Extraction and Characterization of Chitosan from Shrimp Shells

(Egypt : case study)

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Abstract

In the present study we reported the extraction of low cost chitosan's (CHS1, CHS2, CHS3 and CHS4) from shrimp shells by extraction of chitin, then alkaline deacetylation of chitin with strong alkaline solution at different period of time. The different prepared chitosan's (CHS1, CHS2, CHS3 and CHS4) were characterized by FTIR spectroscopy, thermal stability, morphology, crystallography, elemental analysis and degree of deacetylation . The data showed that the prepared chitosan CHS2 has the most thermal stability and the highest degree of deacetylation.

Keywords: Chitosan, Hydrogyl, Shrimp shells, Chitin, Morphology.

1. Introduction

A white hard polysaccharide chitin, which known as 2-acetamido-2-deoxy-D-gluco-pyranose units through (1→4) linkage is extracted from the crustaceans exoskeletons and also from crabs and shrimps **(Minke and Blackwell, 1978; Austin et al., 1989).** The alkaline deacetylation of chitin produces a very useful material chitosan, which known as a copolymer of (1→4) linked 2-amino-2-deoxy-D-gluco-pyranose units, and also it is found naturally in some fungal cell walls. Since it is non-toxic and presents excellent biological properties such as biodegradation in the human body, immunological, antibacterial, and wound-healing activity **(Synowiecki & Al-Khateeb, 2003; Jayakumar, Prabaharan, Reis, & Mano, 2005),** as shown in **(Scheme 1),** chitosan has been widely used in food and pharmaceutical processes and in medical and agricultural drugs **(Kifune, 1992; Kawamura, Mitsuhashi,Tanibe, & Yoshida, 1993; Ravi Kumar, 2000; Sashiwa & Aiba, 2004) (Kandile N. G. , Nasr A. S.;2011).** It can be found also in the skeleton of crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton spp., including coral and jellyfishes **(Shahidi, F.; Abuzaytoun, R.,2005).** Also, the chitin can be

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extracted from various sources to be converted to chitosan by different degree of dacetaylation during using different concentration of NaOH **(Abdo E. S., Nagy Kh. S.A., Elsabee M. Z. ;2008)**

Scheme 1

The aim of the present study is the preparation of low coast chitosan with different degree of deacetylation from wastes of Egyptian shrimp shells to use it as a key material for many applications.

2. Materials and Methods

2.1 Materials

Raw shrimps stated as large size were purchased from Egyptian (Market, Eloubor city, Egypt). Sodium hydroxide (NaOH) (Aldrich, Egypt). Hydrochloric acid (HCl) (Aldrich, Egypt), and acetic acid (Aldrich, Egypt). They were then diluted to the concentration required for the methodology with distilled water. All chemicals were used without further purification.

2.2 Measurements

The infrared spectra were measured on Perkin-Elmer-1430 infrared spectrophoto- meter using the potassium bromide Wafer technique. X-ray diffractograms of polymers were obtained with a

Phillips X-ray radiation unit (Generator PW-1390) and Ni-filtered Cu. Thermogravimetric analysis (TGA) was carried out in a nitrogen atmosphere using a Shimadzo TGA-50H. The morphology of the different hydrogels was investigated using JXA 850 prop micro analyzer scanning electron microscope (SEM). The solubility of the polymers was examined using 0.02 g of polymer in 5 ml solvents at room temperature 25 $^{\circ}$C.

2.3 Methods (Extraction of chitosan)

The extraction of chitosan can be carried out by different four methods under different conditions after removing the loose tissue from the shrimp shells then washed, dried and grind to obtain dry powder. The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with strong alkaline solution at different period of time.

2.3.1 Extraction :

Method 1

i. Deproteinization process

The deproteinization was occurred by heating of 3 gm of shrimp shells powder after adding 2N NaOH with ratio of 12 ml: 1g (w/v) at 70 °C for 4h. The product was neutralized by washing under running tap water. The solid was collected and washed with distilled water. The solid product was dried in vacuum and weighed with analytical balance.

ii. Demineralization process

The dry solid was treated with 10 % HCl (3.25 N) with ratio of 14 ml : 1g (w/v) at room temperature and kept for 4 h.The solid product was collected and washed with distilled water. The solid was then dried.

iii. Deacetaylation

Then the demineralized product was treated with 35% NaOH (8.75 N) with ratio of 14 ml:1g(w/v) at room temperature for 75 h. with stirring. Filter the deacetaylated solid then collected and washed with distilled water. The deacetaylated product was dried in a vacuum to give (1.51gm) and then labeled as **CHS1**.

2.3.2 Extraction

Method 2

i. Demineralization process

The demineralization was carried out by weight 3 gm of shrimp shells powder by using 4 % HCl (1.3 N) with ratio of 14ml: 1g (w/v) at room temperature for 24 h. The product was washed to neutrality under running tap water. The solid was collected and washed with distilled water, then dried in a vacuum.

ii. Deproteinization process

Deproteinization was carried out using 5 % NaOH (1.25 N) with ratio of 12 ml: 1g (w/v) at 90 °C for 24 h. The deproteinized product was collected and washed with distilled water.

iii. Deacetaylation

The product was deacetylated with 70 % NaOH (17.5 N) with ratio of 14ml: 1g (w/v) at room temperature for 75 h. with stirring. The solid was collected and washed with distilled water. The deacetylated product was then dried in a vacuum, producing (2.04gm) and labeled as **CHS2.**

2.3.3 Extraction :

Method 3

i. Deproteinization process

The deproteinization process was carried out by weight 3gm of shrimp shells powder and using 5 % NaOH (1.25 N) with a weight to volume ratio of 1 g : 8 ml (w/v). The solution with shrimp shells was refluxed at 70 °C for 3 h. The product was collected and washed until clear solution. It was then dried in a vacuum. The product was decolorized with pure acetone for 24 h. The product was collected and washed to neutrality, then dried.

ii. Demineralization process

The decolorized product was demineralized by using 1% HCl (0.32 N) with a weight to volume ratio of 1 g : 10 ml for 24 h. at room temperature. The product was collected and washed to give light brown powder.

iii. Deacetaylation

The N-deacetylation of the demineralized product was carried out by using 55 % NaOH (12.5 N) with weight to volume ratio of 1 g : 5 ml at 100 °C for 12 h. The product was washed with distilled water and dried to producing (1.69gm) and then labeled as **CHS3.**

2.3.4 Extraction :

Method 4

i. Demineralization process

Weight 3gm of shrimp shells powder, then treated by 1 N NaOH (4 %) with weight to volume ratio of 1 g:10 ml for 24 h. at room temperature. It was washed and dried in vacuum. The solid from the alkaline treatment were then demineralized by using 1N HCl (3%) with weight to volume ratio of 1 g: 10 ml for 24 h. at room temperature. It was washed and dried in vacuum.

ii. Deproteinization process

The demineralized product was deproteinized by using 1 M NaOH (4 %) with weight to volume ratio of 1 g: 10 ml for 24 h. at room temperature. It was washed and dried in vacuum. The product from deproteinization was decolorized using pure acetone with for 24 h. at room temperature. It was washed and dried in vacuum.

iii. Deacetaylation

From decolorization, the product was then deacetylated by using 50 % NaOH with weight to volume ratio of 1 g: 10 ml for 24 h. at room temperature. The product was washed and dried in vacuum to producing (1.14gm) and then labeled as **CHS4.**

3. Result and Discussion

The major procedure for extraction of chitosan from shrimp shells powder is based on the alkaline deacetylation of chitin with strong alkaline solution via deproteinization, demineralization and deacetaylation of shrimp shells powder at different conditions to give the following chitosan samples : **CHS1, CHS2, CHS3** and **CHS4** respectively.

3.1 Characterization of the prepared chitosan

The chitosan samples: **CHS1, CHS2, CHS3** and **CHS4** were characterized by (FT-IR) to identify the functional groups in chitosan. X-ray diffractometry (XRD) to analyze the crystallinity of the product, thermogravimetric analysis (TGA) to study the thermal stability, the elemental analysis to calculate the degree of deacetylation, Finally, Scanning electron microscope to demonstrate the morphology of the product.

3.1.1 (FT-IR) Spectra

The IR. spectral data for the produced chitosan **[CHS1,CHS2, CHS3, and CHS4]** revealed the following peaks: peak at 3440.9 cm^{-1} , 3396.1 cm^{-1}, 3438.7 cm^{-1}, 3441.5 cm^{-1} is assigned to -OH and -NH stretching vibrations, while the peaks at 2960.8-2890.4 cm^{-1}, 2971.3 cm^{-1}, 2959.7-2890.6 cm^{-1}, 2961.4-2890.5 cm^{-1} is assigned to the aliphatic C-H stretching vibration in the -CH and -CH_2 groups. The amide frequencies consisting of the -C-O bond stretch of the remaining acetamido groups and the N-H bending vibrations of the -NH_2 groups are observed at 1663.5 and 1559.9 cm^{-1}, 1754.1 and 1664.7 cm^{-1} ,1659.5 and 1561.7 cm^{-1} ,1656.3 and 1562.5 cm^{-1} respectively. The peak at 1429.9 cm^{-1}, 1451.1 cm^{-1}, 1418.2 cm^{-1}, 1419.1 cm^{-1} is assigned to -NH_2 deformation. Further bending vibrations are observed at 1379.4 cm^{-1}, 1409.5 cm^{-1}, 1380.5 cm^{-1}, 1381.8 $cm^{-1}$for the C-C-H symmetric bending vibration in the alcohol. Stretching vibrations are also observed at 1317.2and 1156.9 cm^{-1}, 1154.5 cm^{-1}, 1316.0and 1156.8 cm^{-1}, 1316.9and 1156.9 $cm^{-1}$for the C-N stretching vibration and at 1072.7 and 1032.3 cm^{-1} for the -CO stretching vibration of the alcohol groups as shown in **Fig.1**

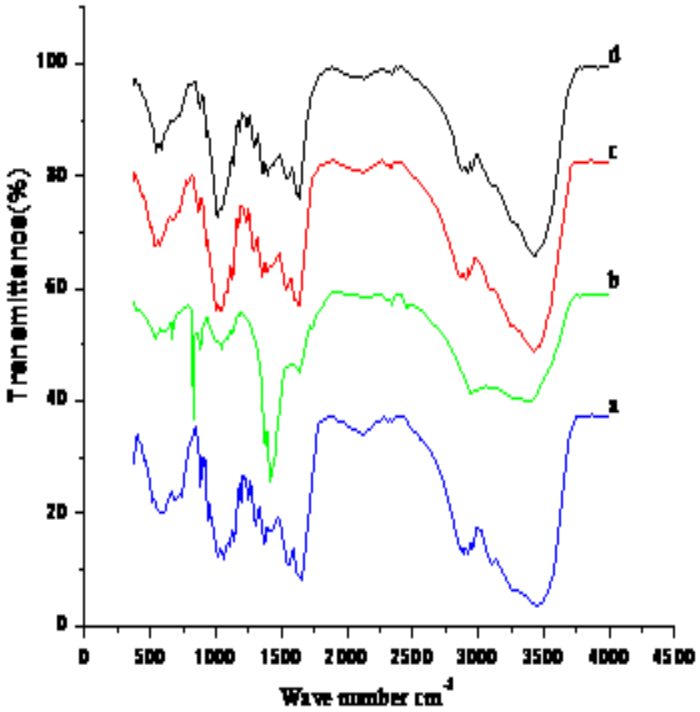


Fig.1. Infrared spectra of (a) CHS1, (b) CHS2, (c) CHS3 and (d) CHS4.

3.1.2 X-ray Difratometry (XRD) Analysis

The X-ray diffraction is used in the characterization of crystalline materials. By studying the X-ray diffraction of the extracted chitosan from the four methods, it can be concluded that the order of crystallinity is of different chitosan samples: [CHS2 > CHS3 > CHS1> CHS4] so the highest crystallinity is shown by chitosan produced from method 2 [CHS2] as shown in **Fig.2**

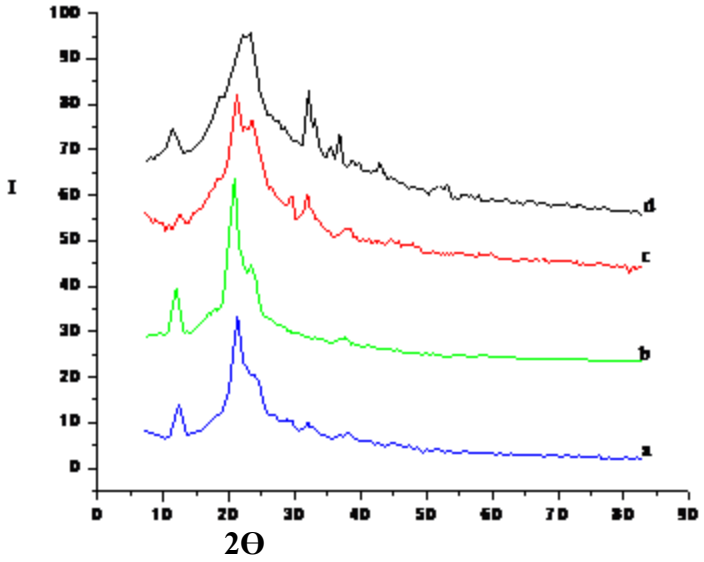


Fig.2. X-ray diffraction pattern for (a) CHS4,(b) CHS1,(c) CHS3and (d) CHS2.

3.1.3 Thermal stability (Thermogravimetric Analysis) (TGA):

The thermograph of the produced chitosans [CHS1 , CHS2 , CHS3, CHS4] were evaluated by using TGA in air at heating rate 10^0C/min and recorded in **Fig (3**) and **Table (1):** It show the following data: the weight loss of the extracted chitosan by the four methods at the beginning may be due to the ease of degradation of the amide groups; however the weight loss in the high temperature range is attributed to the degradation of the main chain.

Table (1): Thermal properties of the extracted chitosan by the four methods

method code	Temp.	Wt. Loss%	Temp.	Wt. Loss%	Temp.	Wt. Loss%
Ex1_CHS	230.0	13.63	690.0	25.17	-	-
Ex2_CHS	250.0	10.82	283.33	5.105	550.0	27.84
Ex3_CHS	225.0	10.89	704.16	53.33	-	-
Ex4_CHS	195.83	9.14	391.66	31.60	629.16	32.60

From the **Table (1),** it can be concluded that: the polymer prepared by method (2) (CHS2), show high thermal stability than the other prepared samples.

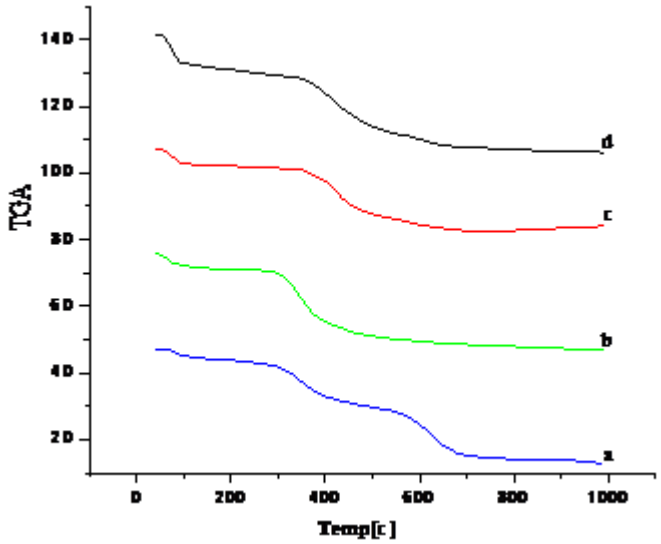


Fig. (3). TGA for (a) CHS1, (b) CHS2, (c) CHS3 and (d) CHS4.

3.1.4 Degree of deacetylation for Chitosan

By using the elemental analysis the percentage of free amino groups on the chitosan can be determined by using the following equation **(Kasaai, Arul &Charlet, 2000)**:

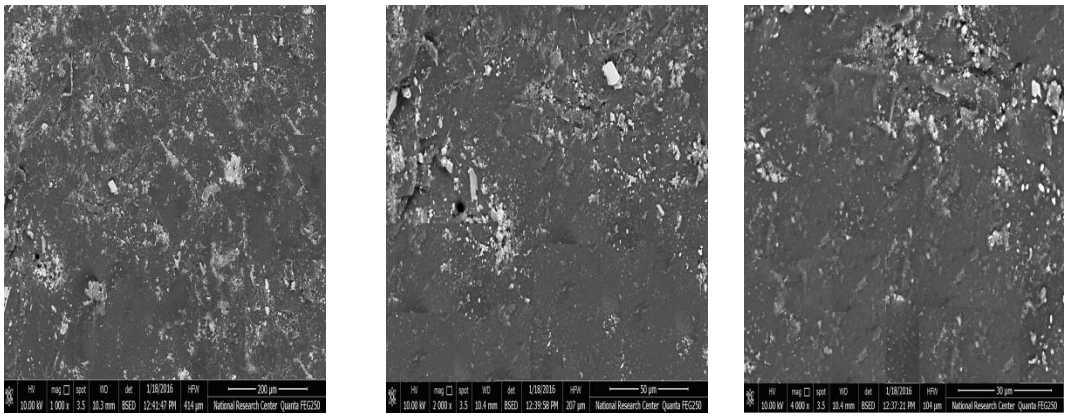
Where 5.145 is related to completely N-deacetylated chitosan ($C_6H_{11}O_4N$ repeat unit) and 6.186 is the fully N-acetylated polymer ($C_6H_{11}O_4N$ repeat unit). The value of degree of deacetylation of chitosan samples was calculated and reported in **Table (2).** The data indicated that the highest degree of deacetylation (DD) shown by the CHS2 , CHS3 .It can be conclude that the degree of deacetylation of chitosan increased by increasing the concentration of the NaOH used in. The elemental analysis and the degree of deacetylation shown in **Table (2).**

Table (2): The elemental analysis, and the degree of deacetylation of chitosan

method code	%C	%H	%N	DD%
CHS1	40.66	5.75	6.65	6.917
CHS2	28.90	3.70	5.51	90.4
CHS3	31.80	2.80	6.01	85.95
CHS4	27.6	3.82	4.30	21.0

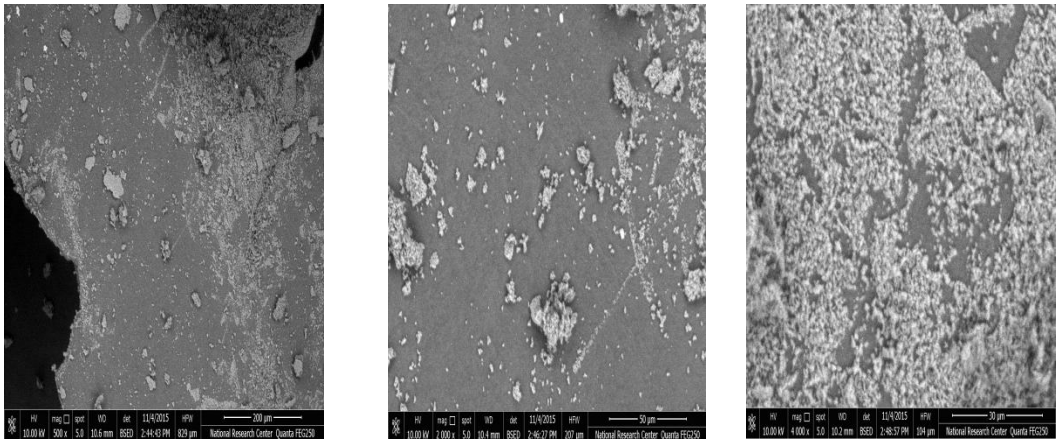
3.1.5 Morphology

The SEM photographs shows the morphologies of the four extracted chitosan as shown in **Figs. (4-7)** which show the morphologies of the extracted chitosan by methods (1,2,3 and 4) at (200, 50, 30μm) respectively. The Figs. show that the different in the shape of each Fig. according to the different method of preparation of each method of chitosan.



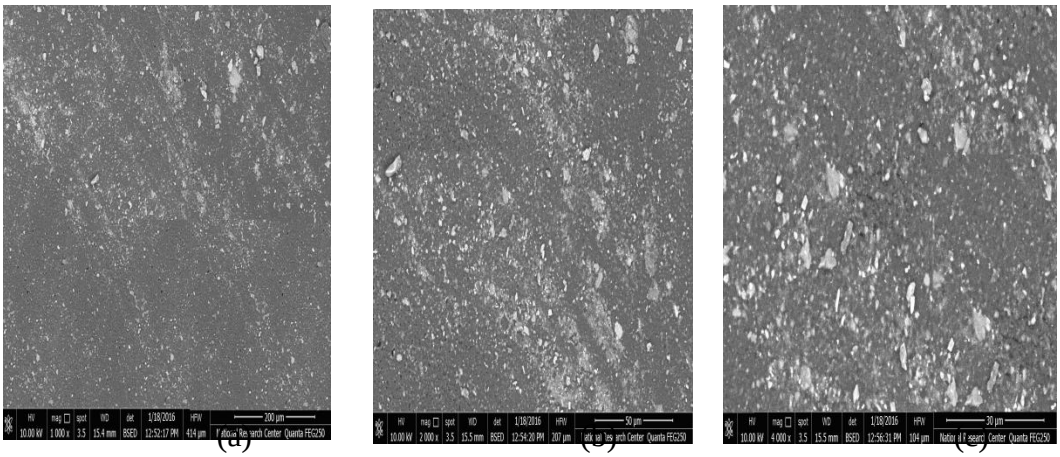
(a) (b) (c)

Fig.4. SEM of CHS1 at (a) 200µm. (b) 50µm. (c) 30µm.



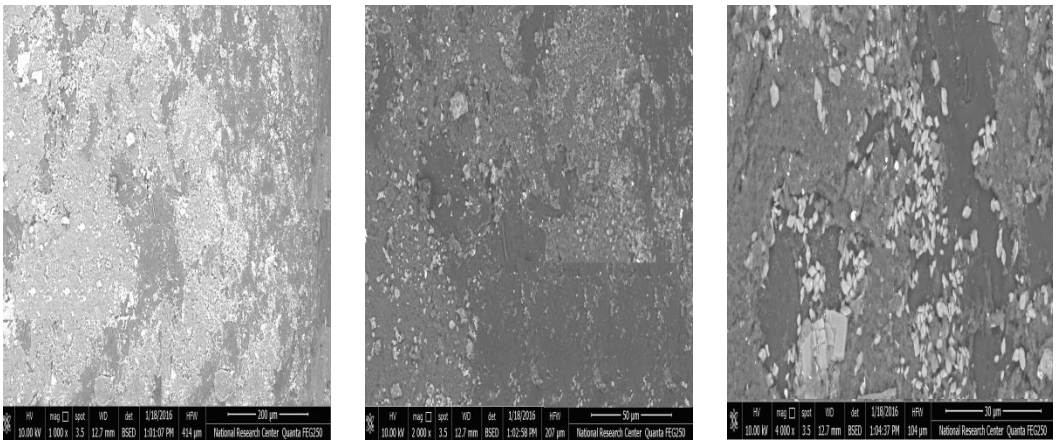
(a) (b) (c)

Fig.5. SEM of CHS2 at (a) 200µm. (b) 50µm. (c) 30µm.



(a) (b) (c)

Fig.6. SEM of CHS3 at (a) 200µm. (b) 50µm. (c) 30µm.



(a) (b) (c)

Fig.7. SEM of CHS4 at (a) 200µm. (b) 50µm. (c) 30µm

4. Conclusions

Chitosan's of different degree of deacetylation can be obtained from deacetylation of chitin in strong sodium hydroxide solution at different period of time after extraction from shrimp shells . Chitosan (CHS2) possesses the highest thermal stability, crystallinity and degree of deacetylation which may be attributed to the increase of the sodium hydroxide concentration, and its morphology shows crystals on its smooth surface.

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الملخص باللغة العربية

إستخلاص وتوصيف الكيتوزان من قشور الجمبري

(دراسة حالة: ج.م.ع)

ياسمين جمال، عبير صلاح ناصر، هويدا طلعت زكي، منصورة إسماعيل محمد، نادية غريب قنديل

- يتضمن هذا البحث كيفية إستخراج الكيتوزان من قشور الجمبري وذلك عن طريق استخراج الكيتين ثم تحويله الى الكيتوزان في وسط قاعدى قوى لنزع مجموعات الأسيتيل مع إختلاف الوقت اللازم للتفاعل.
- تم تحضير الكيتوزان بأربع طرق مختلفه لتحضير **CHS1, CHS2, CHS3 and CHS4**.
- وقد تم توصيف جميع التراكيب الكيميائيه بطرق متعددة مثل:تحليل العناصرعن طريق الوحدات المتكررة لكل بوليمر وكذلك الدراسه الطيفيه لها باستخدام الاشعة تحت الحمراء، والثبات الحراري بإستخدام التحليل الحرارى، ودراسة الشكل البللوري بإستخدام أشعة إكس، كما تم دراسة المورفولوجى للبوليمرات بإستخدام الماسح الإلكترونى الميكروسكوبى.
- أظهرت النتائج أن الكيتوزان الذي تم تحضيره بالطريقة الثانية هو أكثر ثباتا حراريا وأعلي درجه في نزع مجموعة الأستيل.