm calculated to knew the favorability of fluoride ion adsorption on the adsorbent. The value of separation factor,RL, indicates the isotherms shape and the nature of the adsorption process as unfavorable (RL >1), linear (RL =1), favorable (0< RL <1) and irreversible (RL = 0). The result was calculated using the formula as shown below and summarized in the table. The value of RL which is less than 1 implies the adsorption process was deemed favored.

$$\mathrm{RL} = \frac{1}{1 + Cob}$$

Table 4-9 Separation factor at different initial concentrations

Initial concentration, $\boldsymbol{Co}$ (mg/l)	Intercept, $\boldsymbol{b}$	**RL**
10	5.0157	0.0175
15		0.013
20		0.00987

Freundlich Isotherm

The parameters for isotherm models of Freundlich were determined from the linear plot of logarithmic scales. The linear plot of logqe versus logce for the data points given in the table below at constant dosage, contact time and pH with varying concentration results in the values of correlation coefficient (R^2) = 1 and the values of slope and intercept found to be 0.20875 and -0.01279 respectively. The linear plot was drawn using the data points listed given in the table.

Table 4-10 Freundlich isotherm data

Ce	Qe	$1/qe$	Ce/qe	Logq$e$	Logce
0.153	0.65646	1.52332	0.23307	-0.1828	-0.8153
0.87	0.942	1.06157	0.92357	-0.0259	-0.0605
2.44	1.1706	0.85426	2.0844	0.06841	0.38739

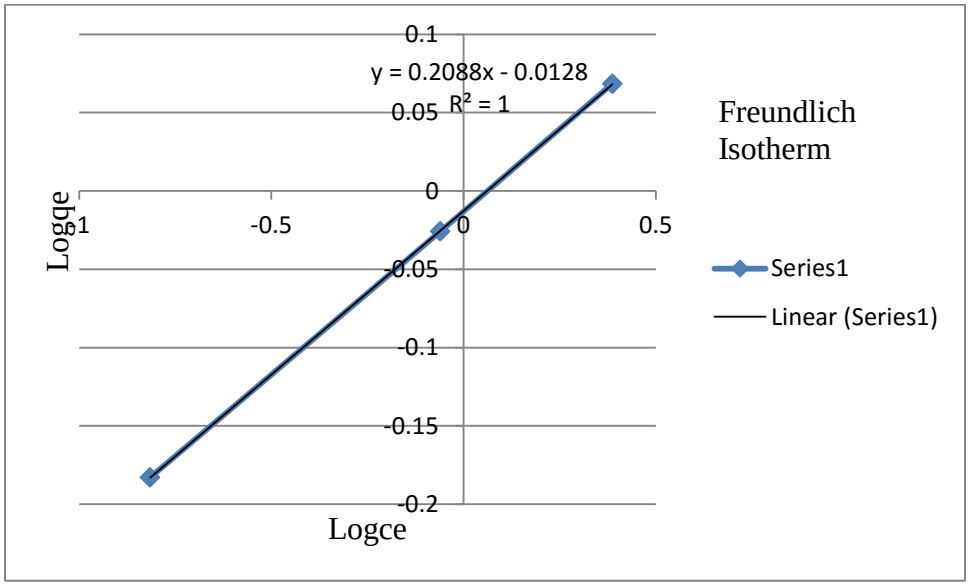

Figure 4-14 Freundlich isotherm for adsorption for 3g adsorbent dosage and pH of 7

The Freundlich isotherm model constants Kf and $1/n$ were determined from the slope and intercept of linear plots of $logce$ versus$logqe$. The results found to be 0.97097 and 4.79; which tells the adsorption capacity and adsorption intensity respectively. Adsorption intensity indicates

the deviation of isotherm from linearity. The value of n = 4.79 which is in the range 1-10 favors the adsorption process of Freundlich isotherm. From the above results for correlation coefficient (R^2) it was justified that the model best fits for Freundlich isotherm model.

4.11. Adsorption kinetics

Adsorption kinetic parameters are useful for the prediction of adsorption rate, designing and modeling adsorption process during the uptake of fluoride on adsorption process. The two kinetic models, pseudo first order and pseudo second studied by varying the concentration of fluoride solution from 10mg/l to 20mg/l at different intervals of time, at constant pH and fixed dosage at room temperature and they were compared with respect to the values obtained for linear regression correlation coefficient (R^2).

Pseudo first order kinetic model

The simplest linear plot of pseudo first order kinetic model drawn using the equation as shown below. The parameters $K1$ and calculated equilibrium adsorption capacity obtained from the slope and intercept of linear plot of $\mathrm{Log}\left(q_e - q_t\right)$ versus$t$ for the data points given in the table4.11. The value of $K1$ = 0.029, q_e = 1.459 and the regression coefficient (R^2) = 0.9642 was obtained. The linear plot was drawn using the data points listed in the table4.11.

$$\mathrm{Log}\,(q_e - q_t) = \mathrm{Log}\, q_e - \frac{k_1 t}{2.303}$$

Table 4-11 data of adsorption kinetics models for pseudo orders

Time (min)	Fluoride concentration (15mg/l)				
	Ce	Qt	Qe	Log (qe – qt)	t/qt
30	9.36	0.39	0.942	-0.258	76.923
60	5.55	0.634	0.942	-0.511	94.637
90	2.805	0.846	0.942	-1.0177	106.383
150	0.87	0.942	0.942	-	159.24
180	0.87	0.942	0.942	-	191.082

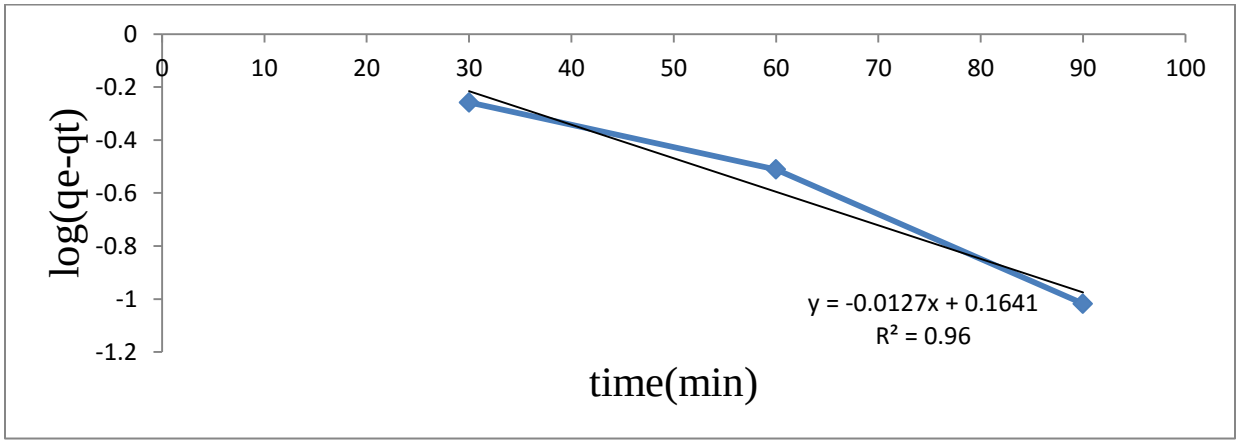

Figure 4-15 linear regression of pseudo first order kinetic model

Pseudo second order kinetic model

The equation for pseudo second order kinetic model is given as shown below. The parameters K2 and calculated equilibrium adsorption capacity obtained from the slope and intercept of the linear plots of t/qt versus$t$ for the data points given in the table4.11. The values determined to be rate constant (K2) = 0.012, qe = 1.316 and the regression coefficient (R^2) is = 0.9796

$$\frac{t}{qt} = \frac{1}{qe^2 K2} + \frac{t}{qe}$$

Figure 4-16 linear regression of pseudo second order kinetic model

From the results obtained above it was concluded that the regression model best fits for pseudo second order kinetic model because of the higher value of regression coefficient(R^2) for second order.

4.12. Regeneration study

The conditions in which maximum removal efficiency were 3gm of adsorbent dosage, pH of 7 and fluoride concentration of 10mg/l with removal efficiency of 98.47% were used to regenerate the exhausted chitosan powder. At these conditions as shown below in the bar graphs; the removal efficiency of the adsorbent decreased from 1st run with 93.4% efficiency to 2nd run with 87.8% and. The decrement in the removal efficiency was explained by the fact that during the regeneration process chloride ions irreversibly occupied adsorption sites thereby the available adsorption sites decreased significantly in the runs observed and from the results obtained there is a possibility to use the adsorbent up to 5 runs of adsorption experiments and minimizes the disposal of the sludge afterwards to the environment.

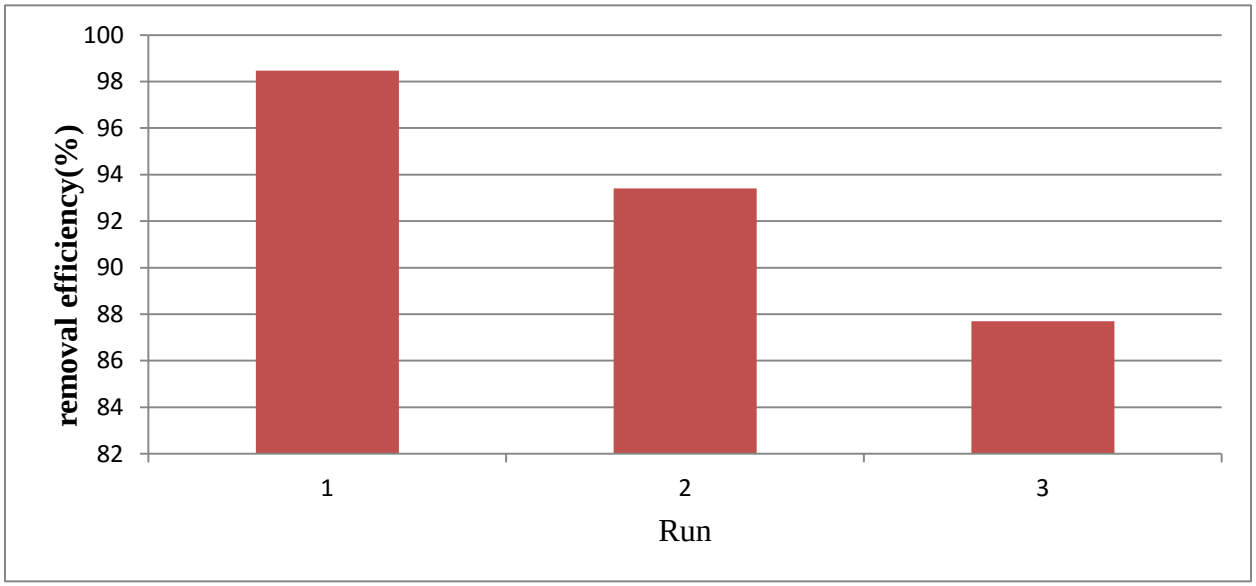

Figure 4-17 removal efficiency for regenerated chitosan

Chapter Five

5. Conclusion and recommendations

5.1. Conclusion

In this study the use of chitosan adsorbent extracted from fish scale became viable to overcome this problem related with fluoride. Chitosan has been extracted from wastes of marine sources which are cheap raw materials and used in various applications. The standard methods employed here for extraction of chitosan from fish scale were demineralization, deproteinization followed by deacetylation. The obtained adsorbent characterized for Physico-chemical analysis and the conformity final made with XRD and IR analysis. From this study on the XRD analysis the crystallinity of chitosan and fish scale were compared and the result revealed the sharp peak chitosan with slight intensity is more crystal. According to the IR analysis the patterns displayed the bands corresponding to the stretching vibrations of OH, NH_2 carbon number 2 of the glycosidic linkage and CO bonds confirmed the formation of chitosan from fish scale. The proximate analysis showed the ash content below 1% indicates the effectiveness of demineralization process and the reduction in protein content was due to deproteinization process. Ground water contamination related with fluoride is a common problem in developing country. Ethiopia as one of developing country faced with this kind of problem especially in the rift valley region which is beyond the permissible limit (1.5mg/l) set by WHO organization and hence results in skeletal fluorosis and dental fluorosis. Therefore the adsorption process is a suitable and cheap technique to remove the fluoride content in which it was obtained in excess amount. To investigate the performance of an adsorbent an aqueous solution of fluoride was prepared and batch adsorption experiment was conducted. It was found that the factors mainly affect the removal efficiency were selected. pH of the solution, adsorbent dosage and concentration of the solution affected significantly. The removal efficiency increased with through with pH and then started to decline after it was reached the equilibrium pH where maximum removal efficiency of fluoride was obtained.

The removal efficiency of fluoride ion also increased with increase in adsorbent dose; however the reverse is true for concentration of fluoride ion. The kinetic studies revealed that the data best fits for pseudo second order regression model and the adsorption process followed Freundlich

isotherm model. Finally regeneration study of chitosan was conducted using 0.1N of NaCl solution which is the most prominent desorption reagent. According to this study done and the results found chitosan adsorbent is determined to be a promising and suitable adsorbent for the removal of fluoride and protects the environment in a way that converting the waste of fish scale into valuable polymer of chitosan simultaneously.

5.2. Recommendations

- It should be further recommended to study the continuous adsorption study to generate data for kinetics and isotherm evaluation and compare with the current batch adsorption study.
- It is essential to perform BET tests for the study of surface area and pore volume of the chitosan adsorbent.
- It is crucial to study the batch kinetic studies at various temperature and agitation speed and determine thermodynamic parameter on adsorption of fluoride by chitosan polymer from fish extract.
- It is also essential to study and compare biological method of chitosan extraction with that of chemical method for the present situation.
- It is suggested to study the extraction process of chitosan from the scale at various conditions of concentration, temperature and reaction time for the steps that acquire.
- It is recommended to analyze the contributing factors of fluoride concentration in water such as total dissolved solids, alkalinity and hardness etc…

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Appendix A. Design expert data

Table A-1 Experimental versus Predicted values

Std	A: fluoride concentration(mg/l)	B: pH of solution	C: adsorbent dosage	Experimental removal efficiency(%)	Predicted removal(%)	Residual
1	10.00	5.00	1.00	94	94.04	-0.043
2	15.00	5.00	1.00	89.2	89.05	0.15
3	20.00	5.00	1.00	79.8	80.34	-0.54
4	10.00	7.00	1.00	96.5	96.58	-0.078
5	15.00	7.00	1.00	91.65	91.15	0.50
6	20.00	7.00	1.00	81.2	82.02	-0.82
7	10.00	9.00	1.00	92.75	92.33	0.42
8	15.00	9.00	1.00	86.2	86.48	-0.28
9	20.00	9.00	1.00	77.6	76.91	0.69
10	10.00	5.00	2.00	94.8	95.35	-0.55
11	15.00	5.00	2.00	92.35	91.31	1.04
12	20.00	5.00	2.00	84.92	83.56	1.36
13	10.00	7.00	2.00	97.3	97.53	-0.23
14	15.00	7.00	2.00	93	93.06	-0.061
15	20.00	7.00	2.00	84	84.89	-0.89
16	10.00	9.00	2.00	92.8	92.92	-0.12
17	15.00	9.00	2.00	86.9	88.03	-1.13
18	20.00	9.00	2.00	80	79.42	0.58
19	10.00	5.00	3.00	95.6	95.89	-0.29
20	15.00	5.00	3.00	92.7	92.81	-0.11
21	20.00	5.00	3.00	85	86.02	-1.02
22	10.00	7.00	3.00	98.47	97.71	0.76
23	15.00	7.00	3.00	94.2	94.20	-2.222E-003
24	20.00	7.00	3.00	87.8	86.99	0.81

25	10.00	9.00	3.00	92.87	92.75	0.12
26	15.00	9.00	3.00	88.7	88.81	-0.11
27	20.00	9.00	3.00	81	81.17	-0.17

Table A-2 Sequential Model Sum of Squares

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	2.153E+005	1	2.153E+005			
Linear vs Mean	809.43	3	269.81	53.78	< 0.0001	
2FI vs Linear	14.77	3	4.92	0.98	0.4226	
Quadratic vs 2FI	90.55	3	30.18	50.92	< 0.0001	Suggested
Cubic vs Quadratic	5.75	7	0.82	1.90	0.1725	Aliased
Residual	4.33	10	0.43			
Total	2.163E+005	27	8010.13			

Appendix B. variation of final concentration with contact time

Variation of final F^- concentration with contact time at 10mg/l initial concentration of fluoride at 3g of adsorbent dosage and pH of 7

Table B-1 Final Fluoride concentration for 10mg/l

Time (min)	F^- concentration	F^- Removal (%)
30	5.46	50
60	2.32	78
90	0.153	98.47
150	0.153	98.47
180	0.153	98.47
220	0.153	98.47

Variation of final F^- concentration with contact time at 15mg/l initial concentration of fluoride at 3g of adsorbent dosage and pH of 7

Table B-2 Final Fluoride concentration for 15mg/l

Time (min)	F^- concentration	F^- Removal (%)
30	9.36	39
60	5.55	63.4
90	2.805	84.6
150	0.87	94.2
180	0.87	94.2
220	0.87	94.2

Variation of final F^- concentration with contact time at 20mg/l initial concentration of fluoride at 3g of adsorbent dosage and pH of 7

Table B-3 Final Fluoride concentration for 20mg/l

Time (min)	F^- concentration	F^- Removal (%)
30	14.12	33.1
60	10.16	52.2
90	7.174	67.13
150	4.58	79
180	0.94	87.8
220	0.94	87.8

Appendix C. XRD data of scale and chitosan

Table C-1 XRD data of chitosan

XRD data of chitosan		
$2\boldsymbol{\theta}$	Peak Height	FWHM (β)
6.92	335.5	0.12
7.1	245.3	0.2
7.3	338.2	0.08
7.51	345.8	0.16
7.77	283.9	0.08
8.08	286.4	0.12
8.25	289.6	0.04
9.05	137.4	0.08
9.3	1000	0.04
9.79	128.4	0.08
10.01	188.3	0.12
10.48	170.8	0.08
11.26	886.2	0.04
11.49	135.1	0.12
14.07	150.1	0.08
14.94	109.2	0.16
16.55	125.7	0.2
20.83	161.3	0.16
25.98	231.6	0.48
26.2	202.4	0.16
29.16	910.4	0.16
31.79	400.6	0.2
31.89	413.2	0.44
32.11	518.5	0.2

32.33	409.8	0.12
32.69	309.1	0.2
39.96	68.8	0.16

Table C-2 XRD data of fish scale

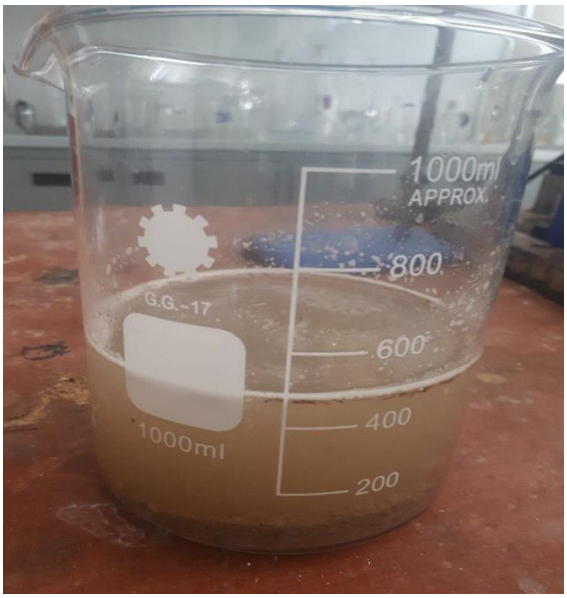
XRD data of fish scale		
	Peak	FWHM
$2\boldsymbol{\theta}$	Height	(β)
6.55	136.1	0.84
7.66	356.1	0.4
8.69	759.4	0.2
29.48	1000	0.2

Appendix D pictures of Laboratory Instruments and Process

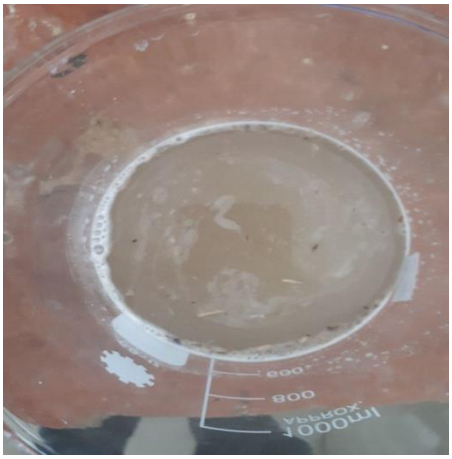
Fish scale

more powdered by mortar and pestle

Scale hammer crusher



Demineralization



Deproteinization process

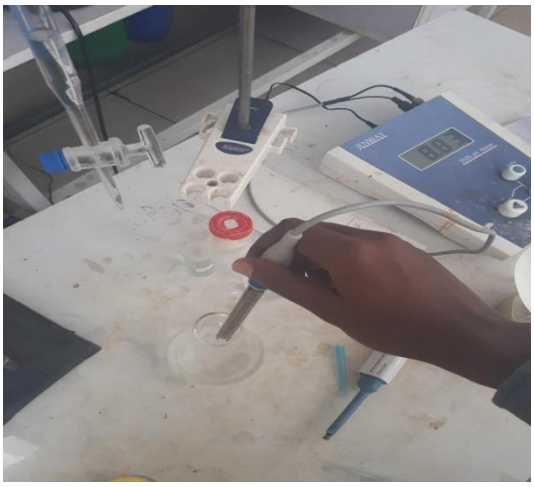
Deacetylation

Chitosan

Centrifuge

Vortex mixer

moisture analyzer



pH meter reading

Prepared solutions of fluoride