

Comparative Studies of the Effect of Inorganic and Organic Acid Treatment on Chitosan Property from Shrimp Shells

O. A Fatoye¹ A. Jimoh² and A. Olugbenga³

Research Scholar ¹, Professor ², Professor³

Department of Chemical Engineering

Federal University of Technology

Minna, Niger State

Nigeria

ABSTRACT

Hydrochloric acid has been the conventional acid of use in shrimp shell demineralization for chitosan synthesis. This inorganic acid was compared to acetic acid; an organic acid in the demineralization stage. Deproteinization and deacetylation stages were carried out using a sodium hydroxide solution to produce chitosan. Characterization using Fourier Transform Infrared Spectroscopy (FTIR) showed the structure and functional groups of the synthesized chitosans, while X-Ray Diffractometry (XRD) and Scanning Electron Microscopy (SEM) were used to investigate the crystallinity and structural differences respectively. From the analysis, chitosan property was compared at the molecular weight, viscosity, solubility and Degree of Deacetylation (DD) (85% when acetic acid was used, and 87.06% when HCl was used). Experimental analysis indicated that acid type did not significantly affect properties of synthesized chitosan.

Key Words: Acetic acid, Chitosan, Demineralization, Degree of Deacetylation.

1. INTRODUCTION

Biodegradable Chitin is the second most abundant natural biopolymer after cellulose [1]. It is a white, hard, inelastic and nitrogenous polysaccharide found in the exoskeleton of crustaceans, such as crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton, including coral and jellyfishes [2].

However, more important than chitin is chitosan, which has a molecular structure similar to cellulose. Presently, chitosan is attracting an increasing amount of research interest in the process industries because of its varied applications in food, agriculture, biomedicine, textile, water treatment and pharmaceuticals [3].

Chitosan is obtained by alkaline deacetylation of chitin giving amino group. It is usually found as polymer chains containing a unique monomer (N acetyl- D-glucosamine or D-glucosamine) [4]. Chitosan and its derivatives are typically obtained from demineralization, deproteinization and deacetylation processes respectively.

In traditional chemical methods of demineralization, the removal of minerals; primarily calcium carbonate with the usage of acids is carried out with the application of dilute hydrochloric acid (HCl) as the conventional method [5]. Among such methods are those of [6].

Exceptions to the above are seen in the methods of [7] and [8] where demineralization was accomplished with 90% formic acid and 22% HCl, respectively, at room temperature.

Most of the conventional methods included drastic treatments that caused modifications, such as depolymerization and deacetylation of native chitin [9].

This process may not be considered as a good chitosan synthesis alternative, since it is expensive and non-environmentally friendly [5].

In order to overcome this problem, other methods have been developed using mild acids to minimize degradation. [10] used ethylene-diaminetetracetic acid (EDTA), [11] applied acetic acid while [12] studied a sulfurous acid process.

[13] reported that the effectiveness of using lactic acid for demineralization of shrimp shells was comparably better than that of using hydrochloric acid since organic acids were less harmful to the environment and they best preserved the characteristics of the purified chitin and by extension chitosan. Also, since organic acids were produced from low cost biomass such as citrus fruits, their resultant organic salts from the demineralization process served effectively as food preservatives and environmentally friendly anti-icing agents.

This research work was aimed at comparing the properties of chitosan prepared after demineralization with acetic acid; an organic acids with that of hydrochloric acid an inorganic acid.

1.2 Statement of The Problem

Chitosan synthesis using conventional harsh inorganic acids such as hydrochloric acid has in spite of its effectiveness posed limitations on the extent to which the polymer can be used in various industrial applications because of the drastic modifications that occur due to the harsh nature of such acids [13]. Hence, the need to investigate the properties of chitosan obtained from chitin and the modifications that occur to these properties when subjected to alternative organic acid treatments, in order for better industrial applications.

1.3 Aim and Objectives

The aim of this research work is to investigate and compare the effectiveness of inorganic acid (HCl) and organic acids (acetic acid) on the properties of chitosan in heavy metal ion removal from waste water.

The activities that will be carried out in pursuance of this aim will include:

1. Production of chitosan from chitin using acetic and hydrochloric acids demineralization treatments respectively.
2. Characterization of the synthesized chitosan particles using various analysis including; X-ray diffraction (XRD), Scanning Electron Microscope (SEM) and Fourier transformation infrared (FT-IR).
3. Determination and comparison of the properties of the organic and inorganically synthesized chitosan including molecular weight, degree of deacetylation, solubility and viscosity.

2. METHOD AND PROCEDURES

2.1 Preparation of Shrimp Shells

Shrimp was obtained from Lagos market and the exoskeleton was manually removed, dried, and ground to pass through a 300 μ m sieve. Acetic acid, sodium hydroxide pellets and hydrochloric acid (AnalaR, BDH) were procured and the two acids were prepared into a 1.0M concentrations.

2.2 Demineralization

100ml of 1.0M acetic and hydrochloric acid solutions were separately introduced into a conical flasks (250ml) and stirred continuously using a magnetic stirrer at ambient temperature for 180 minutes respectively after which the content of the flasks were quickly filtered and washed to neutrality with distilled water to attain neutral pH. The demineralized samples were dried in an oven at 80°C to constant weight.

2.3 Deproteinization

The resulting samples after demineralization were weighed and treated in 3% NaOH at a solute to solvent ratio of 1:10 (w/v) at a temperature of 100°C for 1 hour to dissolve the proteins and sugars thus isolating the crude chitin. Following the deproteinization process, the resulting solutions were filtered and the residues washed thoroughly with distil water until neutrality. The samples were then oven dried at a temperature of 60°C to constant weight to obtain chitin powder. The chitin contents were determined from the weight differences of the pre-treated shrimp shell powder and that obtained after acidic and alkaline treatments.

2.3 Deacetylation of Chitin

Deacetylation of chitin with NaOH to obtain chitosan was carried out by mixing 65% NaOH, at a heating time of 180 minutes and reaction temperatures 90°C respectively. The synthesized chitin samples for each acid treatment were treated with NaOH solution at a ratio of 1:10w/v. After the specified times of reflux, the mixtures were cooled at room temperature. Afterwards the samples were washed with distilled water till the filtrates all reached neutrality. The samples were oven dried at 80°C to obtain a white powdery chitosan.

2.3 Physicochemical Properties of Chitosan

2.3.1 Solubility

0.1g of dried chitosan samples obtained from both acetic and hydrochloric acids demineralization were weighed and dissolved in 10ml of 1% acetic acid followed by stirring and then filtration.

2.3.2 Molecular Weight Determination

The molecular weights of the various samples were determined using the Mark-Houwink equation:

$$[\eta]=KMW^a \quad \text{----- (1)}$$

$$\eta = \frac{\eta-\eta_0}{\eta_0 c} \quad \text{----- (2)}$$

Where MW is the viscosity average molecular weight for the given polymer; K and a are constants, that depend on the polymer type and the chosen solvent. In addition, $[\eta]$ is intrinsic viscosity and $[C]$ is the concentration.

2.3.4 Degree of Deacetylation

The degree of deacetylation of the various chitosan samples obtained was determined using the relationship [14]

$$DD = 118.883 - [40.1647 \times (A_{1655}/A_{3450})] \quad \text{----- (3)}$$

Where A_{1655} and A_{3450} are absorbance bands at 1655 and 3450 from the FTIR spectra.

2.4 Structural Analysis

The structural differences in the synthesized chitosan samples in terms of its functional group, crystallinity and morphology were analyzed using the Fourier Transform Infrared Spectroscopy (FTIR – 8400S spectrophotometer, shimadzu) analysis, Scanning Electron Microscope (SEM – JEOL JSM – 630 J) and X-Ray Diffraction (XRD – X’Pert PRO MRD XL) analysis.

3.0 RESULTS AND DISCUSSION

The composition of fresh shrimp shells from the proximate analysis carried out were shown in table 1.0 respectively.

Table 1.0: Proximate Analysis of Fresh Shrimp shells

Parameters	Composition (%)
Ash	34.8
Fat	0.9
Fibre	1.28
Moisture	8.7
Protein	42.1

The protein content in the shrimp biowaste was found to be the main component similar to several studies including [15]. The quality of chitin and chitosan produced from crustacean shells is partially dependent on the type of raw material used. As observed in this study, the fat and fibre values were generally low as compared to previous studies by [16] and [17] possibly due to the small size of the shrimp samples used and the preparation method of the shell wastes in which all adhering meat was removed and washed. Ash content which is another major component accounts for 34.8% similar to the results of [18]. Furthermore, proximate composition of shrimps, crustaceans and other aquatic organisms has been found to vary with seasonal factors, climatic factors, habitat, and developmental stages [19].

3.1 Solubility Test

Chitosan is a semi-crystalline biopolymer; because of its rigid crystalline structure it is not soluble in most of the solvents like water, alkali or aqueous solution and common organic solvents. At certain pH values under continued stirring, chitosan is soluble in few acids such as hydrochloric, lactic, propionic, tartaric, citric, acetic and formic acids [20]. [11] have stated that lower solubility values suggest incomplete removal of protein. In this present work, chitosan produced from both lactic and hydrochloric acid treatment have been found to be soluble in 1% acetic acid solution and insoluble in water indicating that solubility is not subject to acid type.

Table 2.0 show the properties of synthesized chitosan which include viscosity, molecular weight and Degree of Deacetylation (DD) with acetic and hydrochloric acids demineralization synthesis respectively.

Table 2.0: Properties of Chitosan for Different Acid Treatments

Acid Type	Viscosity(cPs)	Molecular weight (g/mol)	Degree of Deacetylation (%)
Hydrochloric acid	6.7	2.04x10 ⁵	87.06
Acetic Acid	5.1	2.34x10 ⁵	86.02

The properties of chitosan based on various acid type treatments were presented in table 2.0 above. These factors significantly affect the physicochemical behaviour and biological functionality of chitosan, particularly the Degree of Deacetylation. The viscometer was used in the measurement of viscosity with ranges between 5.0–7.0. The differences in viscosities were not

significantly different indicating that acid type was not a varied factor in viscosity determinations. [21] reported that viscosity of chitosan varied considerably depending on the species and the preparation path used.

Using Mark-Houwink equation, the molecular weight of the samples were calculated within the range shown above which were within the values reported by [22]. The importance of the molecular weight is that of its effect on the thermal stability of the end polymer. However, factors including high temperature, reaction time, particle size, dissolved oxygen concentration, shear stress and concentration of alkali may influence the molecular weight of the chitosan.

3.2 Degree of Deacetylation (DD)

The Degree of Deacetylation is an important parameter affecting solubility, chemical reactivity, and biodegradability. Depending on the source and preparation procedure, DD may range from 30% to 95 % [23]. It is rare however to have the production of chitosan with 100% degree of deacetylation. Therefore, commercial chitosan with various degree of deacetylation in the range of 75–85% is commonly found.

The degree of deacetylation (DD) was calculated using the Rout equation. The values obtained were 86.02% when acetic acid was used while that of HCl was 87.06%.

Several studies show that chitosan DD affects both hydrolytic and thermal behaviour of the polymer products [24]. It was found that the more extensive de-*N*-acetylated chitosan sample, the slower the rate of acidic hydrolysis observed during storage [25]. Also, chitosan with higher DD has a less porous structure and lower water-uptake ability, which limits the rate of the degradation process in acidic environment. On the contrary, a slower rate of chitosan thermal depolymerization may be a result of interchain crosslinking between free amino groups, which exerts a stabilizing effect on the polymer's structure [24].

3.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR was used to probe the surface characteristics of the synthesized chitosans. This is the determination of the functional groups and the corresponding specific transmittance peak in each of the synthesized chitosan.

Figures 1.0 and 2.0 represent the FTIR spectra for the chitosan samples of lactic and hydrochloric acid respectively.

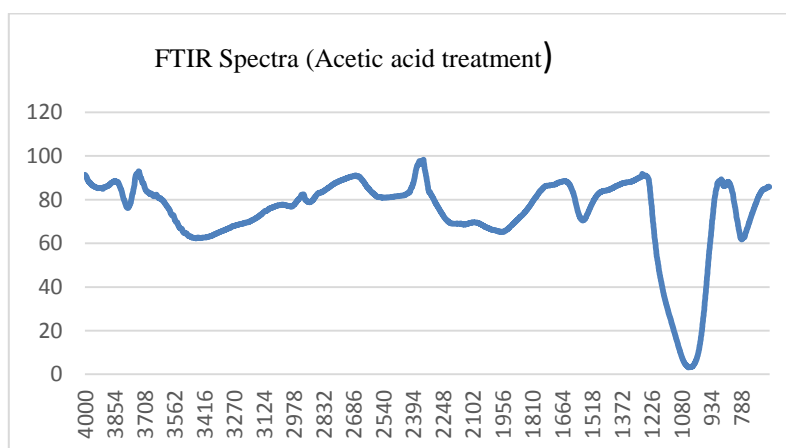


Fig 1.0: FTIR spectra of extracted chitosan

The FTIR spectra of synthesized chitosan presented show that the bands of the chitosan samples produced using both acetic acid and HCl demineralization are typical and follow the trend expected of chitosan.

Generally chitosan shows bands at 3000-3500 cm^{-1} that corresponds to O-H stretching vibrations of hydrolysis and N-H stretching vibrations of free amino groups and band at 1400-1650 cm^{-1} is attributed to the C=O stretching (amide I) of O=C-NHR as reported

by [26]. The research similarly reported unique chitosan characteristic peaks around 1363cm^{-1} attributing to the CH_3 bending vibration and 1645cm^{-1} assigned to the amide group.

The spectra indicated the presence of absorption band observed between 1220 and 1020 cm^{-1} , which represents the free amino group ($-\text{NH}_2$) at C2 position of glucosamine, a major group present in chitosan.

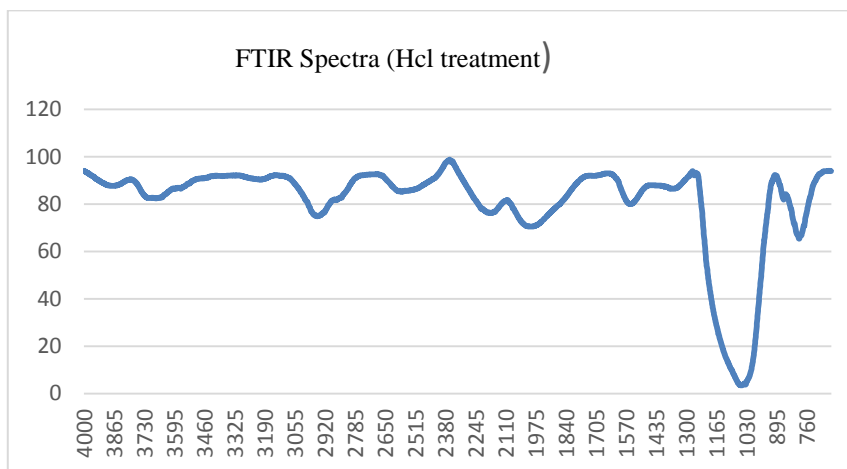


Fig 2.0: FTIR spectra of extracted chitosan

3.4 X-Ray Diffraction Analysis

The X-Ray Diffraction (XRD) patterns of optimally synthesized chitosan in both yield and quality are illustrated in Figures 3.0 and Fig 4.0.

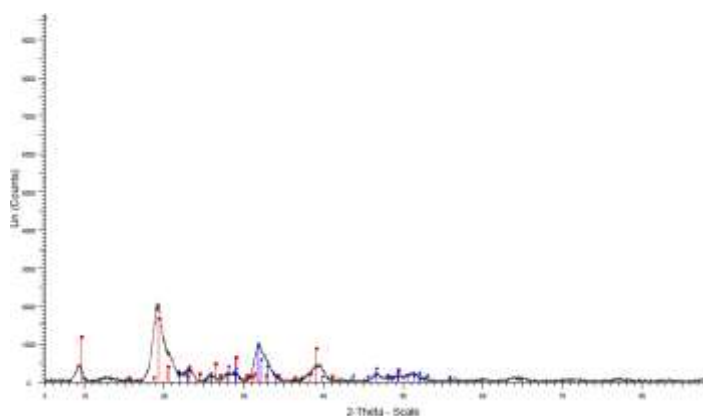


Figure 3.0: XRD Diffractogram of prepared chitosan after 1.0M HCl acid concentration

The XRD pattern of chitosan exhibits broad diffraction peaks at (2θ) at 18.10 and 24.0 which are typical fingerprints of semi-crystalline chitosan. [27] found that fungal chitosan showed two crystalline reflections at 9.7° and 19.9° . It has been reported that the XRD patterns of shrimp chitosan showed two major characteristic peaks at $2\theta = 9.9-10.7^\circ$ and $19.8-20.7^\circ$ [28].

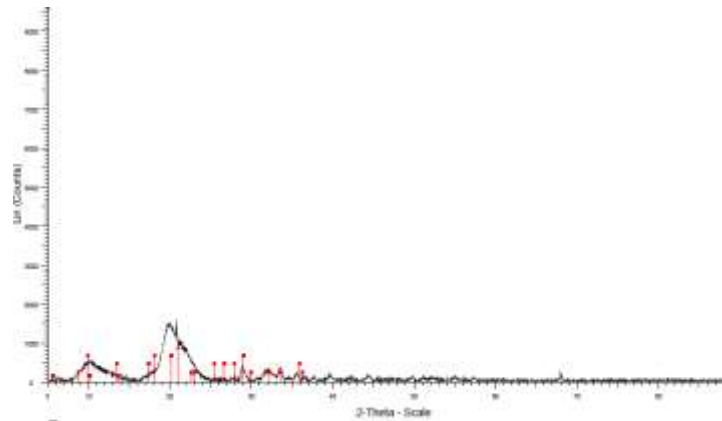


Figure 4.0: XRD Diffractogram of prepared chitosan using 1.0M Acetic acid concentration

It is also reported that the two characteristic crystalline peaks with slightly fluctuated diffraction angles found in the XRD patterns indicated that two types of α - and γ -chitosan exhibited comparable degree of crystallinity and had two consistent peaks of 9-10° and 19-20°.

The XRD analysis indicated that the chitosans have crystalline and amorphous regions, which confirmed the semi-crystallinity of the synthesized chitosans.

3.5 Scanning Electron Microscopy (SEM) Analysis

Figures 5.0 and 6.0 present the SEM micrographs illustrating the surface morphology of chitosan synthesized using acetic acid and hydrochloric acid demineralization respectively.

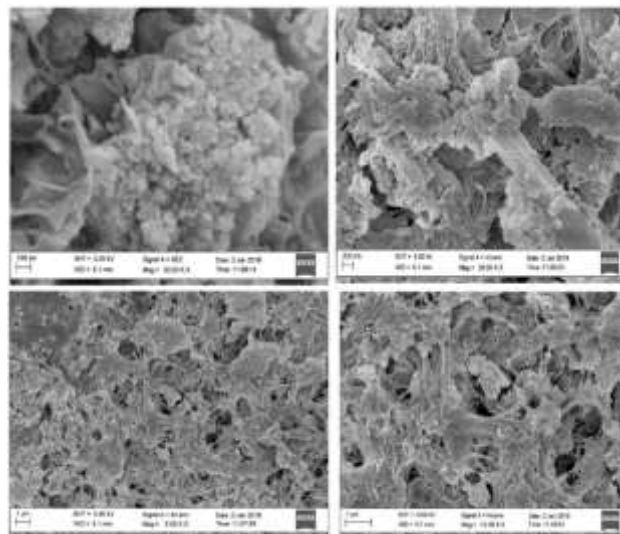


Figure 5.0: The SEM image of chitosan using 1.0M Acetic acid for demineralization

Under the electron microscopic examination, both chitosans showed a non-homogenous and unsmooth surface with straps and shrinkages which correspond to the SEM image of standard extracted chitosan [29].

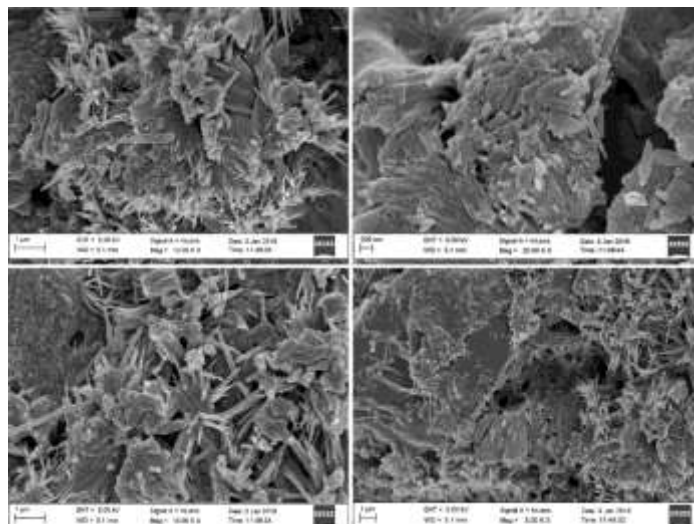


Figure 6.0: The SEM image of chitosan using 1.0M Hydrochloric acid for demineralization.

4.0 CONCLUSION

The research observations indicated that chitosan was successfully synthesized from shrimp exoskeleton after demineralization using acetic and hydrochloric acids respectively. The properties of the synthesized chitosan were found to be independent of acid type as yields of 15.40% and 16.42% were obtained respectively for acetic and hydrochloric acid synthesis. FTIR, XRD and SEM analysis were carried out and the properties of the synthesized chitosans were found to be independent of acid type as indicated by the individual analysis. Hence, acids of organic origins like acetic acid are friendlier to the environment and thus can replace acids of inorganic origins such as HCl in chitosan synthesis.

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