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PREPARATION AND CHARACTERIZATION OF CHITOZAN-ZEOLITE (CS/Z) COMPOSITE

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Abstract

Chitosan–zeolite composite (CS/Z) adsorbent was prepared from chitosan and zeolite 3A. The chitosan (CS) was prepared from shrimp processing waste (shell) using chemical processes. The percentage yield and degree of deacetylation were 13.64% and 87.07% respectively. The matrix formulation was gotten with Response Surface Methodology (RSM) using central composite design. The best matrix ratio of chitosan to zeolite was 1:7. The CS/Z adsorbent was characterized by x-ray diffraction (XRD) pattern and scanning electron microscopy (SEM).

Key words: Chitosan, zeolite, CS/Z adsorbent.

Introduction

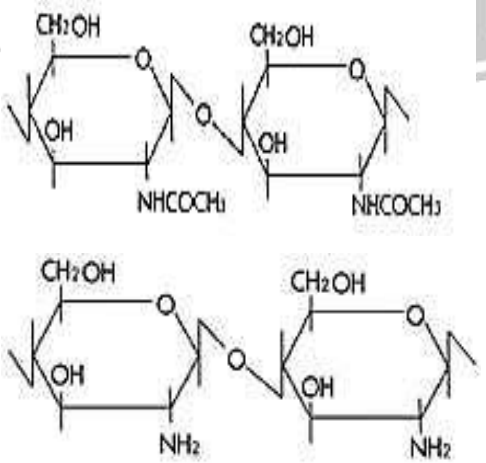
Only with the advent of bio-technology in 1859 was the process of deriving Chitosan from Chitin made possible. Chitosan was discovered in 1859 by Professor C. Rouget. Chitosan has been regarded as a source of potential bioactive material. It is the main and most important derivative of Chitin for industrial consumption, since it is soluble in acidic aqueous systems and, by extension, in human body (Gilbert, 2009). From Chitosan, many more derivatives are obtained, each with its own unique characteristics.

Chitin is the second most abundant natural biopolymer found in nature (No and Meyers, 1989). However, unlike plant fibre, chitosan possesses unique properties including the ability to form films, optical structural characteristics and much more. Generally, the shell of selected crustacean was reported by Knorr (1984) to consist of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin. Chitosan is widely available from a variety of source among which, the principal source is shellfish waste such as shrimps, crabs, and crawfish (Allan *et al*., 1979). It

also exists naturally in a few species of fungi.

Chitosan is a biomaterial which is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as shrimps and crabs) and cell walls of fungi. Chitosan is a linear-polymer of acetylamino-d-glucose. As chitosan is biodegradable, harmless for living things and ease of chemical derivation, besides, it has many amino and hydroxyl groups that can chelate heavy metal ions. Therefore, chitosan presents a very promising starting material for chelating (Karthikeyan *et al*., 2005).

Chitosan having a free amino group is the most useful derivative of chitin (No and Meyers, 1989).



(a) Chitin

(b) Chitosan

Figure 1. Structural units of Chitin (a) and Chitosan (b)

Production of Chitosan

Chitosan is manufactured commercially by a chemical method. It is extracted from crustacean shell waste such as crab, shrimp, lobster, and crawfish. The shells contain approximately 30-40% protein, 30-50% calcium carbonate, and 20-30% chitin on a dry basis (Johnson and Peniston, 1982). These portions vary with crustacean species and seasons (Green and Mattick, 1979).

Production of chitosan from shrimps shell wastes involves four traditional steps namely: Demineralization (DM), Deproteinization (DP), Decolorization (DC), and Deacetylation (DA).

However, the isolation of chitin specifically consists of only two steps: demineralization (DM) and deproteinization (DP), which involves the dissolution of calcium carbonate with 1.0 N HCl and the removal of proteins with 3% NaOH, respectively.

Alternatively, the sequence of demineralization and deproteinization steps can be reversed. In fact many authors have followed the procedure of acidic decalcification after removal of protein (Muzzarelli, 1977). Though the process

normally involves the use of dilute sodium hydroxide and dilute hydrochloric acid for deproteinization and demineralization, respectively, there have been reports indicating several variations of the characteristics of final chitosan products, but this also depends on the crustacean species from which chitin is isolated, and on the production sequence (Cho *et al*., 1998; No *et al*., 2000b; Wu and Bough, 1978).

The demineralized and deproteinized chitin has a light pink color due to the presence of astaxanthin pigment. When bleached product is desired, this pigment can be eliminated during the decolorization (DC) step. The resulting chitin is insoluble in most organic solvents; however, its deacetylated derivative chitosan is soluble in weak acids. The subsequent conversion of chitin to chitosan is generally achieved by treatment with concentrated sodium hydroxide solution (40-50%) at 100ºC or higher for 30 minutes to remove some or all of the acetyl groups from the polymer (No and Meyers, 1995).

Deproteinization

Firstly, the sources such as shrimps or crab shells are washed and grounded in to powdered form and then deproteinized by treatment with 3-5% solution of sodium hydroxide at elevated temperature (65-100^0C) to dissolve the protein present. Reaction time usually ranges from 0.5 to 12 hr depending on preparation methods

Demineralization

Demineralization is usually accomplished by extraction with dilute hydrochloric acid (up to 10%) at room temperature with agitation to dissolve calcium carbonate as calcium chloride.

$[CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 (\uparrow) + H_2O]$

1

Deacetylation

Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. Depending upon the production sequence, deacetylation can be achieved by reaction of demineralized shells or shrimps chitin with 50% NaOH (w/w) solution at 100°C for 30 min in air using a solid to solvent ratio of 1:10 (w/v) (No *et al*., 1989). The N-acetyl groups cannot be removed by acidic reagents without hydrolysis of the polysaccharide, thus, alkaline methods must be employed for N-deacetylation (Muzzarelli, 1977).

Degree of Deacetylation (DD)

Degree of deacetylation (DD) an important property in chitosan production as it affects the physicochemical properties, hence determines its appropriate applications.

Deacetylation also affects the biodegradability and immunological activity (Tolaimate *et al*., 2000). The degree of deacetylation of chitosan ranges from 56% to 99% with an average of 80%, depending on the crustacean species and the preparation methods (No and Meyers, 1995). Chitin with a degree of deacetylation of 75% or above is generally known as chitosan (Knaul *et al*., 1999). Various methods have been reported for the determination of the degree of deacetylation of chitosan. These included ninhydrin test, linear potentiometric titration, near-infrared spectroscopy, nuclear magnetic resonance spectroscopy, hydrogen bromide titrimetry, infrared spectroscopy, and first derivative UV-spectrophotometry (Khan *et al*., 2002). The degree of deacetylation depends on the raw material from which chitin was obtained and the experimental procedure, and controls the fraction of free amino groups that will be available for interactions with metals ions. When the degree of deacetylation of chitin is larger than 40–50%, chitosan becomes soluble in acidic media. The solubilization occurs by protonation of the NH_2 groups on the C_2 position of the d-glucosamine unit, although the distribution of acetyl groups along the chain may modify solubility.

The carbon/nitrogen ratio will be used in determining the degree of deacetylation of the chitosan sample using the Kasaai equation (Abdou *et al*., 2008)

$$DDA\% = \frac{6.857 - C/N}{1.7143} \quad 2$$

Definition of zeolite

Zeolite is an aluminum silicate that occurs naturally or by synthesis and it belongs to the class of mineral known as "tectosilicates". The most common of natural Zeolites are phillipsite, chabazite; clinoptilolite, analcine, erionite and mordenite among others, while for synthetic Zeolite are Zeolite A, X, Y and ZSM-5 as the most common ones. Zeolites, both natural and synthetics are used commercially because of their unique characteristics like adsorption, ion-exchange, molecular sieve and catalytic properties (Breck, 1974).

By the conventional definition, zeolites are microporous aluminosilicates. This definition, according to Ajayi (2012) has been modified within the past years and currently isomorphously substituted materials, like for example gallosilicates, titanosilicates or aluminophosphates, are also called zeolites or zeolitic materials. Microporosity (pores with diameters below 2 nm) is an intrinsic feature of all these

materials and is caused by channels and cavities within the crystal structures of zeolites. The wide application of zeolites on environmental management is based on its unique properties. Thus zeolites contain cations like Na^+, K^+, or $NH4^+$ after synthesis. The negative net–charge caused by the trivalent aluminium cations are balanced by the aforementioned cations which are coordinated tetrahedrally by oxygen ions. When solutions containing other metal cations are in contact with zeolite containing sodium ions (Zeolite 4A), the sodium ions can be exchanged with the other ion provided the ions are within the zeolite pore size (molecular sieve).

Response Surface Methodology (RSM) is a combination of mathematical and statistical techniques used to determine the optimum operational condition of the process or to determine the region that satisfies the operating specifications. In this study, RSM will be used to determine the best matrix formulation of chitosan and zeolite in terms of Chromium (VI) ion removal.

MATERIALS AND METHODS

Synthesis and characterization of Chitosan-Zeolite Composite

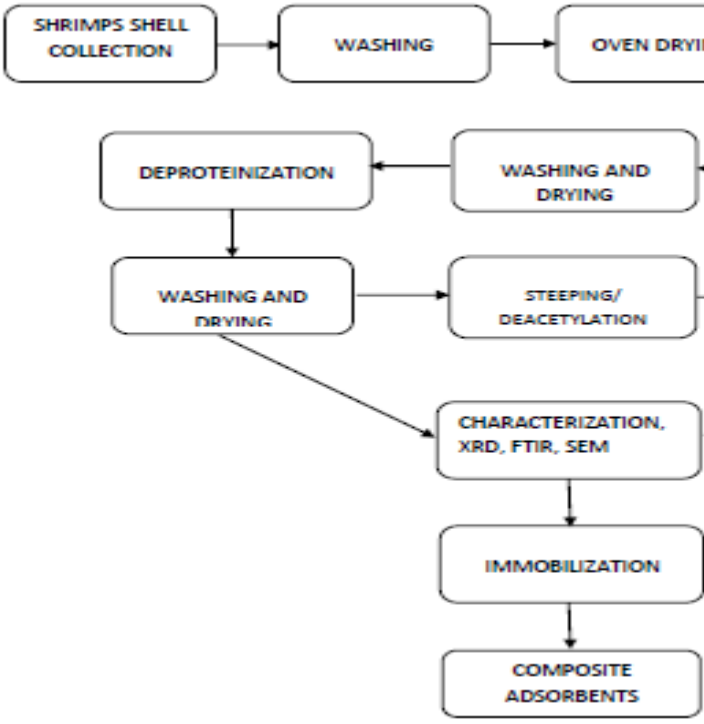


Figure 2: Block representation of the production of chitosan

Design of Experiments

The Response Surface Methodology (RSM) was applied using Design Expert 7.0.0 program design. The experimental design as a function of the selected process variables was carried out using central composite design (CCD). In order to obtain the required data, the range of values of each variable (mass of chitosan and zeolite) was determined. The mass of chitosan and zeolite were chosen for the independent variables. For variables (n = 2) and two levels (low (-) and high (+)), the total number of experiments was 13 determined by the expression: 2^n +2n + 5. The

percentage removal was selected as the response for the combination of the independent variables. Experimental runs were randomized to minimize the effect of unexpected variability in the observed responses.

The results were analyzed by applying the response plot. For RSM, the most commonly used second –order polynomial equation developed to fit the experimental data can be written as:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x^2 + \sum \sum \beta_{ij} x_{ij} x_j + \varepsilon \quad 3$$

Where Y is the response (yield); β_0 is the intercept coefficient, β_i is the linear terms, β_{ii} is the squared terms and β_{ij} is the interaction terms and x_i and x_j are the uncoded independent variable (Ghadge *et al*.,2006).

Table 1. CCD for two independent variables used in this study

Run /Order	Chitosan (g)	Zeolite 3A (g)	Ratio
1	0.55	8.00	1:15
2	0.55	8.00	1:15
3	0.20	8.00	1:40
4	0.80	11.0	1:14
5	0.30	5.00	1:17
6	0.55	8.00	1:15
7	0.55	8.00	1:15
8	0.55	8.00	1:15
9	0.55	12.24	1:22
10	0.55	3.76	1:7
11	0.90	8.00	1:9
12	0.80	5.00	1:6
13	0.30	11.0	1:37

Preparation of Chitosan Immobilized on Zeolite (CS/Z)

The synthesized chitosan with different masses from Table 1 were dissolved in 30 mL of 5% (v/v) HCl in different beakers respectively, stirred for 2h at 200 rpm. Different masses of zeolite from Table 3.4 were added to the corresponding solution as presented in the table. Chitosan is precipitated onto zeolite by the drop wise addition of 1N NaOH until neutralization occurs. The beads formed was washed with distilled water and dried in the oven at 85^0C for 18 h and sieved. In this study the best matrix formulation of the composite in terms of % removal was determined.

Characterization of Samples

Functional Group Analysis

The chitosan was analysed by FT-IR in the wavelength between 4000cm^{-1} and 400cm^{-1} and in solid state using KBr pellet method. The FT-IR spectra were normalized and major vibration bands were identified associated with the main groups

XRD Analysis

Room temperature low angle X-ray diffraction (XRD) pattern of the of the samples of chitosan and zeolites were studied using X-ray powder diffractometer using a Ni – filtered Cu Kα X – ray radiation source. 1 g of each samples were air dried properly, homogenized, and placed on the sample holder of the machine for scanning. The average bulk compositions were determined.

The samples were analyzed using the reflection-transmission spinner stage with the Theta-Theta settings. Two-Theta starting position was 0.00483 and ends at 75.96483 with a two-theta step of 0.026 at 3.57 seconds per step. Tube current was 40 mA and the tension was 45 VA. Fixed Divergent Slit size of 1^0 was used and the goniometer radius was 240 mm.

The intensity of diffracted X-rays was continuously noted as the sample and detector rotate through their respective angles.

Scanning Electron Microscopy (SEM)

Electron microscope Philip XL40 was used to carry out this experiment and it was carried out under a vacuum condition and at 5 bar pressure. The essence of utilizing SEM is to determine morphology of the sample. The samples were mounted over sample holder (stubs) aided by double sided tape. Bio Rad coating systems were to further coat the sample with gold and this was carried out at 10^{-1} mbar with 30mA of current flow or 75 ^{o}. The samples were then placed into SEM instrument for scanning. Tungsten filament was utilized as electron source and SEM micrograph was recorded with 10 Kv revolution to obtain 1000 X, and 2000 X enlargement.

Result and Discussion

Chitosan Yields

The percentage yield of chitosan was determined by taking the dry weight of shrimp shells before treatment and the dry weight of prepared chitosan in percentage. The percentage yield of chitosan was calculated from the weight of chitosan obtained as a percentage of the shrimp before deacetylation.

The prepared chitosan had a percentage yield of 13.64 %, which was compared to the percentage yield obtained by Brzeski (1982) who reported 14% yield of chitosan. Huthman (2013) reported

Characterization

Solubility Test for chitosan

In common, it is justified that main physical differences between chitin and chitosan is the ability of chitosan to be soluble in organic acid such as acetic acid. Chitosan

with higher content of protonated amino group are readily to form well-ordered arrangement in Van der Waals force and hydrogen bond which exceed its tendency for intramolecular chemical bonds (Zhang *et al*., 2012, He, Chen and Dong, 2001).

The prepared chitosan was found to be soluble in 0.1M of acetic acid and hydrochloric acid respectively and insoluble in water.

Properties of Produced Adsorbents

Table 4.4 and 4.5 present the properties of produced adsorbents

Table 4.4: Properties of produced chitosan

Properties	Values
Colour	White
Buoyancy in Oil/water	Floats
Surface	Smooth
Sorption	Oleophilic and hydrophobic
Solubility in water (%)	Insoluble
Solubility in Acetic acid (%)	100
Viscosity (cPs)	5
Molecular wt. (g/mol)	1.559×10^5
Surface Area (m^2/g)	269.4
*p*H	7.2
Particle size (nm)	3.57
DDA (%)	85.70

X- ray Diffactometry Analysis (XRD) of chitosan, zeolite and CS/Z

The X-ray diffractogram of prepared chitosan, zeolite and CS/Z are illustrated below. Figure 3 shows the x-ray spectrum of prepared chitosan. The XRD analysis indicated that chitosan have crystalline and amorphous region, which confirmed the semi-crystallinity of chitosan. The XRD pattern exhibited strong diffraction peaks in the scattering range (2θ) at 18.3^0 and 24^0, also a weak peak at 10^0. This is comparable with the pattern of shrimp chitosan with peaks at 10° and 21° 2θ (Islam *et al*., 2011) and peak at, 9.60^0, 19.52^0 and 21.12^0 (Rumengan *et al.*, 2014).

In figure 4, the X-ray diffraction studies of zeolite 3A presents high crystallinity. The plot shows all the characteristic peaks matching with X-ray diffraction pattern of Zeolite 3A by Rondon *et al*., 2001. This means that the purchased commercial Zeolite 3A from Lemandou Chemicals, China was well refined. The X-ray diffraction analysis indicates the degree of crystallinity of the commercial Zeolites 3A sample.

Figure 5 shows the X-ray diffraction pattern of Zeolite 3A by Rondon *et al*., 2001, which is used as standard. Figure 4.1 and 4.2 confirmed the semi-crystallinity of chitosan and the crystallinity of zeolite.

Figure 6 shows the X-ray diffractogram of chitosan-zeolite composite (CS/Z). The introduction of zeolite 3A particles into matrices increased the crystallinity of the chitosan and the thermal stability, although it decreases the flexibility. The X-ray diffractogram of CS/Z sample showed some characteristic peaks of both components (zeolite and chitosan) and the differences may be justified by the incorporation of zeolite crystals in the chitosan matrices

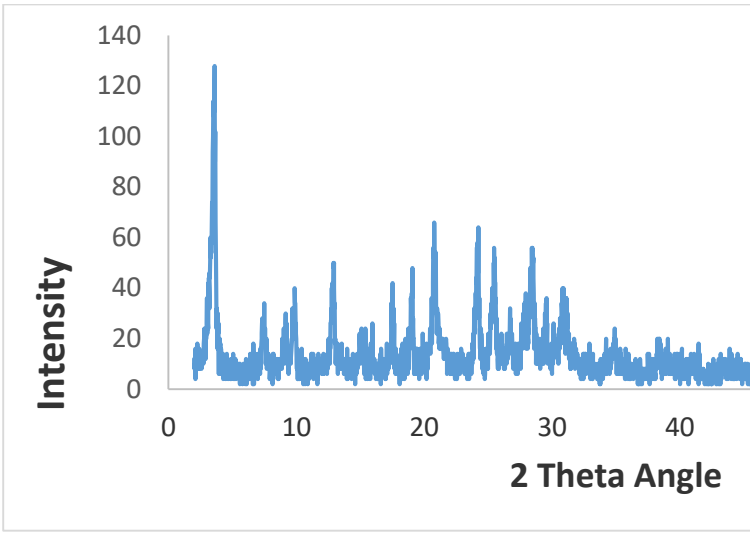


Figure 4: XRD diffractogram of Zeolite 3A

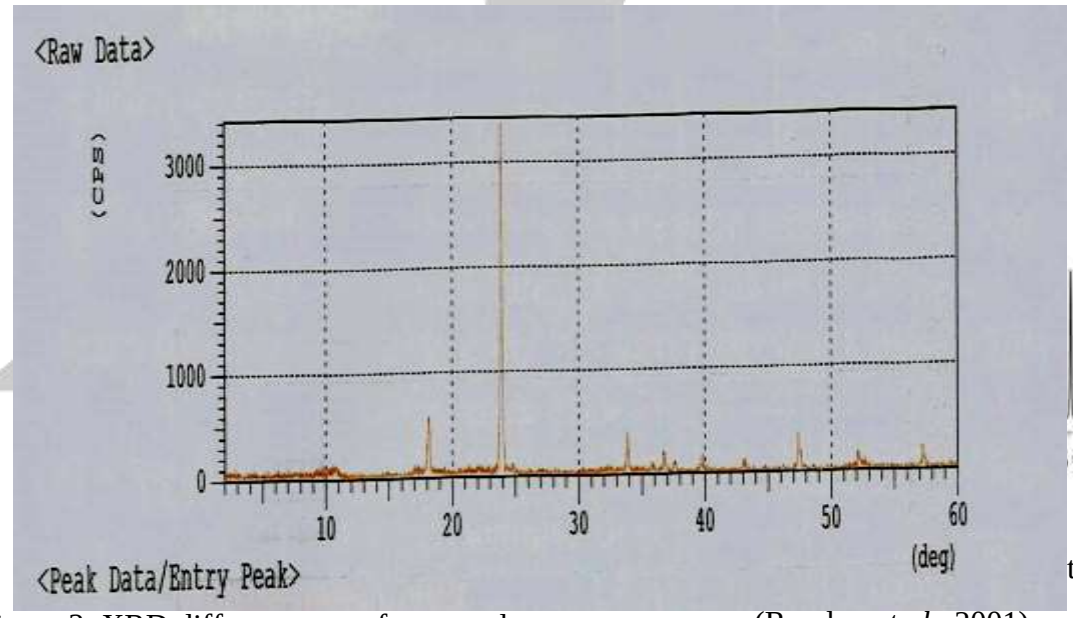


Figure 3: XRD diffractogram of prepared chitosan

te 3A

(Rondon *et al*., 2001)

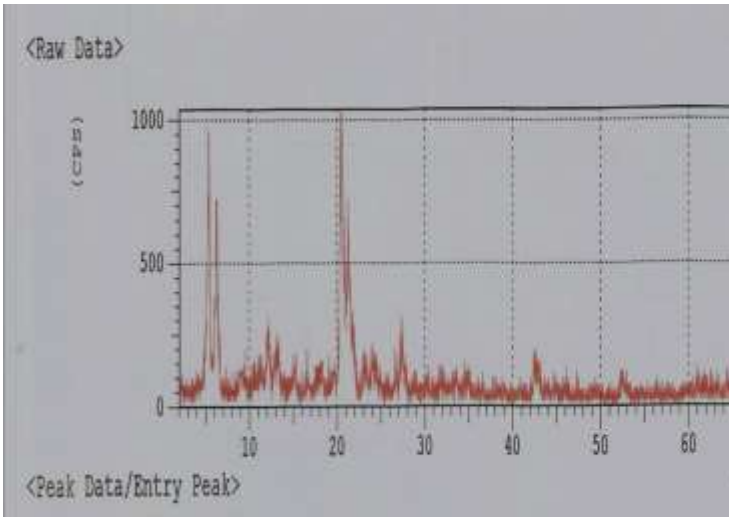


Figure 6. XRD diffractogram of prepare chitosan-zeolite composite (Best matrix)

SEM Analysis

Figure 7a present the morphology of the surface of the synthesized chitosan. Micrograph of synthesized chitosan has rough close-fitting surface morphology with minimum residues Isa *et al.*, (2013). Chitosan showed non homogenous and non-smooth surface with straps and shrinkage

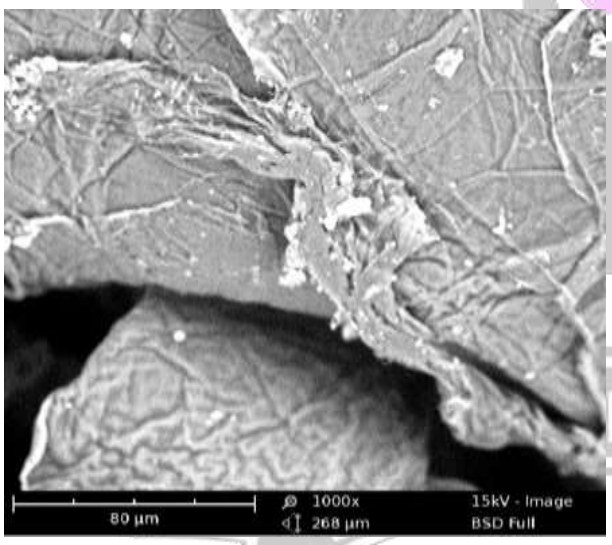


Figure 7a: SEM image of prepared chitosan

The SEM micrograph (figure 7 b and c) of the CS/Z after adsorption of Cr (VI) ion shows a darker area, compared to before, which indicates the presence of Cr(VI) ion.

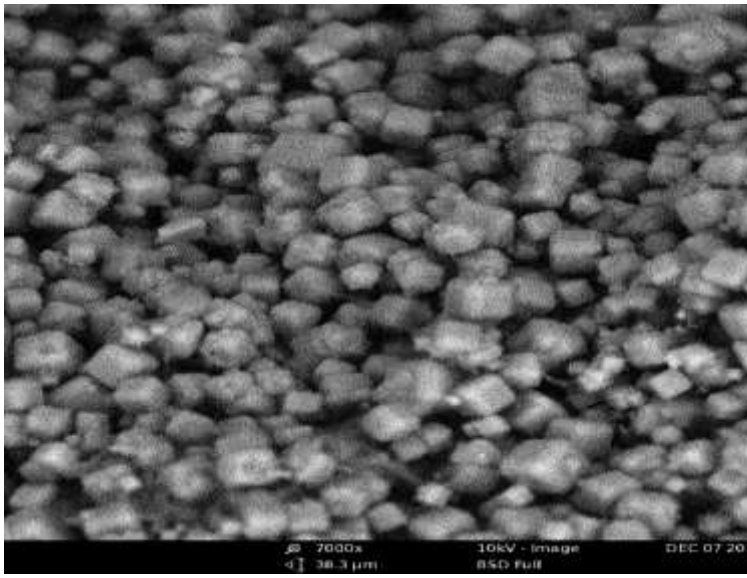


Figure 7b: SEM image of CS/Z before adsorption

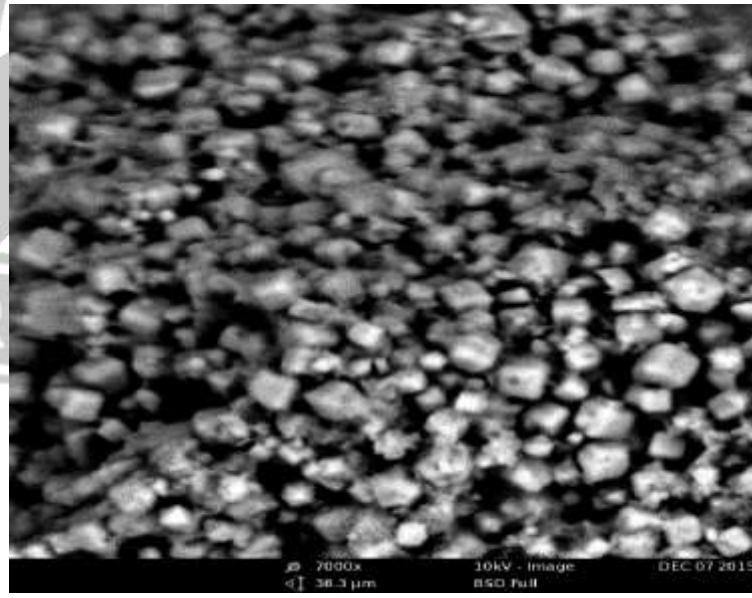


Figure 7c: SEM image of CS/Z after adsorption

FT-IR Analysis

Figure 8 present the FT-IR of synthesized chitosan

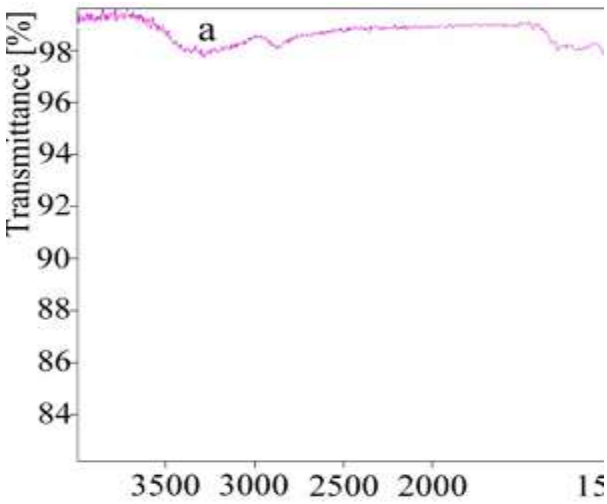


Figure 8: Fourier Transform Infra-red of Chitosan synthesized.

The expected functional group in chitosan, a derivative of chitin after deacetylation the free amino, the primary and secondary hydroxyl and the ketones groups which have the band width peak values. The wide band of –NH2 at 3442.76 cm–1 corresponded to N-H stretching vibrations of free amino groups. Likewise, the band observed at 2950.29 cm–1 corresponded to CH stretching vibrations. The band at 1050.50 cm–1 is as a result of stretching primary hydroxyl groups of tertiary O-H stretching. The band at 1630.70 cm–1 corresponded C=O stretch of the carbonyl group, a structural feature of chitosan and the occurrence of deacetylation. This characteristic feature of carbonyl group overlap is not alone sufficient to determine the extent of deacetylation because there are ranges in frequency of carbonyl compound due to difference in classes which may be primary, secondary or tertiary group

Conclusions

The study observation indicate that chitosan has been successfully prepared from shrimps with a yield of 13.64 percent, semi crystallized structure and 64 % degree of deacetylation. The composite adsorbent (CS/Z) was successfully synthesized and characterized by using XRD, FT-IR and SEM analysis. The best matrix formulation ratio of chitosan to zeolite 3A was 1:7.

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