REVIEW ARTICLE JPAR 2018

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Enzymatic preparation of low molecular weight chitosan

Anita Sharma M, I. Noorul Alam*, A.N. Rajalakshmi, V. Gopal

College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Punducherry, India.

Received: 20.02.2018 Revised: 23.02.2018 Accepted: 26.02.2018 Published: 28.02.2018

ABSTRACT: Chitosan is a natural polysaccharide, a deacetylated derivative of chitin. It is composed of β-(1-4) linked 2-amino-2-deoxy–D-glucopyranose and 2-acetamido-2-deoxy–D–glucopyranose. Chitosan has been considered as a functional biopolymer, which is used in various industrial applications such as food & nutrition, pharmaceuticals, cosmetics industries and agricultural industries. Due to its high molecular weight with fibre like structure, containing abundant hydrogen bonds, chitosan is only soluble in dilute acid, insoluble in water and tough to be assimilated by human bodies, prevents the widespread use of chitosan. Several methods have been used to lower the molecular weight of chitosan, like chemical depolymerisation by acid hydrolysis or redox reaction using O_3, $NaNO_2$, H_2O_2, Physical depolymerisation using Ultrasonic radiation or hydrodynamic shearing and Enzymatic depolymerisation. However chemical depolymerization has several drawbacks, including harsh conditions of hydrolysis, low yield of product etc. Enzymatic hydrolysis is more advantageous since it requires only mild condition to produce high yield product and also it retains the original molecular structure. Enzymatic degradation can takes place either with the use of specific enzymes like chitinase and chitosanase, but they are most expensive. Hence non-specific enzymes like cellulase, pectinase, pepsin, papain, protease, and amylase are most widely used for cost effective high yield production of low molecular weight chitosan (LMWC). This article discusses about the various enzymatic methods used in the preparation of low molecular weight chitosan.

Corresponding author *
Mr. Noorul Alam
Asst. Prof., Dept. of Pharmaceutics,
College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry, *India.*
Tel: +91 9843779210
E-mail: ceuticsalam@gmail.com.

Key words: Chitosan, Low molecular weight chitosan (LMWC), Enzymolysis, Enzymatic depolymerization.

INTRODUCTIONS:

Chitosan is a natural polysaccharide composed of β-(1-4) linked 2-amino -2-deoxy –D-glucopyranose and 2-acetamido-2-deoxy –D –glucopyranose. It is non-toxic, biodegradable and biocompatible. Chitosan is commercially produced by deacetylation of chitin, which is extracted from the exoskeleton of crustaceans (Such as shrimp, lobster, crabs and fish) and cell walls of fungi. It is widely used in various industrial applications [1-4]. Due

to its large molecular weight and the structure containing abundant hydrogen bonds, chitosan is only soluble in dilute acid, insoluble in water, and tough to be assimilated by human bodies, which hinders its application in pharmaceutical preparations drastically. If chitosan is degraded to low molecular weight less than 10000, its water solubility is greatly increased, conducive to human intestinal digestion and absorption. Low molecular weight, water soluble chitosan, oligomers of β-1,4- linked 2-amino-2-deoxy-D-glucopyranose (GlcN) and 2-acetamido -2-deoxy-D-glucpyranose (GlcNAc) has many special biological, chemical and physical properties such as antifungal, antibacterial and antitumor activities which are different from ordinary chitosan[5].

Low molecular weight inhibits the growth of bacteria and fungi[6-11] exerting antitumor activity[9,12,13], anti-atherosclerotic effects[14] acting as immune potentiating effectors[15], and eliciting pathogenesis – related proteins in higher plants[16]. Low molecular weight chitosan with an average molecular weight in the order of 5-20 kDa seem to have enhanced functional-biochemical significance compared to chitosans of higher molecular weights. According to Kondo, *et al*. [17] 20kDa chitosans prevent progression of Diabetes mellitus and exhibit higher for lipopolysaccharides than 140kDa chitosan. Low molecular weight chitosans of 5-10 kDa showed stronger growth inhibitory effect on several pathogens including *Fusarium oxyporum*, *Phomopsis fukushi*, *Alternaria alternate*. Felt et al and Ikeda et al demonstrated that LMWC, with an average molecular weight more than 5kDa prevented the rise of serum cholesterol of rats fed cholesterol-enriched diets for 14 days[18,19]. Low molecular weight chitosan also reduced the incidence of early pre-neoplastic markers of colon carcinogenesis[20], Suzuki et al found that chito-hexamer suppressed Sarcoma – 180 and Meth-A tumour growth in mice[21].

Degradation of chitosan into low molecular weight chitosan includes various methods like physical, chemical and enzymatic degradation. Chemical degradation can be carried out by acid hydrolysis or redox reaction using O_3, $NaNO_2$, H_2O_2, however has some defects, including harsh hydrolysis conditions, low yields, and chemical modification of the glucose rings. Physical degradation of chitosan requires special equipment and the resulting molecular weight cannot be controlled [22-24]. Enzymatic methods offers more advantages such as mild and specific reaction condition, high yield of product, no glucose ring modification and more facile to be controlled[3,24-26]. Most importantly enzymatic hydrolysis retains original biological properties of chitosan. Hence enzymolysis is more relevant and advantageous for the preparation of Low molecular weight chitosan from the practical point of view.

ENZYMOLYSIS OF CHITOSAN:

Enzymatic degradation process of chitosan for the production of low molecular weight chitosan can be carried out by either through specific enzymes or by through non specific enzymes[22,23].Specific enzymes which very effectively reduce the molecular weight includes chitinase and chitosanase[27-29]. But their practical application is limited, due to their high cost and limited availability. Hence most commonly non specific enzymes are used for the degradation process of chitosan. Non specific enzymes used in depolymerization of chitosan includes α-amylase[30,31], Cellulase[32-34], Pectinase[35-38], Pepsin[24], Papain[39,40], neutral Protease[41] and Lipase [42].

Preparation of LMWC using Complex Enzymes:

Huafei, *et al.* [43] prepared low molecular weight Chitosan by complex enzymes. The complex enzymes composed of commercial α-amylase, cellulase and pectinase. The enzymatic degradation for 2 h. under the conditions of zymolyte ratio 1:5 (m/m), pH 5.3 and temperature 56˚C resulted in low molecular weight Chitosan ranged from 1000 to 4000 Da and production yield reached more than 90% with good water solubility.

Preparation of LMWC using Proteolytic enzymes:

Vishnu, *et al.*[44] prepared depolymerised Chitosan by using Proteolytic enzymes. Chitosan solution (1% dissolved in 1% acetic acid and pH adjusted with 0.1N HCl/NaOH) was treated with enzymes (papain, pepsin and pronase) in the ratio 100:1 (w/w), incubated for different periods at optimum conditions (temperature of 30-45˚C and pH ranges from 1.5-7). The reaction was arrested by heat denaturing the enzyme and adjusting the pH of the reaction mixture to 7.0 using 2N NaOH. The precipitate (LMWC) obtained after centrifugation was washed and dialyzed against water using a membrane having M_w cut-off 2KDa and freeze dried. Molecular weight of low molecular weight Chitosan was determined by Gel Permeation Chromatography and

HPLC. The depolymerization reaction obeyed Michaelis-Menten kinetics and K_m and V_{max} values indicated higher affinity of pepsin towards chitosan. The chitosanolytic products were low molecular weight chitosans (LMWC, a major product), chitooligomers (COs) as well as monomers. Low molecular weight chitosans had molecular weight in the range, 4.1–10.0 kDa depending on the reaction time.

Preparation of LMWC using α-Amylase:

Shengjun [45] prepared water soluble Chitosan using α-amylase. About 1% w/w of chitosan was dissolved in 1% v/v aqueous acetic acid and pH was adjusted to 5.0 using 1M NaOH. 20 mg of α-amylase was added into a reactor containing 500 ml of chitosan solution and then maintained in a thermostatic water bath at 50˚C for 4 h. The aliquots of reaction mixture were periodically with drawn, filtered and heated at 95˚C for 15 min to terminate the reaction. The aliquots of hydrolysate were neutralized with 1M NaOH, filtered concentrated to 16% (w/v) and precipitated with ethanol. The precipitate was then filtrated through a pre weighed Whatman GF/A filter, dried at 60˚C for 3 h and finally crushed the percentage recovery of water soluble Chitosan was calculated. Under these conditions, the average molecular weight of chitosan decreased to 730 Da.

Preparation of LMWC using Pepsin:

Foua, *et al*.[46] optimized and characterized the chitosan enzymolysis by Pepsin. Response Surface morphology was launched to investigate the influence of process variables on the degree of hydrolysis (DH) followed by a Box-Behnken design approach. The optimized conditions were a 10.0-g/l chitosan concentration of pH 4.0 at 50^{o}C, a 110-mg/l pepsin concentration and enzymolysis time of 70 min, where the predicted value of the DH was 91 %. Based on the characteristic analyses by FTIR, UV-VIS and SEM, hydrolyzed product LMWCs almost retained the backbone of the chitosan macromolecular structure. The breaking of the C-O-C glycosidic bond led to chain scission and the formation of carbonyl groups. Therefore, the author concluded that this degradation method was feasible, convenient and potentially applicable.

***Preparation of LWMC using Pepsin with PQCI*:**

Tao, *et al*. [47] degraded chitosan by pepsin using Piezoelectric Quartz Crystal Impedance Analysis Technique (PQCI). Chitosan solution (15 ml) was added to a 25 ml detection cell; the solution was first stirred for 5 min at 45˚C and then sonicated for 3min to eliminate any gas bubbles. After that, the PQCI sensor was immersed in the solution and the detection cell was placed into a thermostatic bath to incubate at ambient temperature. After the temperature reached the measurement temperature, 20 µl of the stock pepsin solution was injected into the detection cell and the variation of the impedance response parameters was monitored by an HP4192A impedance analyzer in real time. The investigated solution was stirred during the degradation process. The degradation conditions were investigated in detail. The results show that the optimal pH value and temperature were 4.6 and 55˚C, respectively. PCQI analysis technique is perhaps a potential method for determining the degree of deacetylation. When compared to other methods, PQCI analysis technique can not only avoid cumbersome and tedious operation, but also monitor the degradation process in real time.

Preparation of LMWC using crude cellulose:

Xie, *et al*.[48] depolymerised Chitosan using crude cellulase when accurately weighed chitosan was dissolved in 1% (v/v) of acetic acid solution. One percent (w/v) of enzyme solution was then added to the chitosan solution. The reactions were conducted by incubation at different temperatures in a temperature-controlled water bath shaker. The reaction time was varied to obtain material with different degrees of polymerization. At the end of the specific time period, the reaction was stopped by boiling the mixture for 10 min to denature the enzyme. Then the enzyme was removed by filtration under reduced pressure. The apparent viscosity, intrinsic viscosity and viscosity average molecular weight were calculated. The optimum conditions for enzymatic hydrolysis were investigated. In the selected conditions (temperature 50^{0}C, pH 5.0 and enzyme to substrate ratio of 1:5), Chitosan was hydrolyzed for 1,4, 8 and 24 h, its viscosity –average molecular weights were found to be 3. 49 × 10^4, 1.18 × 10^4, 5.83 × 10^3, and 1.13 × 10^3 respectively.

Preparation of LMWC using papain:

Zeng, *et al*.[49] enzymolysed Chitosan using papain. The enzymolysis of the chitosan were performed by applying response surface methodology (RSM). Papain solution (1.0 g/l) was obtained by dissolving the enzyme in PBS buffer (0.1 mol/l, pH 7.0). Stock solution of chitosan

(18.0 g/l) was prepared in 0.2 mol/l acetate buffer solution and it was diluted into the concentration varied from 0 to 12.0 g/l. According to the experimental design, different concentrations of papain were added into the chitosan solution. The enzymolysis was performed by employing a shake flask method with 150 ml flask in a temperature-controlled water bath. After 50 min, the reaction was quenched by heating treatment at 100° C for 10 min. The reaction mixture was adjusted to pH 7.0 with 0.2 mol/l NaOH, and then centrifuged at 3000 rpm for 4 min, resulting in a precipitate of low molecular weight chitosan (LMWC) and a supernatant containing both chitosan oligosaccharides and monomers. The supernatant was used for HPLC analysis and the soluble reducing sugars (SRSs) measurement. The precipitate (LMWC) was lyophilized and weighed. All the experiments were carried out in triplicate under the same condition and average values are reported. In the kinetic experiments, the reaction was monitored at regular intervals and the SRSs content was detected. The optimized conditions were chitosan substrate concentrations 7.98 g/l., pH 4.55 and temp. 44.44^0 C.

Preparation of LMWC using Extracellular enzymes:

Shadia, *et al*.[50] prepared low molecular weight chitosan using extracellular enzymes. The extracellular enzymes preparations (EEP) produced by *B. alvei* strain was performed according to the method as described by Novikov and Mukhin[51]. To the Stock (200 ml) solution of chitosan (1%) in a 0.2M sodium acetate buffer at pH 5.5, 50 ml of the EEP was added. The reaction mixture was incubated at 37°C for 3 h (without stirring). The degree of chitosan hydrolysis was measured by viscosity reduction of the chitosan solution using a Brookfield viscometer type LV (at 50 rpm). After measuring the reduction in viscosity of the chitosan solutions, LMWC was obtained. The optimum temperature for the enzymatic hydrolysis was around 40 ^{0}C and the optimum pH was 5.5. Low molecular weight water soluble chitosan with 5 kDa was obtained.

CONCLUSION:

Low molecular weight chitosan is a topic of growing interest, because of its water solubility, advantage and wide applications in various fields. Hence various methods are adopted for high yield and cost effective production of low molecular weight chitosan. Considerably chemical, physical and enzymatic methods of hydrolysis are followed. But enzymolysis is considered superior because of its various advantages like retain of original structure (no ring modification) and mild reaction conditions giving high yield. Therefore enzymatic hydrolysis of chitosan could be done effectively and efficiently using various specific enzymes like chitinase and chitosanase and non specific enzymes like cellulase, pectinase, papain, pepsin, amylase.

ACKNOWLEDGEMENT:

Author wish to thanks Authorities of Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry, for providing Library facility for literature survey.

Table 1. Different approaches of enzymolysis of Chitosan.

Sl. No	Sample & Molecular Weight	Enzymes used	Degradation conditions	Molecular weight of LMWC
1	Chitosan (DD- 92%), Mol. Wt. 100000 Da.	Complex enzymes composed of commercial α-amylase, cellulase & pectinase.	Zymolyte ratio 1:5 (m/m), pH 5.3 and temperature 56°C	1000 to 4000 Da.
2	Chitosan (produced from shrimp chitin)	Proteolytic enzymes (papain, pepsin and pronase)	Temperature 30-45°C and pH ranges from 1.5-7. Substrate enzyme ratio 100:1 (w/w).	4.1 to 10.0 kDa
3	Chitosan (DD-93.5%) Mol. Wt. $41x10^4$ Da)	α-amylase	Temperature 50°C pH 5.5. 20 mg α-amylase/500 ml of reaction mixture.	730 Da (Avg)
4	Chitosan (DD- 91%)	Pepsin	Temperature 50°C pH 4.0. 110-mg/l pepsin, 10.0-g/l chitosan	$9 – 25x\ 10^2$ Da
5	Chitosan (DD-84%) Mol. Wt.5.18×10^5 Da)	Crude Cellulase	Temperature 50°C pH 5.0. Enzyme to substrate ratio 1:5.	Viscosity – avg mol wt. 1.13×10^2 to 3.49×10^4 Da
6	Chitosan (Mol wt 88000 Da)	Extracellular Enzymes Preparation (EEP- *Bacillus alvei*)	Temperature 40°C pH 5.5. 50 ml of the EEP (200 ml) solution of chitosan (1%)	5 – 20 kDa

REFERENCES:

1. Ravi Kumar MNV. A review on chitin and chitosan application. React Funct Polym, 2000; 46(1): 1-27.
2. Jayakumar R, Menon D, Manzoor K, Nair SV, Tamura H. Biomedical applications of chitin and chitosan based nanomaterials—a short review. Carbohydr Polym, 2010; 82(2): 227–232.
3. Kim SK, Rajapakse N. Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review. Carbohydr Polym, 2005; 62(4): 357–368.
4. Li J, Du Y, Liang H. Influence of molecular parameters on the degradation of chitosan by a

commerciall enzyme. Polym Degrad Stab, 2007; 92(3): 515–524.
5. Kang B, Dai YD, Zhang HQ, Chen D. Synergetic degradation of chitosan with gamma radiation and hydrogen peroxide. Polym Degrad Stab, 2007; 92: 359-362.
6. Xu JG, Zhao XM, Han XW, Du YG. Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. Pesti Biochem Physiol, 2007; 87: 220–228.
7. Hu Y, Du YM, Yang JH, Tang YF, Li J, Wang XY. Self-aggregation and antibacterial activity of N-acylated chitosan. Polymer, 2007, 8: 3098–3106.
8. Chang WT, Chen YC, Jao CL. Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. Bioresour Technol, 2007; 98: 1224–1230.
9. Wang SL, Lin HT, Liang TW, Chen YJ, Yen YH, Guo SP. Reclamation of chitinous materials by bromelain for the preparation of antitumor and antifungal materials. Bioresour Technol, 2008; 99: 4386–4393.
10. Chung YC, Chen CY. Antibacterial characteristics and activity of acid-soluble chitosan. Biores Technol, 2008; 99: 2806–2814.
11. Tsai GJ, Zhang SL, Shieh PL. Antimicrobial activity of a low-molecular-weight chitosan obtained from cellulase digestion of chitosan. J Food Prot, 2004; 67: 396–398.
12. Qin CQ, Du YM. Enzymic preparation of water-soluble chitosan and their antitumor activity. Int J Biol Macromol, 2002; 31: 111–117.
13. Qin CQ, Zhou B, Zeng LT, Zhang ZH, Liu Y, Du YM, Xiao L. The physicochemical properties and antitumor activity of cellulose-treated chitosan. Food Chem. 2004; 84: 107–115.
14. Je JY, Kim EK, Ahn CB, Moon SH, Jeon BT, Kim BK, *et al.* Sulfated chitooligosaccharides as prolyl endopeptidase inhibitor. Int J Biol Macromol, 2007; 41: 529–533.
15. Zaharo DA, Rogers CJ, Hance KW, Schlom J, Greiner JW. Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination. Vaccine, 2007; 25: 2085–2094.
16. Agarawal GK, Rakwal R, Tamogami S, Yonekura M, Kubo A, Saji H. Chitosan activates defense/stress response(s) in the leaves of *Oryza sativa* seedlings. Plant Physiol Biochem, 2002; 40: 1061–1069.
17. Kondo Y, Nakatan A, Hayashi K, Ito M. Low molecular weight chitosan prevents the progression of low dose streptozotocin induced slowly progressive diabetes mellitus in mice. Biol Pharm Bull, 2000; 23: 1458-1464.
18. Felt O, Carel A, Bachni P, Buri P, Gumy R. Chitosan as tear substitute: a wetting agent endowed with antimicrobial efficacy. J ocul Pharmacol Ther, 2000; 16: 261-270.
19. Ikeda I, Sugano M, Yashida K, Sasaki E, Iwamoto Y, Hatono K. In: Kritchevsky D, Bonefiled C. Editors. Dietry fibre in health and disease. USA: Eagen press; 1995. pp. 45-53.
20. Torzas TL, Kendall CWC, Sugano M, Iwamoto Y, Rao AV. The influence of high and low molecular weight chitosan on colonic cell proliferation and aberrant crypt foci development in CFI mice. Food Chemical Toxicol, 1996; 34: 73-77.
21. Suzuki K, Milkami T, Okawa Y, Tokoro A, Suzuki S, Suzuki M. Anitumor effect of hexa- N-acetylchitohexaose and chitohexaose. Carbohydr Res, 1986; 151: 403-408.
22. Tsao CT, Chang CH, Lin YY, Wu MF, Han JL, Hsieh KH. Kinetic study of acid depolymerization of chitosan and effects of low molecular weight chitosan on erythrocyte rouleaux formation. Carbohydr Res, 2011; 346(1): 94–102.
23. Yoon HG, Kim HY, Lim YH, *et al.* Thermostable chitosanase from Bacillus sp. Strain CK4: cloning and expression of the gene and characterization of the enzyme. Appl Environ Microbiol, 2000; 66(9): 3727–3734.
24. Roncal T, Oviedo A, De I, Armentia L, Fern AL, Villar MC. High yield production of monomer-free chitosan oligosaccharides by pepsin catalyzed hydrolysis of a high deacetylation degree chitosan. Carbohydr Res, 2007; 342(18): 2750–2756.
25. Wu S. Preparation of water soluble chitosan by hydrolysis with commercial α-amylase containing chitosanase activity. Food Chem, 2011; 128(3): 769–772.
26. Xie Y, Hu JG, Wei Y, *et al.* Preparation of chitooligosaccharides by the enzymatic hydrolysis of chitosan. Polym Degrad Stab, 2009; 94: 1895-1899.
27. Cheng CY, Li YK. An Aspergillus chitosanase with potential for large scale preparation of chitosan oligosaccharides. Biotechnol Appl Biochem, 2000; 32: 197-203.

28. Ogawa K, Chrispinas OA, Yoshida N, Inoue J, Kariya K. Chitosanase, Its manufacture, and manufacture of chito-oligosaccharides. Jpn, Kokai Tokkyo Koho, 2001; 6(9): 975-982.
29. Santos ES, Guirardello R, Francon TT. Purification of chitosanase from *Bacillus cereus*. In: Muzzarelli RAA, editor. Chitin Enzymology. Italy: Ace; 2001.
30. Yu HY, Wang S. Study on enzymatic production of low molecular weight of chitosan. Food Sci, 2008; 29(8): 464-466.
31. Zhang WQ, Xia W, Xu H, *et al.* Reaction characteristics of chitosan hydrolysis catalyzed by non-specific enzymes. J Funct Polym, 2003; 16(1): 44-48.
32. Lin SB, Lin YC, Chen HH. Low molecular weight chitosan prepared with the aid of cellulase, lysozyme and chitinase: characterisation and antibacterial activity. Food Chem, 2009; 116: 47-53.
33. Xia WS, Liu P, Liu J. Advance in chitosan hydrolysis by non-specific cellulases. Bioresour Technol, 2008; 99: 6751-6762.
34. Han YP, Lin Q. Preparation of oligochitosan by cellulase depolymerization with membrane technology. Chemical World, 2007; (2): 98-101.
35. Kittur FS, Kumar ABV, Tharanathan RN. Low molecular weight chitosan preparation by depolymerization with *Aspergillus niger* Pectinase, and characterization. Carbohydr Res, 2003; 338: 1283-1290.
36. Sardar M, Roy L, Gupta MN. A smart bioconjugate of alginate and pectinase with unusual biological activity toward chitosan. Biotechnol prog, 2003; 19: 1654-1658.
37. Ipsita R, Meryam S, Munishwar NG. Hydrolysis of chitin by PectinexTM. Enzyme Microb Technol, 2003; 32: 582-588.
38. Cabrera JC, Custsem PV. Preparation of chitooligosaccharides with degree of polymerization higher than 6 by acid or enzymatic degradation of chitosan. Biochem Eng J, 2005; 25: 165-172.
39. Li L, Guo SY, Cai MY. Characteristics of chitosan degradation by papain. China Univ Sci Tech, 2003; 31: 71-75.
40. Lin H, Wang H, Xue CH, Ye M. Preparation of chitosan oligomers by immobilized papain. Enzyme Microb Technol, 2002; 31: 588-592.
41. Li j, Du YM, Yang JH, Feng T, Li AH, Chen P. Preparation and characterization of low molecular weight chitosan and chito-oligomers by a commercial enzyme. Polym Degrad Stab, 2005; 87: 441-448.
42. Lee DX, Xia WS, Zhang J. Enzymatic preparation of chito-oligosaccharides by commercial lipase. Food Chem, 2008; 111: 291-295.
43. Huafei X, Zhengyu J, Jinhui H, Cuirong Z. Preparation of low molecular weight chitosan by complex enzymes hydrolysis. Int J Chem, 2011; 3(2): 229-235.
44. Vishnu KAB,Tharanathan RN. A comparative study on depolymerization of chitosan by Proteolytic enzymes. Carbohydr Polym, 2004; 58: 123-129.
45. Shengjun W. Preparation of water soluble chitosan by hydrolysis with commercial α-amylase containing chitosanase activity. Food chem., 2011; 128; 769-772.
46. Bi Foua C, Alain G, Hong-Yan Z. A Dan Pan. Optimization and characterization of chitosan enzymolysis by pepsin. Bioeng, 2016; 3: 17-22.
47. Han T, Wanzhi W, Youan M, Shufen Z, Jianjun X. Study on degradation characteristics of chitosan by pepsin with piezoelectric quartz crystal impedance analysis technique. Anal Sci, 2005; 2(1): 1057-1061.
48. Yu X, Ya W, Jingang H. Depolymerization of chitosan with a crude cellulase preparation from *Aspergillus Niger*. Appl Biochem Biotechnol, 2010; 160: 1074-1083.
49. A-Dan P, Hong YZ, Gohi BF, Claude A,Yu-Qin L. Enzymolysis of chitosan by papain and its kinetics. Carbohydr Polym, 2016; 135:199-206.
50. Shadia M, Abdel A, Foukia EM. Preparation of low molecular weight chitosan by extracellular enzymes produced by *Bacillus alvei*. J Appl Sci Res, 2008; 4(12): 1755-1761.
51. Novikov V, Mukhin V. Chitosan depolymerization by enzymes from the hepatopancreas of the crab *Paralithodes camtschaticus* Prikl. Biokhim Microbiol, 2009; 39: 530-535.

Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Anita, *et al*. Enzymatic preparation of low molecular weight Chitosan. J Pharm Adv Res, 2018; 1(1): 27-32.