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Extraction of Chitosan from Kentish Snail Exoskeleton Shell's, *Monacha cantiana* (Montagu, 1803) for the Pharmaceutical Application

Eman Hussein Abed[1,*], Nadia Imad AL Ameen[2], Liqaa Ali Jazaa[2]

[1]*Department of Ecological Biotechnology, Environment and water Directorate, Ministry of Sciences & Technology, Baghdad, IRAQ;* [2]*Science College for Women, Ministry of Research and Higher Education, Baghdad, IRAQ.*

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Abstract: Chitin and chitosan are a natural polysaccharide prepared by processing Kentish snail exoskeleton's (species *Monacha cantiana*) which involves by partial deacetylation of chitin. Chitosan considered the second most abundant natural polymer. Many researchers have found that chitosan very important bioactive polymer as biodegradable and nontoxic material which made wide applicability in conventional pharmaceutics as a potential formulation excipient. This study is interested in the extraction of chitosan, with low molecular weight and suitable, for pharmaceutical industry. The crude chitin was collected from exoskeleton of *Monacha cantiana* specimens to obtain chitosan. The current study was synthesized from chitosan usage for the pharmaceutical industry.

Keywords: *Chitin, extraction, chitosan , pharmaceutical industry, biodegradable.*

Introduction

Chitin is a white colour, hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well every bit in the inner structure of invertebrates (ASTM, 2003). The waste containing of these natural polymers was a major source of surface contamination in coastal regions. Chitin is the second most natural polysaccharide after cellulose on earth and is composed of β (1-4) - linked 2-acetamido-2deoxy-β-D-glucose 1(N-acetyl glucosamine) (Dutta *et al.*, 2002). Figure 1 shows Chitin and Chitosan chemical structure.

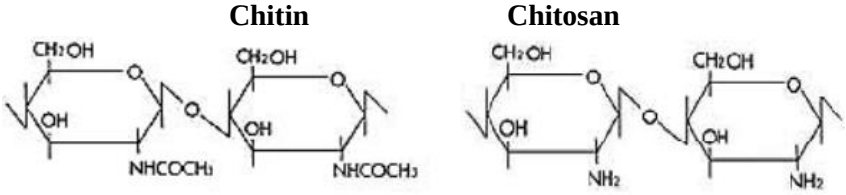

Figure 1. Structure of Chitin and Chitosan

The structure of chitosan is a straight-chain composed of D-glucosamine and N-acetyl-D-glucosamine obtained by the partial deacetylation of chitin, It's structure was similar to cellulose, which is composed of only one monomer of glucose (Muzzarelli, 1997). Reaction of chitosan is considerably more versatile than cellulose due to the presence of NH_2 groups. Chitin and chitosan are biopolymer according to the degree of deacetylation (DD) determining by the proportion of D-glucosamine and N-acetyl- D-glucosamine (ASTM, 2003).

Chitosan was defined as solubility material, biodegradability, reactivity, and adsorption of many substrates depending on protonated amino groups present in the polymeric chain, proportion of acetylated and non-acetylated D-glucosamine units (Kurita, 2006). Solubility of chitosan distinguished by amino groups with pKa smaller than 6.2 (Klug *et al*.,1998), and after stirring in acids such as hydrochloric, acetic, perchloric and nitric acid, in addition insoluble in organic solvents and water (Guibal,2004). Chitosan is considered as the most valuable polymer for biomedical and pharmaceutical applications due to its biodegradability, biocompatibility, antimicrobial, non-toxicity, and anti-tumor properties.

Nanoparticles, microspheres, hydrogels, films, and fibers are typical chitosan based on biomedical and pharmaceutical applications, such as nasal, ocular, oral, parenteral and transdermal drug delivery

**Corresponding: E-Mail: suaad_b1978@yahoo.com, Tel: 07804524950;*

(Kumar, 2000). Also medical applications of chitin and chitosan are inhibiting fibroplasia in wound healing and promote differentiation tissue (Le *et al*., 1997). In addition its potential applications in wastewater treatment and removing metal ions (Lim & Hudson, 2003).

Engineering tissue referred to replace unwell body parts with biodegradable and elastic tissues for contractile tissues such as blood vessels and heart valves, so Cross-linked chitosan hydrogels can prepared by reaction between the N-sussinyl-chitosan and aldehyde group of oxidizedhyaluronic acid. In capsulation of bovine chondrocytes within chitosan hydrogel supported cell survival and the cells for tissue regeneration due to its ability to form under mild conditions (Kathuria *et al*.,2009; Tan *et al*.,2009). The research goal to study extraction chitin from kentish snail exoskeleton shells *Monacha cantiana* (Montagu, 1803) (Pulmonata: Hygromiidae) for pharmaceutical application (Ali *et al*., 2012).

Materials and Methods

Sample preparation

Specimens of land snails were collected from (Al- Jadiriyiah) site irrigated with plenty of mostly citric trees shed (33 17' 03" N, 44 23' 30" E) ; vegetables and palm trees from Al- Zaafaraniayh site and citric trees(33 14' 32" N44 27' 54" E), and removing from the animal (Figure 2). The land snail shell collected are placed in Ziploc bags and refrigerated overnight approximately 1500 gm of crushed land snail shell wet samples were placed on foil paper and measured using a balance. Land snail shell was crushed into smaller pieces using a meat tenderizer. And drying for 4 days at 65°C, then measured and divided into four equal parts for efficient material handling (Toan, 2009).

Figure 2. Map of collection sites

Crude chitin was extracted after washing crushed shells then, placed on beakers and soaked with boiling sodium hydroxide solution (2-4% w/v) for one hour in order to dissolve proteins and sugars (Lertsutthiwong *et al*., 2002), then snail shell samples were removing and allowed to cool for 30 minutes at room temperature before crushed to pieces about (0.5-5.0) mm by using meat tenderizer (Lamarque *et al*., 2005).

Demineralization

The grounded shells were demineralizing by using 1% HCl for four times, and alloweded to soak for 24 hrs to remove the minerals (mainly calcium carbonate) Trung *et al*. (2006), then treated with 50 ml of a 2% NaOH solution to decompose the albumen into water soluble amino-acids. The remaining chitin was washed with deionized water, and then drained off. The chitin was further converted into chitosan by the process of deacetylation (Huang *et al*., 2004).

Deacetylation

The deacetylation process is carried out by adding 50% NaOH then boiled at 100°C for 2 hrs. Samples were cooled for 30 min at room temperature, then washed continuously with the 50% NaOH

and filtered in order to retain the solid matter, (chitosan). The samples were left uncovered and dried at 110°C for 6 hrs. in oven to obtain a creamy white chitosan (Muzzarelli & Rochetti ,1985).

Chitosan analysis using acid hydrolysis and HPL

Fifty mg of chitosan samples was weighed into thick-wall glass (digestion tube), then added 2ml of 1% acetic acid solution and mixing in vortexes until forming a consistent gel. Chitosan was hydrolyzed by adding 10ml concentrated HCL and heated for 105 °C, then the designation mixture was cooled to room temperature, and added 1ml to solution of sodium borate 3.8gm, and 30ml DDW, completed volume to 50ml by using 0.2M borate buffer(pH 7.0).

Chitosan was derivatived by mixing 1ml of diluted neutralizing solution with 1ml of 10gm/ml Fmoc-Osu in acetonitrile, then reaction was allowed to completion at ambient temperature for at least 4 hrs. without agitation. Stander was diluted with 3ml HPLC mobile phase (0.05%TFA/CAN, 1:1(V/V)) for analysis.

Chitosan concentration calculated: As following equation:

$$\frac{\text{Standard C \%}}{\text{Sample C\%}} = \frac{\text{Area of Standard}}{\text{Area of Sample}}$$

$$\frac{\text{St. C \%}}{\text{Sa. C\%}} = \frac{11076.250}{5167.384}$$

$$\text{St. C \%} = \frac{0.003 \times 11076.250}{5167.384}$$

$$\text{Sa. C \%} = 0.006430$$

Results and Discussion:

The synthesis of chitosan involves various chemical steps such as preparation of chitin from the crude shells removal proteins following demineralization to remove carbon and other salts present in the crude, and deacetylation process. The regular chitosan is obtained by above steps. Buta polymer of pharmaceutical grade has to fall in the region of its predetermined quality aspects and usually commercial chitins are prepared by a first step of deproteinisation followed by a second step of demineralization. The FT-IR studies of chitosan from standard commercial species designed in (Figure 3-5).

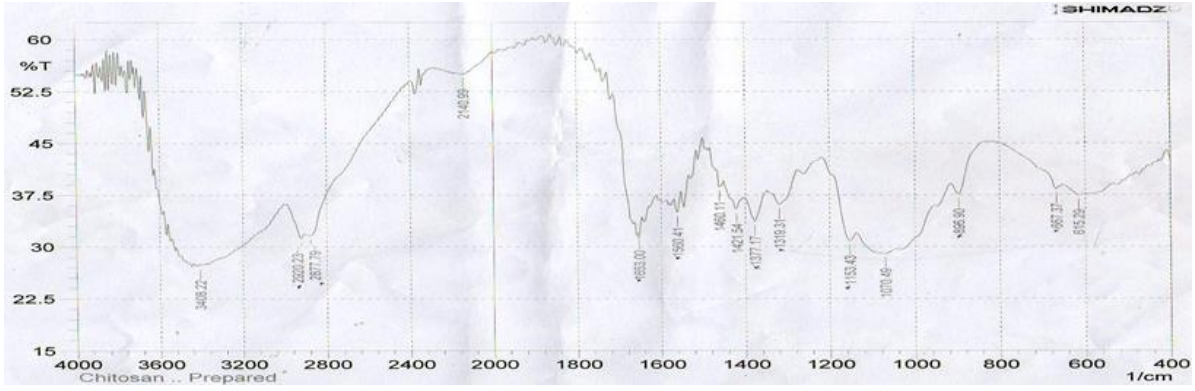

Figure 3. Chitosan sample analysis by FT-IR

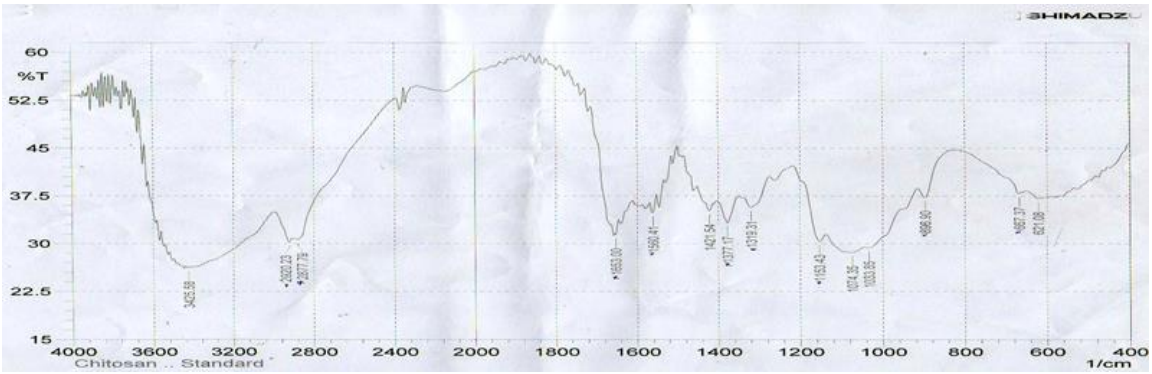

Figure 4. Chitosan Standard analysis by FT-IR

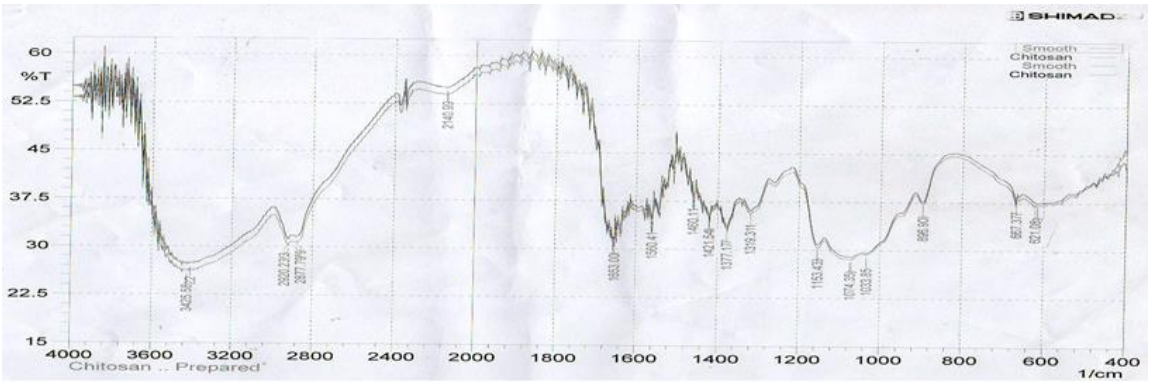

Figure 5. Chitosan sample and Standard analysis by FT-IR

The major absorption band for both standard and sample are observed between 2877.79 and 2920.23 cm^{-1}. Results showed that the same absorption band was observed at 2920.23, 2877.79, 1653.00, 1560.41, 1377.17, 1319.31, 1153.43, 896.90 and 667.37cm^{-1} which confirms the structure of chitosan in (Figure 6 & 7).

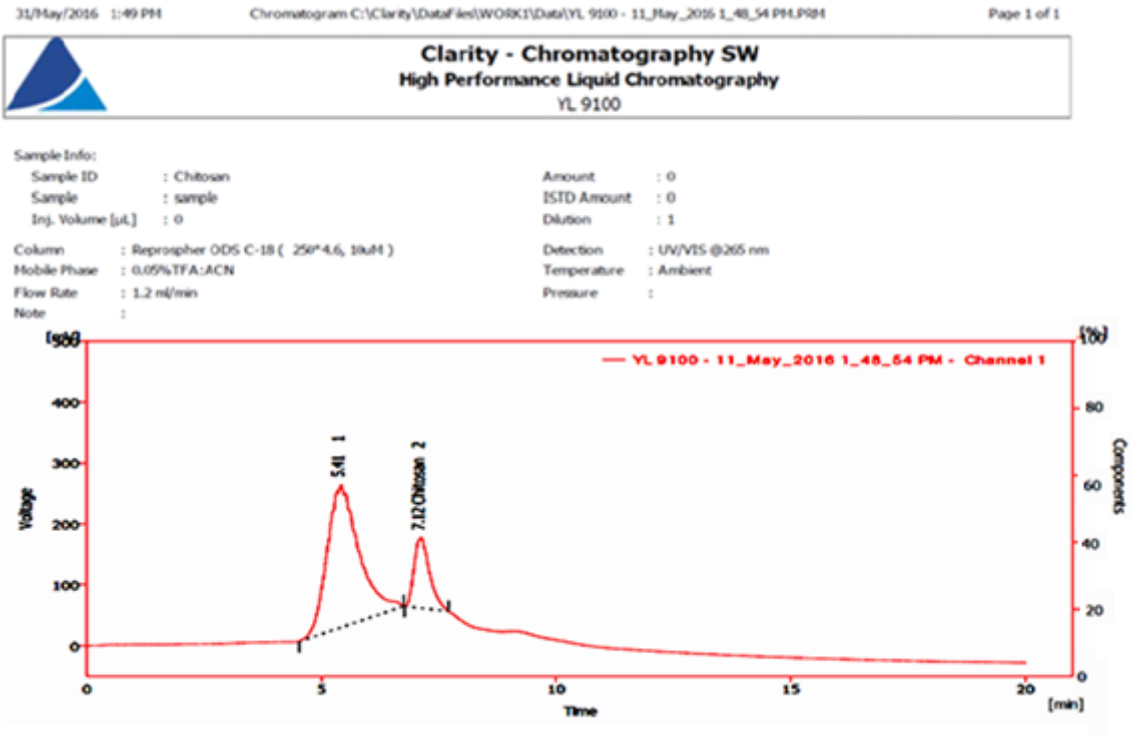

Figure 6. Chitosan sample analysis by HPLC

	Reten. Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)	Compound Name
1	5.407	11076.250	234.259	80.7	66.9	0.72	
2	7.123	2646.801	115.961	19.3	33.1	0.35	Chitosan
	Total	13723.051	350.220	100.0	100.0		

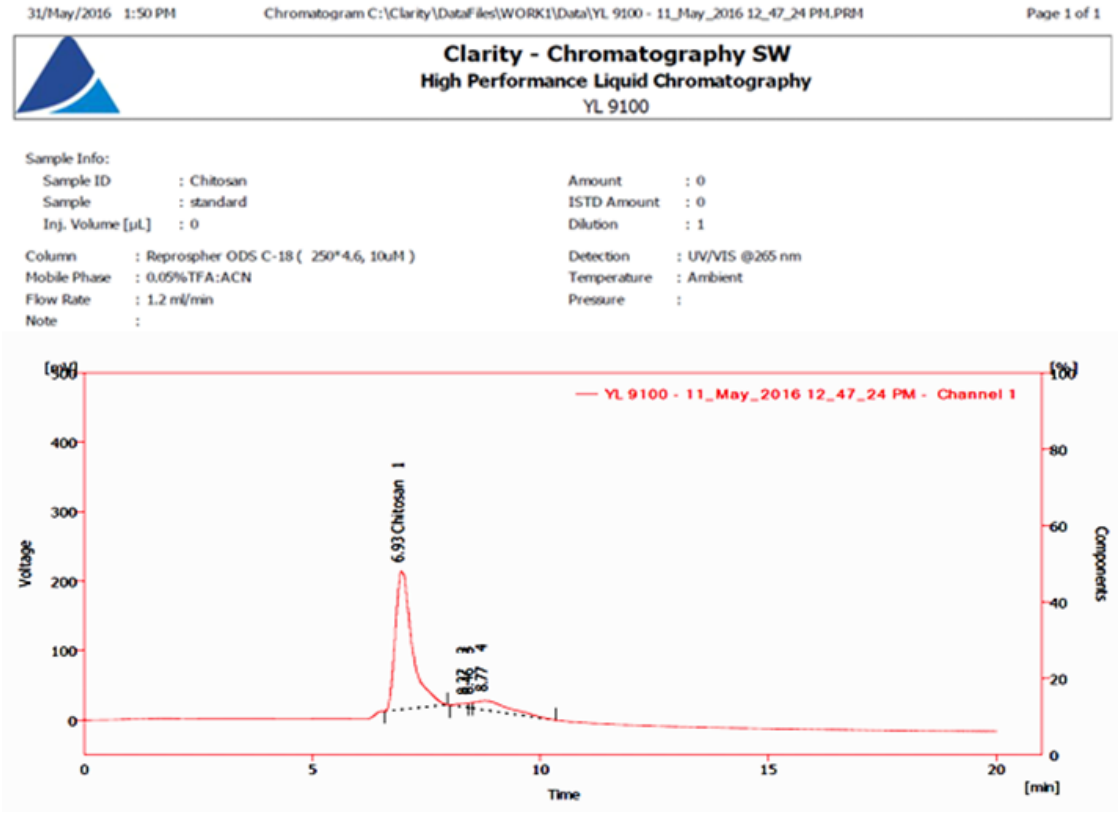

Figure 7. Chitosan standard analysis by HPLC

	Reten. Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)	Compound Name
1	6.932	5167.394	199.783	85.7	87.7	0.37	Chitosan
2	8.365	83.291	6.261	1.4	2.7	0.40	
3	8.465	44.825	7.869	0.7	3.5	0.10	
4	8.765	735.239	13.767	12.2	6.0	0.60	
	Total	6030.739	227.690	100.0	100.0		

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