# Investigation of Biomaterial Characteristics of Chitosan Produced from Crab Shells

**Esther O Babatunde**
University of Ilorin Faculty of Engineering and Technology
**Joshua O Ighalo**
University of Ilorin Faculty of Engineering and Technology
**Sunday A Akolo**
Federal University of Technology Minna School of Engineering and Engineering Technology
**Adewale George Adeniyi** ( adeniyi.ag@unilorin.edu.ng )
University of Ilorin https://orcid.org/0000-0001-6615-5361
**Lydia Adepoju**
University of Ilorin Faculty of Engineering and Technology

**Research**

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# Abstract

Chitosan is a biomaterial that can be obtained from certain parts of aquatic fauna like scales and shells. They are cheap, readily available and environment-friendly complexing agents for heavy metals. In this study, crab shell was used as a source of chitosan and compared with commercial chitosan. The yield was 22.75% and 71% degree of de-acetylation. Solubility test showed that it will dissolve within 30 minutes in 0.1 M HCl. The FTIR indicated the presence of –OH functional group at wavelength 1350 $cm^{-1}$ and R-$NH_2$ at 3450 $cm^{-1}$. SEM revealed that the locally developed chitosan has a rough surface characterized with holes, and has a porous spongy structure. Electron Dispersive Spectroscopy (EDS) was used to examine the presence of elements on the chitosan. Results showed the presence of C, N, O and Na. Usually, Hydrogen is usually present in organic materials but EDS cannot detect its' presence. XRD revealed a low crystallinity of the chitosan obtained.


# 1. Introduction

Chitosan has been regarded as a source of potential bioactive material, but it also has several limitations to be utilized in biological system, including its poor solubility under physiological conditions. Therefore, to overcome these limitations, researchers focused on the derivatisation of chitosan by chemical modifications and partially hydrolysed chitosan by enzymatic actions as it contains various reactive functional groups[1–5]. The main factors which may affect the chitosan properties are its molecular weight and degree of de-acetylation (DD)[6]. These factors enable the researcher to formulate different grades of chitosan which differ primarily in molecular weight and degree of de-acetylation.

Chitosan is the de-acetylated form of chitin, which is a linearpolymer of acetyl amino-d-glucose. Chitosan (poly-1,4-D-glucosamine), which is another polysaccharide biopolymer derived from chitin, also has a high affinity for transition metal ions in a way that the amine groups on chitosan serve as a chelation site for the metal ions [7, 8]. Chitosan is biodegradable, non-toxic and easily of derivatised, and has many amino and hydroxyl groups that can chelate heavy metal ions[4, 6, 9–12]. Chitosan are less expensive, easily available and environment-friendly complexing agents for heavy metals. It has been reported as a heavy metal adsorbent in several studies [13–21].

Chitosan can be obtained from wastes of marine fauna such as scales [4, 10, 11, 14, 22–24] and shells [25, 26]. In this paper, we studied the preparation of chitosan from crab shells and compared the characteristics with those of commercial chitosan.

# 2. Materials And Methods

# 2.1 Collection of Sample

Crab shell was obtained from Lagos lagoon (Nigeria). The crab was washed with tap water to remove possible foreign materials present (dirt and sands). The commercial chitosan was from Sigma-Aldrich in

Germany (purity 99%).

## 2.2 Production of Chitosan

Isolation of chitosan from crab shell wastes involves four traditional steps; de-proteinisation (DP), de-mineralization (DM), de-colourization (DC), and de-acetylation (DA). The wet crab was washed and dried followed by grinding and sieving to a particle size of 750 µm, and then placed in a plastic bottle for storage at ambient temperature until used. De-proteinisation (DP) was then carried out on the crab shells. Four hundred grams of crab shell was place in a solution of 3.5% NaOH (w/v) for 2 h at 65 °C, solid: solvent (1:10, w/v), then the solid was separated from the liquid and washed with distilled water until absence of colour in the medium which represents the absence of protein. The next step was to demineralise the shells. The deproteinised shell was placed in 1 N HCl for 30 minutes at room temperature, solid: solvent (1:15, w/v). Subsequently, the liquid was decanted and the solid was washed with distilled water until neutral pH, the remaining was dried at 50 °C for 12 hr and the product was chitin.The chitin was decolourized with 0.315% sodium hypochlorite (NaOCl) (w/v) for 5 minutes at room temperature solid: solvent (1:10, w/v) was poured into the vessel containing the solid and the suspension was agitated until the pigmentation of the solid disappeared. The white solid (chitin) was washed and dried at 50 °C for 12 hr in the oven.The de-acetylation of chitin was carried out by mixing chitin with 50% NaOH for 30 min at 121$^{o}$C, solid: solvent (1:10, w/v). The mixture was washed with distil water several times to remove residual sodium hydroxide, until pH 7 was achieved. The chitosan was dried in an oven at 50 ºC for 18 hr.

## 2.3 Characterization of Chitosan

The solubility test was determined by placing 0.2 g of chitosan in 0.1 M of Hydrochloric acid and stirred till complete dissolution of chitosan. The chitosan was analysed by Fourier Transform Infrared Spectroscopy (FTIR) in the wavelength between 4000 $cm^{-1}$ and 400 $cm^{-1}$ and in solid state using KBr pellet method. The FITR spectra were normalized and major vibration bands were identified associated with the main chemical groups. Room temperature low angle X–ray diffraction (XRD) patterns of the chitosan were studied using X-ray powder diffractometer using a Ni – filtered Cu Kα X–ray radiation source. The relative intensities were recorded within the range of 10° – 90° (2θ) at a scanning rate of $5°min^{-1}$. The surface morphology of the chitosan was observed with scanning electron microscopy and fibermetric image and pore histograms. Other characterisation tests were Raman spectroscopy (RS), Scanning Electron Microscopy (SEM) and Electron Dispersive Spectroscopy (EDS).

# 3. Results And Discussion

## 3.1 Yields of Locally Produced Chitosan

During the demineralization process excessive undesirable foams are produced due to the $CO_2$ generation ($CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O$) which was also reported by No and Hur [27].The

demineralized and de-proteinized chitin has a light pink colour due to the presence of astaxanthin pigment; this pigment was eliminated during the decolourization step to yield cream white chitin powder which was also obtained by No and Meyers [11]. Yield was calculated as the dry weight of chitosan obtained from 400 g of crab shell. Chitosan yield was 22.75% which is comparatively higher than those reported in literature.Fernandez-Kim [23]reported 16.7–18.8% yield of chitosan from crawfish and No and Meyers [11] reported approximately 23% of chitin from crab shell.Brzeski [26]reported about 14% yield of chitosan from krill andAlimuniar and Zainuddin [3] was 18.6% from prawn waste.

# 3.2 Characterization of Chitosan

## 3.2.1 Solubility Test

It is commonly justified that main physical differences between chitin and chitosan is the ability of chitosan to be soluble in organic acid such as acetic acid or dilute hydrochloric acid. Chitosan with higher content of protonated amino group readily form well-ordered arrangement in Van der Waals force and hydrogen bond which exceed its tendency for intramolecular chemical bonds[21, 28]. The developed chitosan and the commercial chitosan both dissolved in 0.1 M hydrochloric acid within 30 minutes demonstrating excellent solubility.

## 3.2.2 Fourier Transform Infrared Spectrometer (FTIR)

Fourier Transform Infrared Spectrometer (FTIR) was used to probe the surface characteristics of the chitosan. the peak appearing was assigned to various functional group according to their respective wave number as reported in literatures hydroxyl group (OH) peaks appears at wavelength of 1350 $cm^{-1}$ the Amines group (R-$NH_2$) peaks appears at 3400–3500 $cm^{-1}$. The FTIR spectrum of Figs. 3 and 2 for commercial and locally developed chitosan respectively wererelating with FTIR absorption bands. A wide absorption band at 3438 $cm^{-1}$ for Figs. 3 and 2 respectively indicates the presence of –OH stretching while peaks at absorption band featuring bending vibration of N-H from R-$NH_2$ was observed at 3450 $cm^{-1}$ while C-H was displayed with stretching vibration of 2916.1 $cm^{-1}$, 2858.3 $cm^{-1}$and bending vibration of 1415.7 $cm^{-1}$, 1375.2 $cm^{-1}$. It was observed that the peaks were at the same frequency for both commercial and local developed chitosan but the locally developed chitosan has high absorbance as compared to commercial.

## 3.2.3 Raman Spectroscopy (RS)

Raman spectra were obtained for half of the films from deposition A before and after neutralization for determining chitosan's functionality. Raman spectra were analyzed for fingerprint and group frequency peaks. Group frequency peaks tend to occur above 1500 $cm^{-1}$, while fingerprint modes are unique to the specific molecule and are usually found below 1500 $cm^{-1}$ [29]. Before starting the analysis, the chemical structures of chitosan were examined for the groups they contained in order to know what to expect. The functional groups identified are presented in Tables 1 and 2 for the commercial and locally developed chitosan respectively. Examining the molecules, chitosan contains 5 methine (C-H) groups per repeat unit and 1 methylene ($CH_2$) group. According to Wojtkowiak and Chabanel [29] methyl $CH_3$ bending can be

found at 1460 ± 10 $cm^{-1}$ and $CH_3$ deformation at 1375 ± 10 $cm^{-1}$. These peaks are presented in the Figs. 4 and 5 for both commercial and locally developed chitosan repectively.

Table 1
Wave numbers of the bands observed in the Raman spectra for commercial chitosan and their assignment to the respective normal vibrations.

| RS | Assignments |
|---|---|
| 3362w | v(OH)HB |
| 3308w | |
| 2932vs | $v(CH_3)$ |
| 2885vs | $v(CH_2)$ |
| 2818shm | $v(CH_3)$ |
| 2743w | v(CH) |
| 1654w | v(CO) |
| 1591 m | |
| 1458 m | $\delta(CH) + \omega(CH_2) + \delta(OH)$ |
| 1411 m | $\delta(CH_3) + \delta(CH)$ |
| 1325 | v(CN) + δ(CH) |
| 1263 | $v(C\text{-}O) + \delta(CH) + \rho(CH_2)$ |
| Φ,pyranoid ring; v, stretching; δ, in-plane bending vibrations; γ, ω, out-of plane bending; HB, hydrogen bond | |

Table 2
Wave numbers of the bands observed in the Raman spectra for locally developed chitosan and their assignment to the respective normal vibrations.

| RS | Assignments |
|---|---|
| 1146 | v(C-O-C) + v(ϕ) |
| 1114 | v(C-OH) + v($C-CH_2$) |
| 1093 | ρ(CH) + ρ($CH_2$) + ρ($CH_3$) |
| 1044shm | ρ($CH_3$) + δ(CH) + δ(OH) |
| 991shm | v(ϕ) + δ(CH) |
| 936 m | v(CN) |
| 896 m | v(ϕ) + ρ($CH_2$) |
| 703w | ω($NH_2$) + δ(ϕ) |
| 566shm | γ(NH) + γ(C = O) + ω($CH_3$) |
| 493 m | γ(CO-NH) + δ($C-CH_3$) |
| 479 m | γ(COC) |
| 444 m | γ(OH + γ(ϕ) |
| 424 m | γ(OH + γ(ϕ) |
| 285 m | δ(C-NH-C) + γ(OH) |
| Φ,pyranoid ring; v, stretching; δ, in-plane bending vibrations; γ, ω, out-of plane bending; HB, hydrogen bond | |

Raman spectroscopy is very helpful for distinguishing amines from alcohols because the N-H stretch is distinctly stronger than the O-H stretch [29]. Also, hydrogen bonding has less of an effect on amines than alcohols, which changes the spectra completely. There is one primary amine in the chitosan repeat unit the NH bend occurs at 1500 $cm^{-1}$.The bands from the range 500–1500 $cm^{-1}$ can be assigned tothe vibrations: δs($CH_3$,$CH_2$) at 1429 $cm^{-1}$, δs($CH_3$,$CH_2$) at 1360–1372 $cm^{-1}$, δ(CH) at 1319 and 1336 $cm^{-1}$v(C-C) and v(C-O) inthe range 1200–1300 $cm^{-1}$, δ(ϕ -OH) at 1163 $cm^{-1}$, vas(C-O-C)in the range 1000–1160 $cm^{-1}$, γ(CH) in the range 850–1000 $cm^{-1}$, and δ(ϕ) in the range 500–720 $cm^{-1}$(Socrates, 2001).

## 3.2.4 Scanning Electron Microscopy (SEM)

The morphology of commercial and locally developed chitosan from Figs. 7 and 6 was obtained using the scanning electronic microscope at four different magnifications. The locally developed chitosan has

a rough surface characterized with holes, and has a porous spongy structure while commercial chitosan has similar characteristic as the locally developed chitosan but with less holes and pores.

## 3.2.5 Electron Dispersive Spectroscopy (EDS)

Electron Dispersive Spectroscopy (EDS) is a test to examine the presence of elements through amplitude of wavelength for the x-ray emitted after the electron was hit by the electron beam. For the emission of x-ray, the atoms must contain minimum of K-shell and L-shell where the electron is allowed to dislodge from shell to shell. Therefore, hydrogen being the only elements in the periodic table with only K shell is not detectable with EDS[30, 31]. Figure 10 is the EDS image of commercial chitosan, the spots represented with + in the Figureshows the points whose elemental composition are presented, Fig. 11a represent spot 2 and Table 3 presents the weight composition of the elements. Figures 11b and 11c depicts the spectrum of spot 3 and spot 6 respectively and Tables 4 and 5 presents the elemental composition of spot 3 and spot 6 for commercial chitosan. Figure 12 is the EDS image of locally developed chitosan and Figs. 13a and 13b represents spot 2 and spot 6 respectively and Tables 6 and 7 presents the elemental compositions for spot 2 and 6. Tables 3 and 4 contains Fluorine which is not part of elemental weight composition of chitosan and also Tables 6 and 7 contains Sodium which might be present as a result of inadequate washing during synthesis stage.

Table 3
Elemental weight composition of spot 2 for commercial chitosan

| Atomic number | Element symbol | Element name | Confidence level | Concentration percentage | Certainty percentage | Error percentage |
|---|---|---|---|---|---|---|
| 7 | N | Nitrogen | 100 | 47.8 | 99.2 | 0.8 |
| 8 | O | Oxygen | 100 | 27.6 | 97.4 | 2.6 |
| 9 | F | Fluorine | 100 | 14.7 | 96.7 | 3.3 |
| 6 | C | Carbon | 100 | 9.9 | 99.2 | 0.8 |

Table 4
Elemental weight composition of spot 3 for commercial chitosan

| Atomic number | Element symbol | Element name | Confidence level | Concentration percentage | Certainty percentage | Error percentage |
|---|---|---|---|---|---|---|
| 7 | N | Nitrogen | 100 | 49.6 | 99.1 | 0.1 |
| 8 | O | Oxygen | 100 | 26.6 | 97.3 | 2.7 |
| 9 | F | Fluorine | 100 | 12.8 | 96.4 | 3.6 |
| 6 | C | Carbon | 100 | 11 | 99.2 | 0.8 |

Table 5
Elemental weight composition of spot 6 of commercial chitosan

| Atomic number | Element symbol | Element name | Confidence level | Concentration percentage | Certainty percentage | Error percentage |
|---|---|---|---|---|---|---|
| 7 | N | Nitrogen | 100 | 40.3 | 97.5 | 2.5 |
| 6 | C | Carbon | 100 | 31.6 | 93.4 | 0.7 |
| 8 | O | Oxygen | 100 | 28.1 | 95.3 | 4.6 |

Table 6
Elemental weight composition of spot 2 for locally developed chitosan

| Atomic number | Element symbol | Element name | Confidence level | Concentration percentage | Certainty percentage | Error percentage |
|---|---|---|---|---|---|---|
| 8 | O | Oxygen | 100 | 39.7 | 98.3 | 1.5 |
| 11 | Na | Sodium | 100 | 38 | 99 | 1 |
| 6 | C | Carbon | 100 | 12 | 93.4 | 1.1 |
| 7 | N | Nitrogen | 100 | 10.3 | 97.5 | 2.4 |

Table 7
Elemental weight composition of spot 6 for locally developed chitosan

| Atomic number | Element symbol | Element name | Confidence level | Concentration percentage | Certainty percentage | Error percentage |
|---|---|---|---|---|---|---|
| 8 | O | Oxygen | 100 | 39.6 | 98.3 | 1.6 |
| 11 | Na | Sodium | 100 | 38 | 99 | 1.2 |
| 6 | C | Carbon | 100 | 14.9 | 93.4 | 0.9 |
| 7 | N | Nitrogen | 100 | 13.5 | 97.5 | 2.2 |

### 3.2.6 X-Ray Diffraction (XRD)

The XRD spectrum of chitosan has low crystallinity. X-ray diffraction pattern for pure chitosan has peaks at 2θ = 10° and 20.09° for pure chitosan confirms the semi crystalline nature[32]. The XRD pattern of commercial and locally developed chitosan are shown in Figs. 14 and 15 respectively. Both showed broad diffraction at 2θ = 20° and that symbolizes semi crystalline chitosan. This was supported byYen, Yang and Mau [25] as the two characteristic crystalline peaks of chitosan at 9–10° and 19–20° with comparable crystallinity.

## Conclusion

Chitosan has been successfully prepared from crab shell with a yield of 22.75% and 71% degree of de-acetylation.The commercial and locally developed chitosan were characterized by solubility test which

dissolve within 30 minutes in 0.1 M HCl. The FTIR indicate the presence of –OH functional group at wavelength 1350 $cm^{-1}$and R-$NH_2$ at 3450 $cm^{-1}$.SEM revealed that the locally developed chitosan has a rough surface characterized with holes, and has a porous spongy structure.Electron Dispersive Spectroscopy (EDS) was used to examine the presence of elements on the chitosan. Results showed the presence of C, N, O and Na. Usually, Hydrogen is usually present in organic materials but EDS cannot detect the its' presence. XRD revealed a low crystallinity of the chitosan obtained.

## Declarations

# Availability of data and materials

Not Applicable

# Competing interests

NIL, the authors declare that there are no conflicts of interest and the article does not contain any studies involving human or animal subjects.

# Funding

NIL, no funding was received

# Authors' contributions

**Babatunde Esther O:** did the experimental work in the laboratory

**Ighalo Joshua O** did the methodology set up and discussion of results

**AKOLO Sunday A**: edited the whole manuscript

**Adeniyi, Adewale George** did the setting out of the background to the study, wrote the introduction.

**Adepoju Lydia:** assisted in the laboratory work

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# Figures

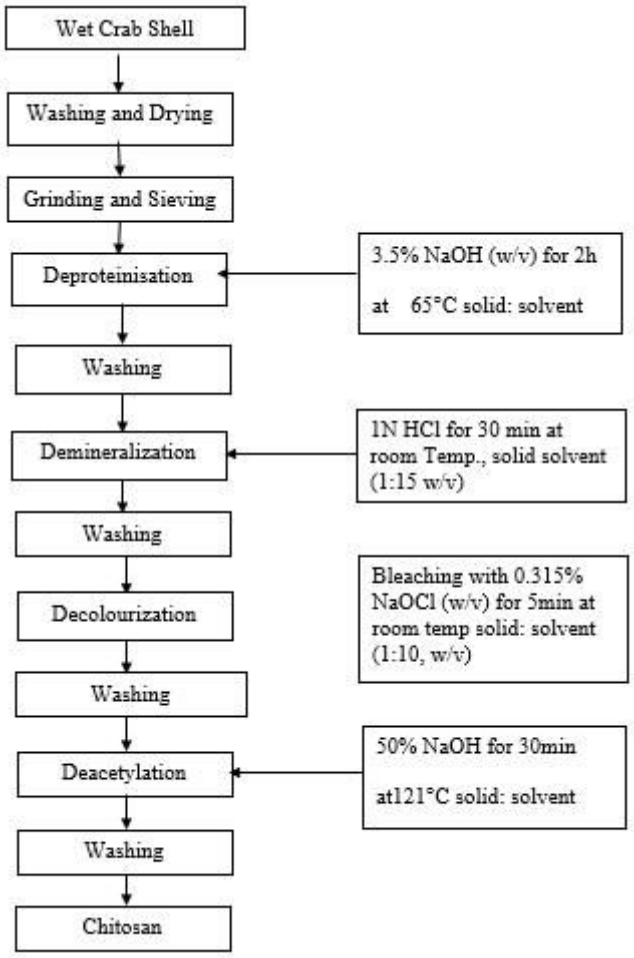



**Figure 1**

Summary of processes involved in the production of chitosan from crab.

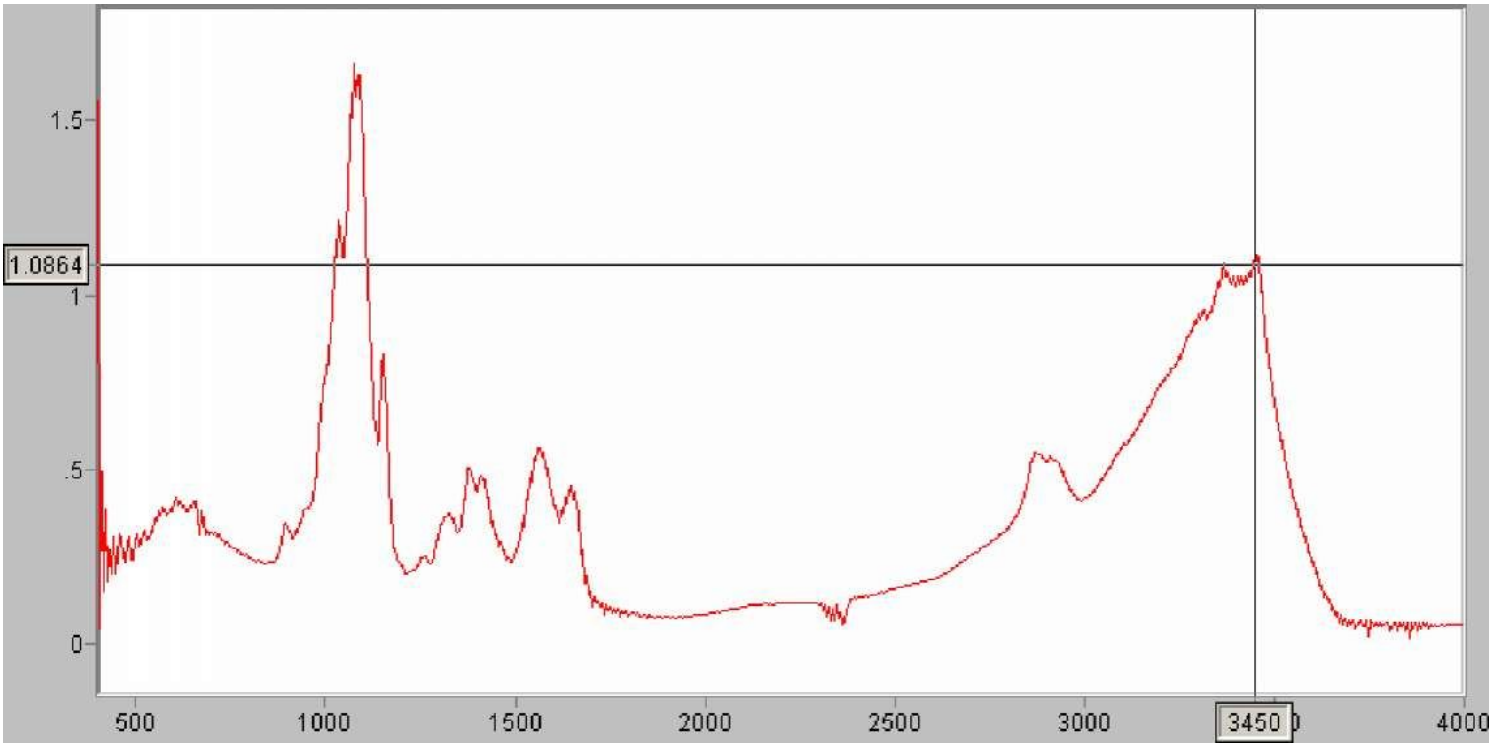



**Figure 2**

FTIR of locally developed chitosan

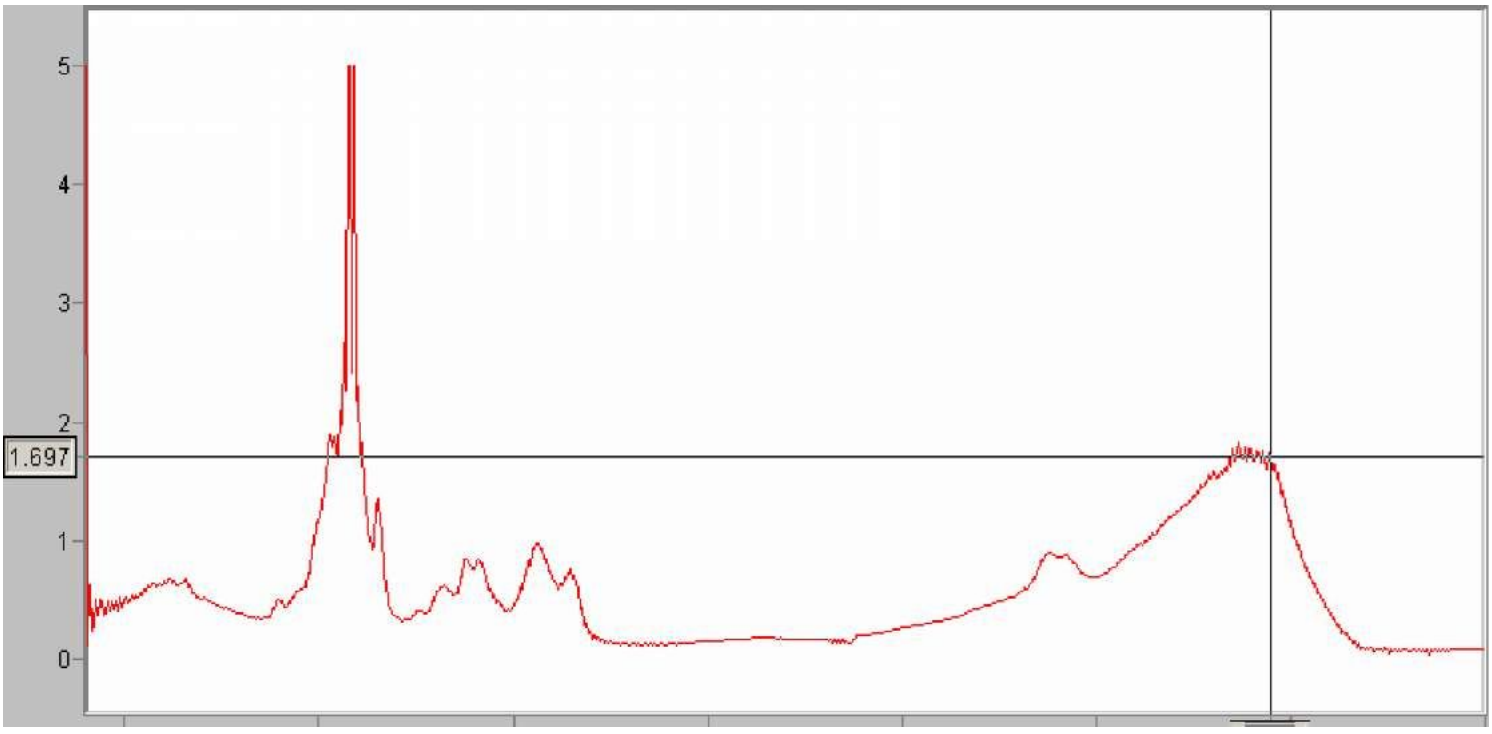
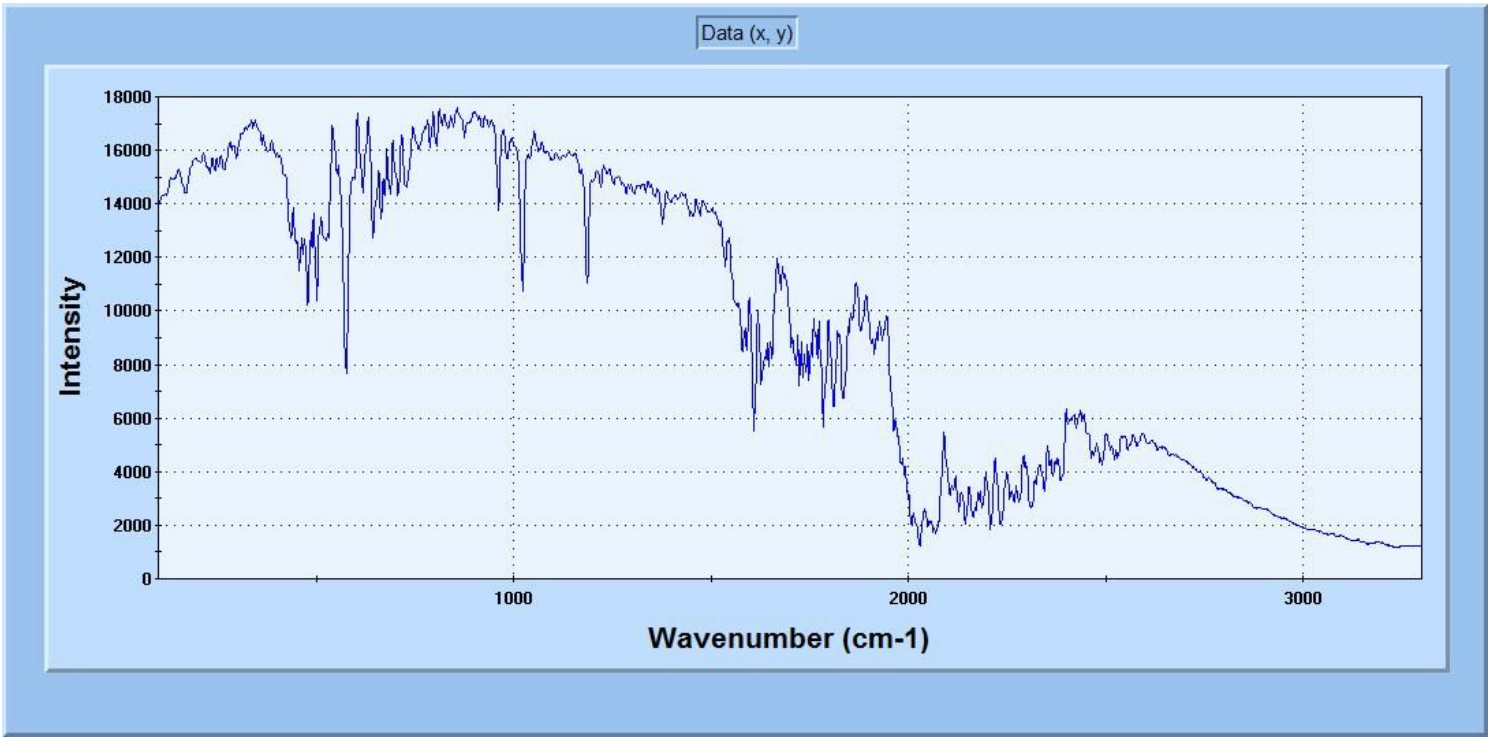


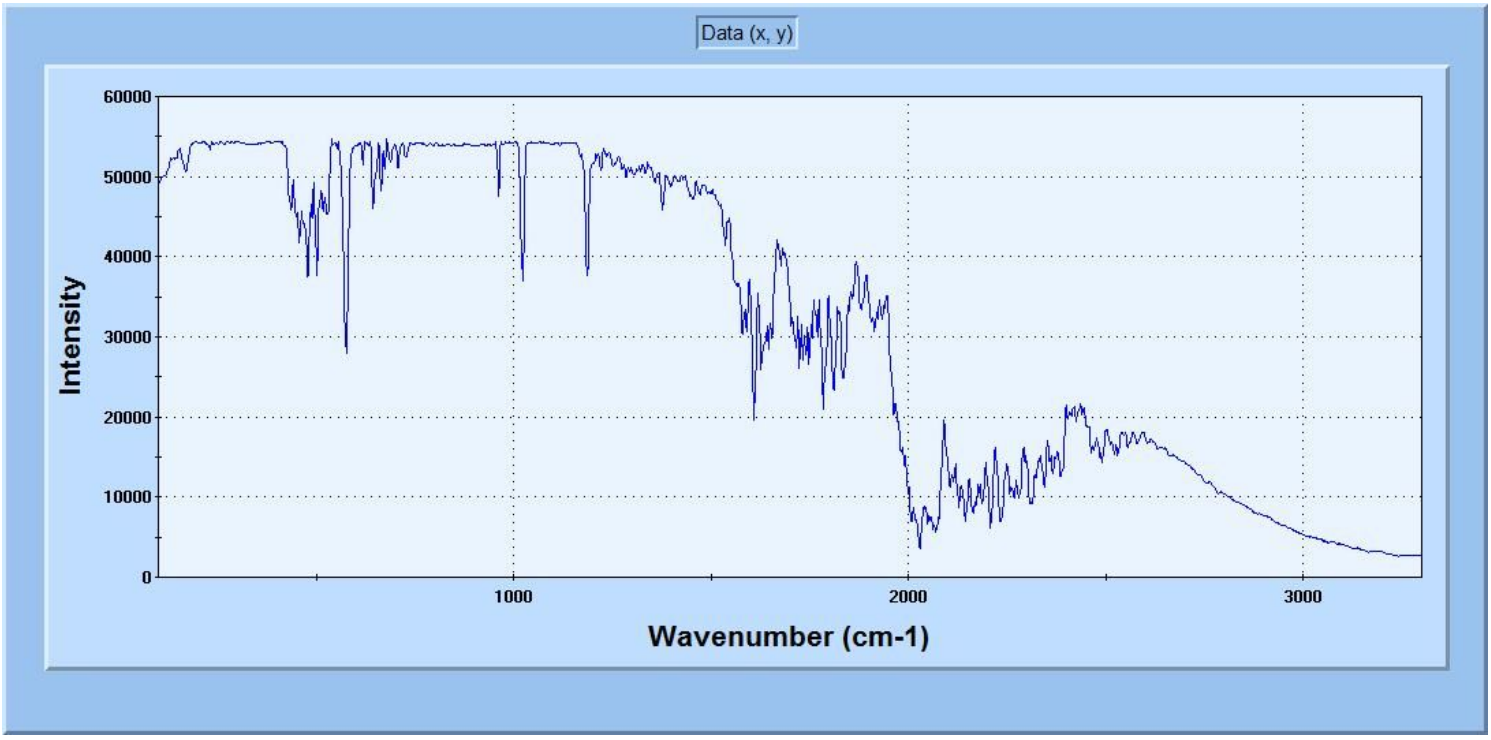

**Figure 3**

FTIR of commercial chitosan



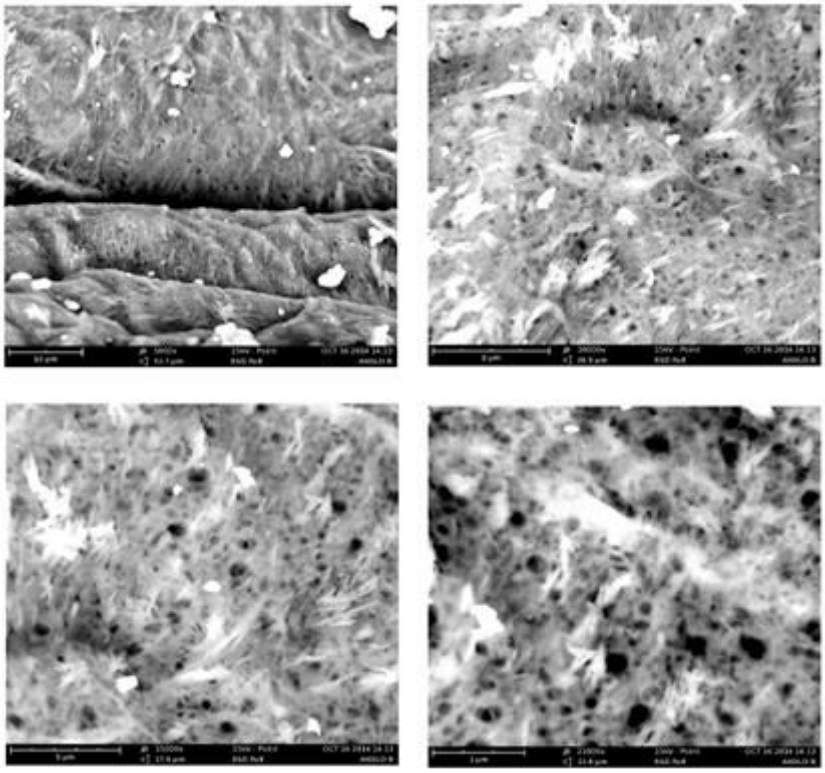

## Figure 4

Raman spectroscopy of commercial chitosan



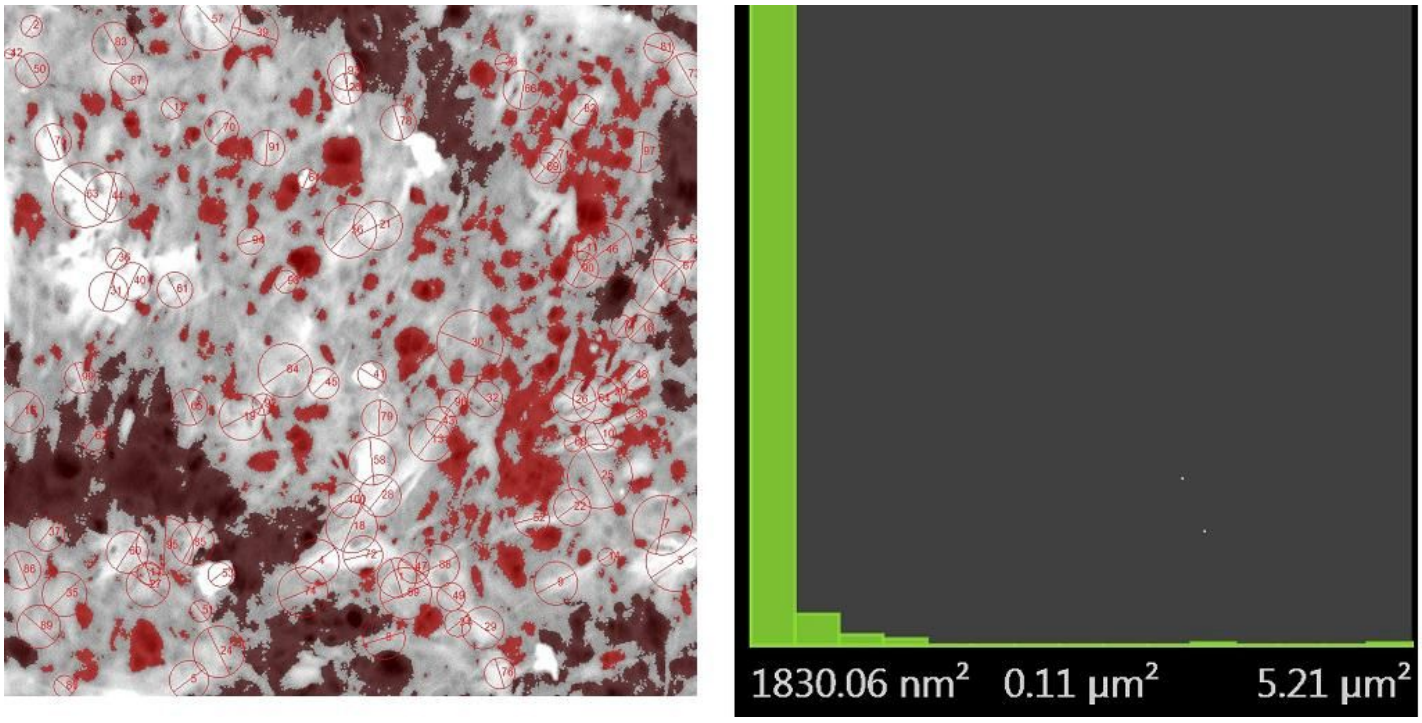

## Figure 5

Raman spectroscopy of local developed chitosan



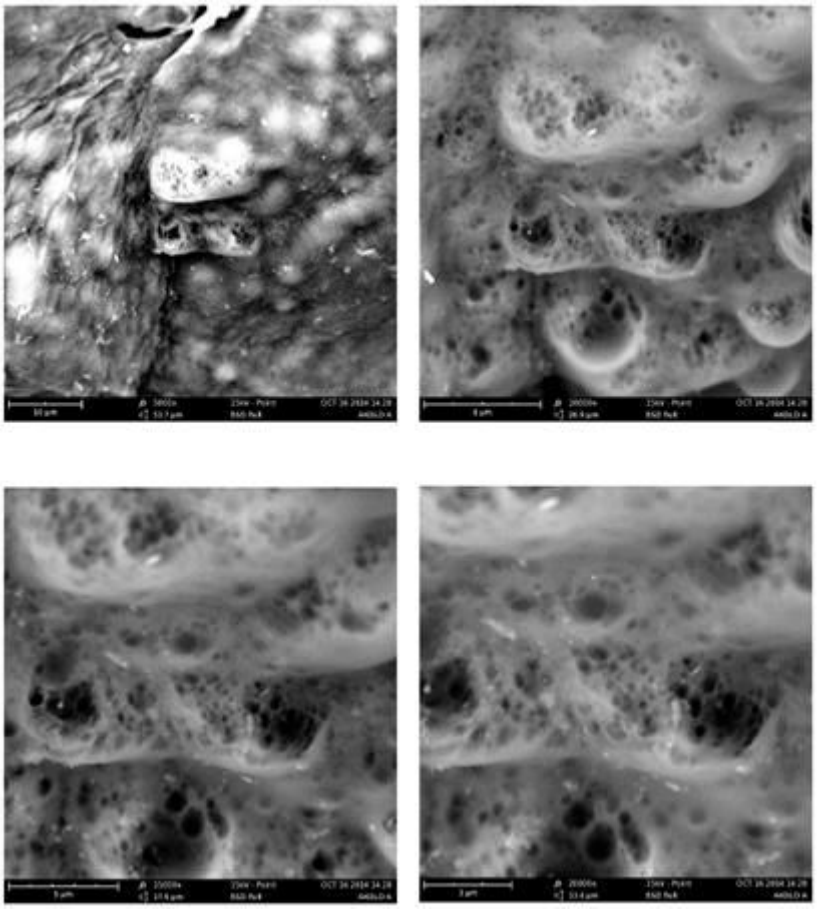
## Figure 6

SEM image of locally Developed Chitosan



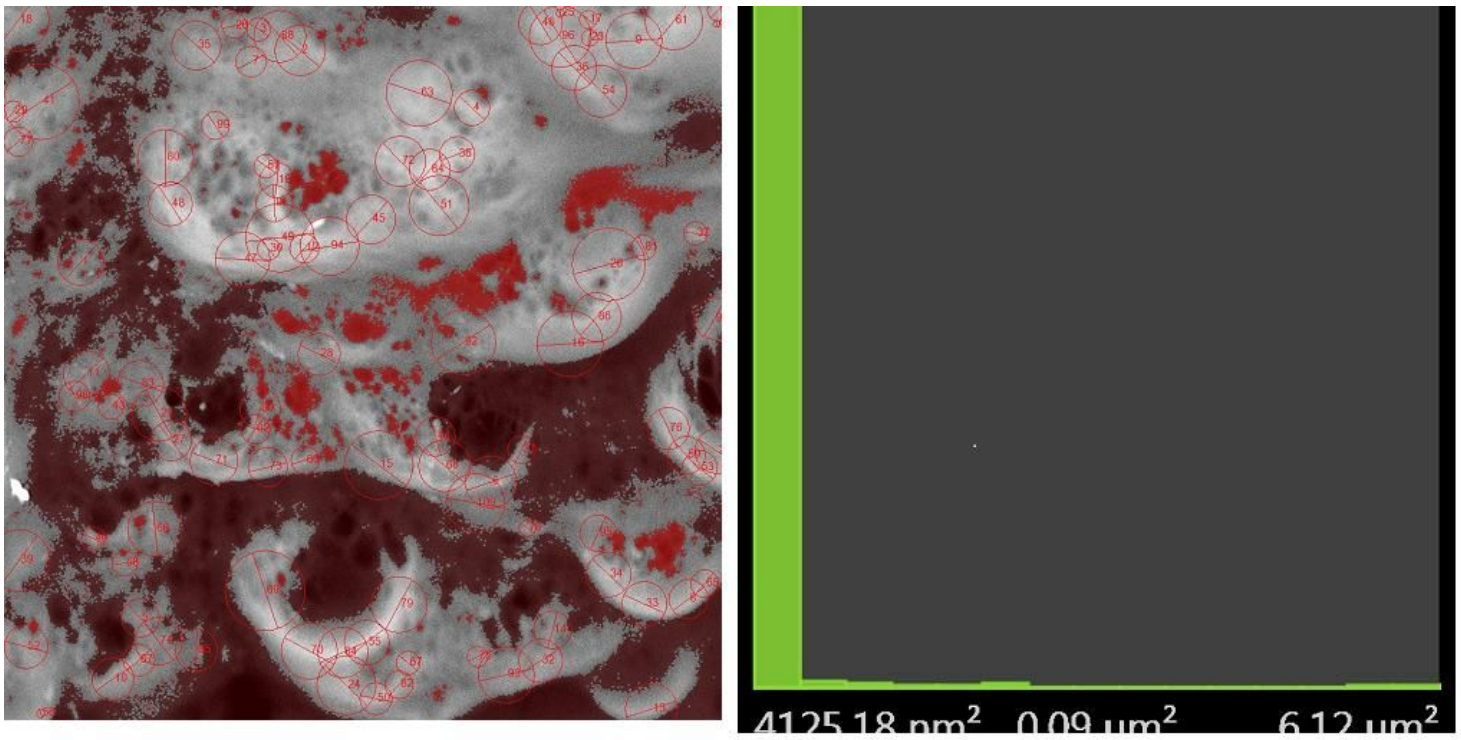

## Figure 7

Fibermetric Image and Pore Histogram of local developed chitosan



## Figure 8

SEM image of commercial Chitosan




## Figure 9

Fibermetric Image and Pore Histogram of commercial chitosan

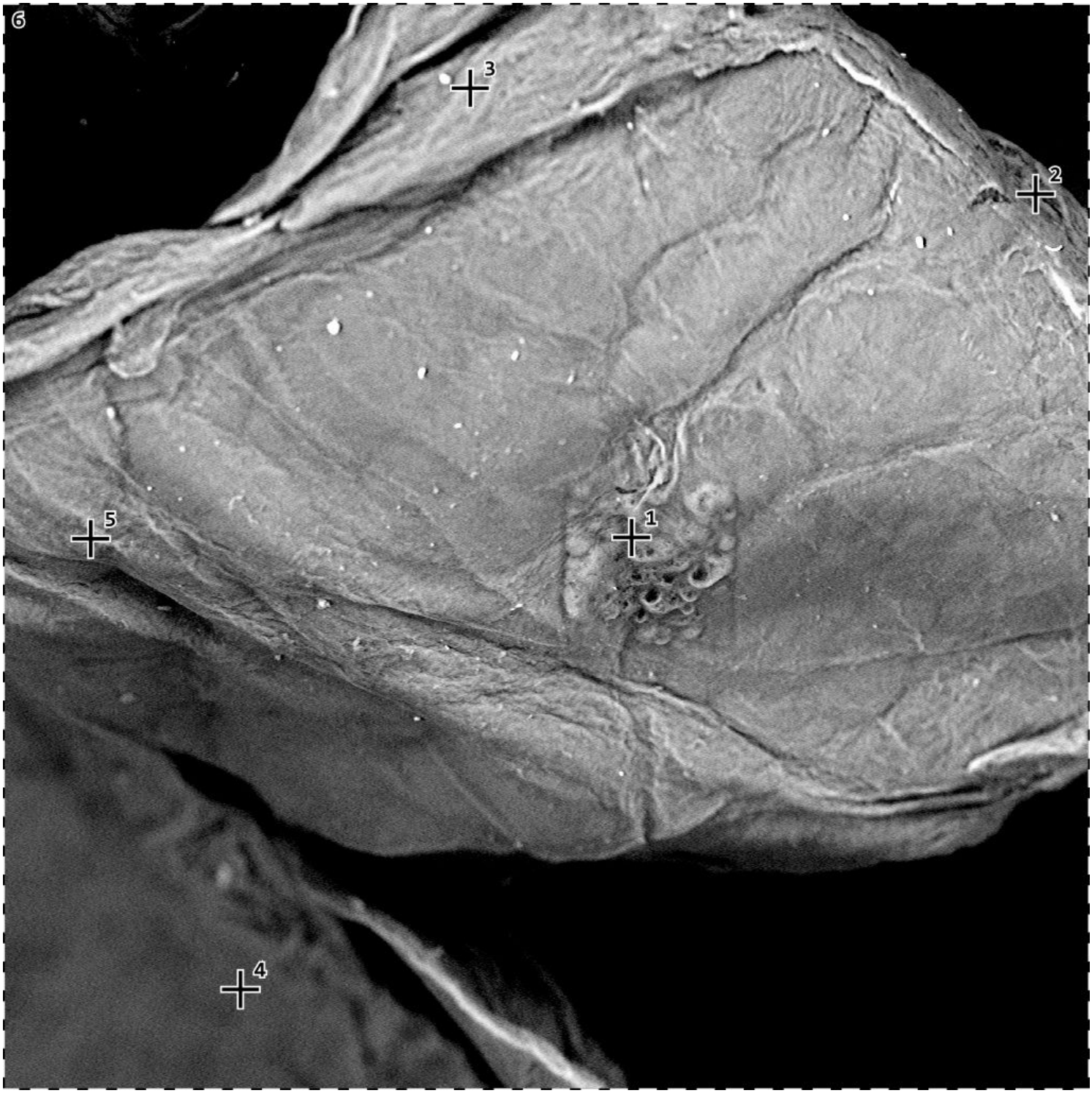
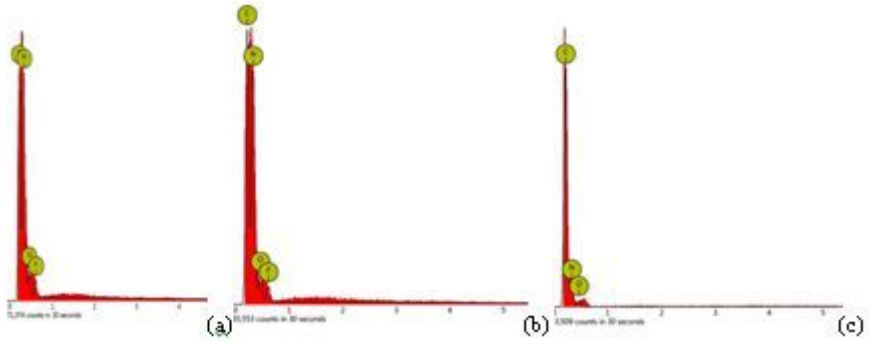


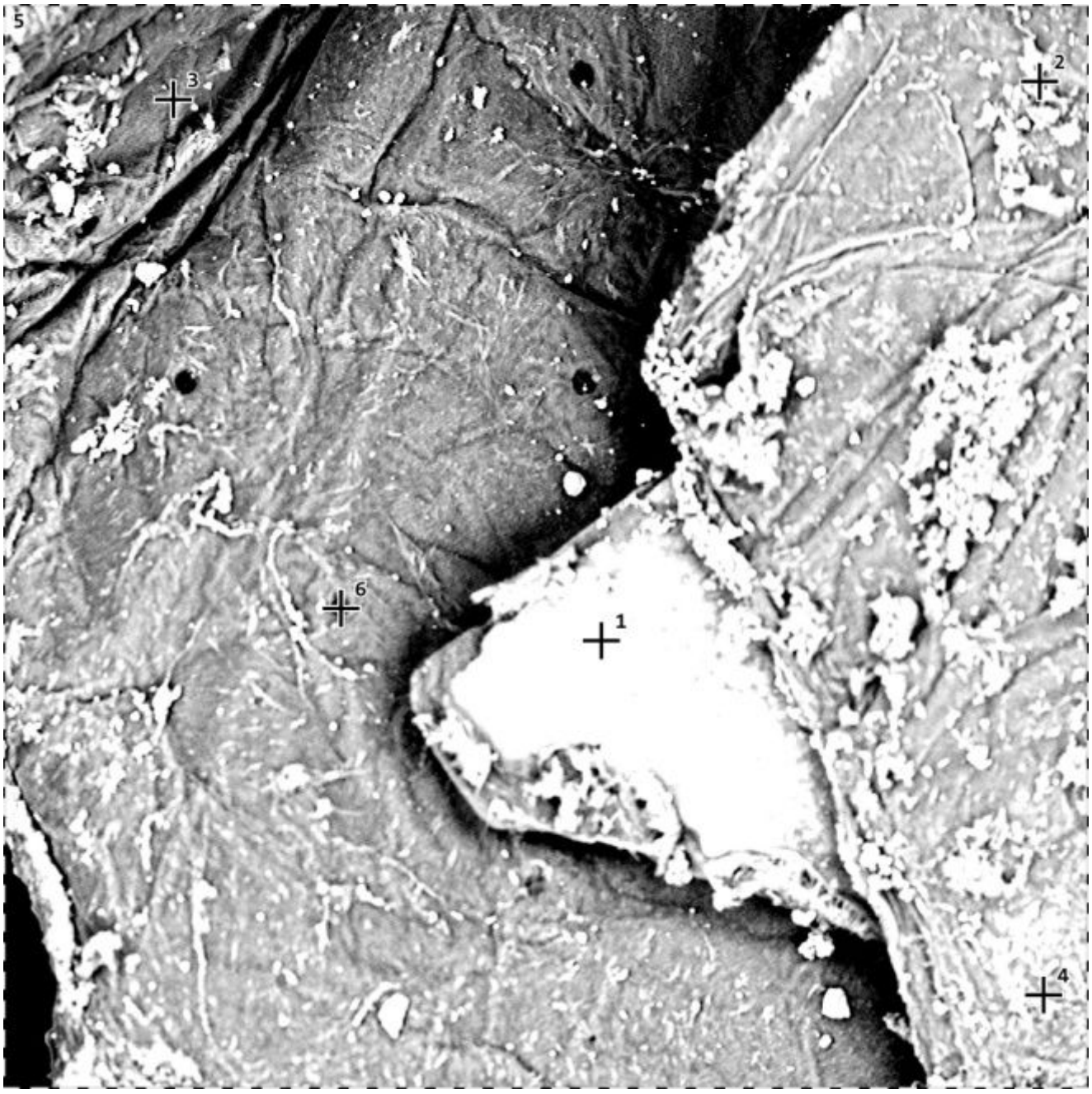

**Figure 10**

EDS image for the commercial chitosan



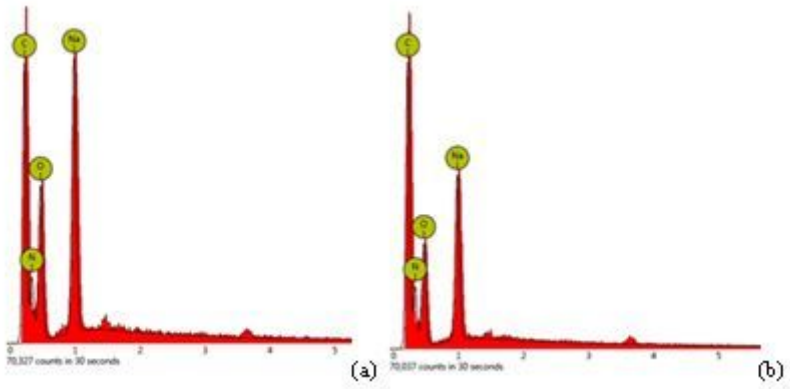

## Figure 11

a(spot 2), b (spot 3) and c (spot 6): EDS spectrum for commercial chitosan



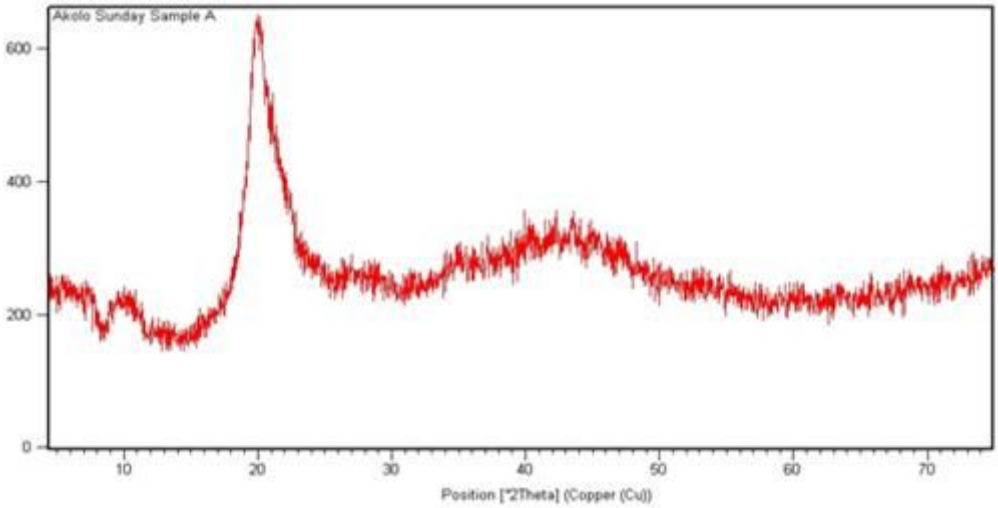
## Figure 12

EDS image for locally developed chitosan



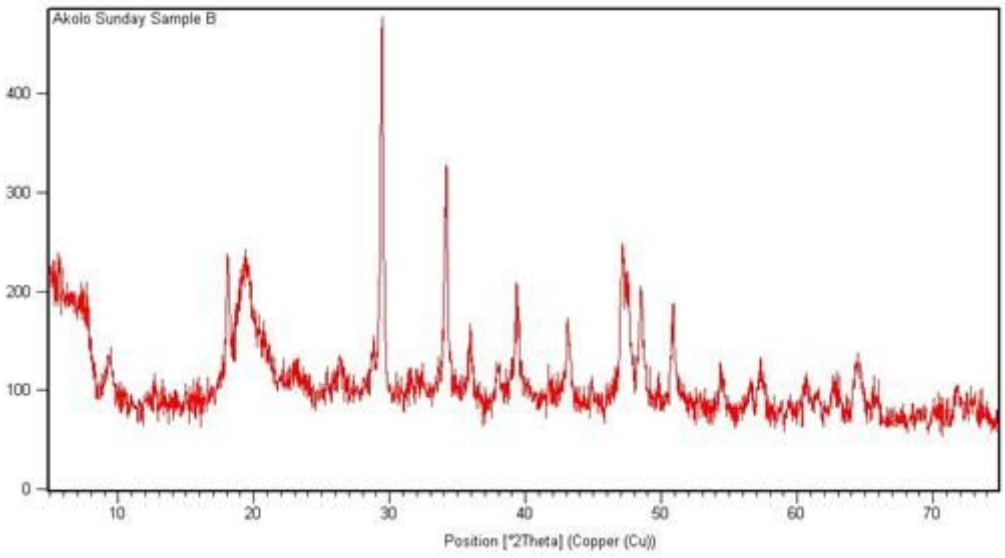

## Figure 13

a (spot 2) and b (spot 6): EDS spectrum for locally developed chitosan




## Figure 14

XRD for commercial chitosan




## Figure 15

XRD for locally developed chitosan