INFLUENCE OF CENTRIFUGATION ON THE MOLECULAR PARAMETERS OF CHITOSAN SOLUBILIZED IN WEAKLY ACIDIC AQUEOUS SOLUTIONS

D. DIMONIE, S. O. DIMA, M. PETRACHE*
National R&D Institute for Chemistry and Petrochemistry – ICECHIM, 202 Spl. Independentei, Bucharest, Romania

Due to centrifugation, the molecular mass distribution of chitosan is changed from monomodal to bimodal. Because of centrifugation, the polydispersity index of population with molecular weight ($M_w$) higher than $10^3$-$10^4$ g/mol becomes close to 1 and the polydispersity index of the population with $M_w$ lower than $10^2$-$10^3$ g/mol becomes very wide. After centrifugation, the proportion of chitosan main populations of small molecules and macromolecular chains depend on solution acidity, as a result of the influence of protonation degree on the macromolecules conformation. The obtained results can be understood as a possible small scale mechanical destruction of chitosan in weakly acidic aqueous solution in centrifugal conditions, which decreases the proportion of population with high $M_w$ in favour of small molecules and oligomers.

(Received September 18, 2013; Accepted December 3, 2013)

Keywords: Hydrogels, Chitosan, protonation, Molecular weight, Polydispersity index

1. Introduction

Chitosan is a polycationic, hydrophilic, binary heteropolymer prepared through fully or partially N-deacetylation of chitin with NaOH [1, 2]. Chitosan consists of β (1-4)2-acetamido-2-deoxy-β-D glucopyranose (N-acetyl glucosamine) and 2-amino-2-deoxy-β-D-glucopyranose (D-glucosamine) units, randomly or block distributed throughout the macromolecules, the units distribution depending on the chitin source and the processing method to derive the biopolymer (Fig.1) [3-8].

![Chemical structure of chitin and chitosan](image)

Fig.1 Chemical structure of “ideal” chitin (A), “ideal” chitosan (B) and “real” chitin and “real” chitosan (C) (Chitin p > 50 %, chitosan q > 50 %) [8]

The chitin sources and the biopolymer preparation method influence structural parameters like number of amino and N acetyl amino units, molecular weight, deacetylation degree (DD), polymer chain arrangement, and others, such as purity, parameters that make differences between various grades of commercially available chitosan [7, 9-11]. Because the

*Corresponding author: avy_marius2005@yahoo.com
distribution of amino and N acetyl amino units is, generally, random, it is easy to generate conformational features through intra- and inter-molecular hydrogen bonds [12, 13]. For example, due to intra-residue hydrogen-bonding between the carbonyl oxygen of the N-acetyl group and the H6 in the following unit, rigid length of 220Å can be found on the chitosan macromolecules [13, 14]. The intra- and inter-molecular hydrogen bonds generate rather stiff molecules which makes chitosan a semi-crystalline polymer (showing polymorphism dependent by its physical state), thermo - mechanical degradable and insoluble in water, alkali and common organic solvents [2, 3, 7]. Increasing DD values lead to a more extended conformation and an even stiffer chain [15]. Its high viscosity and low solubility, especially in case of types with high molecular weights, are the main limitations in the use for several applications [2]. Depending on the various sources and preparation methods, chitosan is normally polydisperse [3], with molecular weights from around 50 g/mol up to 2·10^6 g/mol [16-23].

To determine the chitosan molecular weights, different methods have been proposed. One of them is the dilute solution viscometry applied for the determination of intrinsic viscosity of a polymer solution, this being related to the viscosity average molecular weight, Mv [24]. This viscosity technique is not an absolute technique, requiring calibration experiments with samples of known molecular weights. Multi-angle static laser light scattering (MALLS) is the most accurate technique for determining the molecular weights, the absolute molecular weights in this case, by detecting how samples scatter light at different discrete angles. This technique was applied for determination of chitosan degradation in 0.1 M acetic acid solution by microwave radiation [24] and corroborated with gel permeation chromatography (GPC). GPC is a type of size exclusion chromatography (SEC) that separates analytes on the basis of molecule size and that is also employed to determine the molecular weight of natural and synthetic polymers [24-27]. GPC separates analytes based on the size or hydrodynamic volume (radius of gyration) of the analytes. This differs from other separation techniques which depend on chemical or physical interactions to separate analytes.

The stability of chitosan solution has been extensively studied [28, 29]. The stability of dilute chitosan solution in formic acid and lactic acid were better than in acetic acid and hydrochloric acid [29]. Although dissolution of chitosan in dilute acetic acid is the base of most applications of chitosan, only limited data are available on the chitosan degradation in organic acid [28].

The paper is devoted to study the influence of centrifugation on the molecular parameters of chitosan solubilised in water with various acidity degrees.

2. Experiments

1.5 % chitosan solutions, (Sigma Aldrich, P code 448877 -50 G, 81.76 % DD) in bidistilled water and 0.05 M – 0.15 M acetic acid (Sigma-Aldrich, P Code 24,285 – 3) were prepared at room temperature, by polymer adding in small portions and stirring with 100 rpm for 4 hours. Air bubbles were eliminated by keeping the solution at room temperature for 2 hours. To remove dust and other impurity traces, after 24 hours from preparation, each solution were centrifuged at 3000 rpm for 30 min and then were conditioned by microfiltration on polytetrafluoroethylene microfilters with 0.2 μm pore size. Freshly prepared solutions were used in all experiments.

Each solution was characterized as follows:

- Weight average molecular weight (Mw) and distribution of molecular weights (DMW) were evaluated using an Agilent 1200 Series GPC with refractive index detector, equipped with a Polymer Laboratories aquagel OH MIXED 8 μm column, dimensions L x Di= 300 x 7.5 mm and a G1310A-ISO HPLC Pump. As mobile phase was used a solution of acetic acid – water (1 mL/min flow rate) at 25°C, 50-60 bar, and 20 μL injection volume. The calibration curve of molecular weights was build with 12 polyethylene oxide/glycol standards, ranging from 10^6 to 1·215’000 g/mol. Polydispersity index (PI) PI = Mw/Mn. There were recorded the dependence of the population concentration on the Mw and the relationship between the mass fractions (W (logM), in constant molar mass increments) and Mw. Solution pH was carried out in triplicates using a pH meter INOLAB pH 730 type;
**Solution conductivity** was measured on a conductometer Seven Easy – Mettler Toledo type. Experiments were carried out in triplicates and the average and standard deviation values were reported.

### 3. Results

Experimental results have demonstrated that by centrifugation about 4-5% insoluble contents are removed from each solution and the solutions pH decreases after separation process and the molecular weights ($M_w$) have different values compared with the values before centrifugation.

The biggest change of $M_w$ determined by centrifugation was observed in the case of chitosan solubilization in 0.05 M acetic acid solution (fig. 2 and 3). The solution pH is 5.59 before and 5.57 after centrifugation. The population of molecules with $M_w = 10^2\text{-}10^4 g/mol$ represents about 15% before and 23% after centrifugation and those of molecules having $M_w = 10^3\text{-}10^4 g/mol$ is 16% before and 22.5 % after the separation process. The proportion of oligomers with $M_w$ between $10^4 g/mol$ and $10^5 g/mol$ is 52% before and decreases at 36% after centrifugation. The population of macromolecules with $M_w$ higher than $10^5 g/mol$ is 18% before and 15% after centrifugation. After centrifugation, the molecular species with $M_w 10^3\text{-}10^6 g/mol$ are mono-disperse because the PI is about 1 and those of molecules with $M_w$ between $10^7\text{-}10^8 g/mol$ are strongly poly-dispersed because PI is 7 and decreases to 1 (Fig. 2b). If before centrifugation the DMW is mono-modal, after centrifugation the distribution becomes bimodal containing two populations, un-equal as proportion, one with $M_w 10^7\text{-}2\cdot10^8 g/mol$ of about 17% and another with $M_w 2\cdot10^4\text{-}4\cdot10^5 g/mol$ of approx. 62% (fig. 2a, fig. 3a). The GPC theory explains that small molecules enter into the small pores of separation column, while big molecules enter only inside the big pores, so the retention time is lower (fig 3b) [30].

**Fig. 2** Dependency of the proportion of populations (a) and of polydispersity index (b) on $M_w$ for chitosan solubilised in 0.05 M acetic acid aqueous solution, before and after centrifugation

**Fig. 3** The distribution of molecular weights (a) and the elution diagram (b) for chitosan solubilised in water with 0.05 M acetic acid before and after centrifugation
The 0.075M acidic aqueous solution has a 5.07 pH before and 4.92 pH after centrifugation. If chitosan is solubilised in this solution then, because of centrifugation, the population of molecules with Mw up to $10^2$ g/mol increases from 5% to 17% and those of molecules with Mw $10^2$-$10^3$ g/mol rises from 17% to 20%. Also the population of the macromolecules with Mw ranged as $10^3$-$10^5$ g/mol decreases from 31% to 28%, those of macromolecules with Mw $3\cdot10^5$-$10^6$ g/mol diminishes from 10% to 4%, and population of macromolecules with Mw higher than $10^6$ g/mol drops from 4% to 1% (fig 4a). The population of molecules with Mw $10^5$-$10^4$ g/mol and those of macromolecules with Mw $10^0$-$3\cdot10^5$ g/mol remain almost at the same level after centrifugation (28% and 31%). PI varies, before and after centrifugation between 1 - 4, especially for molecules with Mw up to $10^5$ as well for the chains with Mw $3\cdot10^5$-$10^6$ g/mol which have smaller PI of about 1.2-2 (fig 4b). The DMW are changed from mono-modal before to bimodal after centrifugation, when coexist two populations, almost equal as proportion, one of molecules with Mw between $10^2$ g/mol and $4\cdot10^3$ g/mol of about 26% and another one with Mw between $2\cdot10^4$-$3\cdot10^5$ g/mol (fig 5a) for approx. 28%. The retention time in the separation column of high macromolecules is lower than that of small molecules which are easy eluted because of the retention into its pores (fig.5b).

![Fig.4 Dependency of the proportion of populations (a) and of polydispersity index (b) by Mw for chitosan solubilised in 0.075 M acetic acid aqueous solution, before and after centrifugation](image)

![Fig.5 The distribution of molecular weights (a) and the elution diagram (b) for chitosan solubilised in water with 0.075 M acetic acid before and after centrifugation](image)

The 0.1M acetic acid aqueous solution with 1.5 % chitosan has 4.6 pH before and 4.58 pH after centrifugation. The centrifugation of this solution determines the growth from 4 to 6% the population of molecules with Mw up to $10^2$ g/mol and from 16% to 20% those of molecules with Mw $10^2$-$10^4$ g/mol. The fractions of chain with Mw $10^5$-$3\cdot10^5$ g/mol which is approx. 10% and those of the chains with Mw ranged as $3\cdot10^2$-$10^6$ g/mol which represent almost 6% remain almost unchanged. However, the proportion of populations of oligomers with Mw $10^2$-$10^3$ g/mol is diminished from 31% to 26%, those of chains with Mw $10^4$-$10^5$ g/mol is reduced from 33% to...
27.5% and the population of macromolecules with Mw higher than $10^6$ g/mol is decreased from 3% to 1% (fig. 6a). PI is ranged as 1 - 3.2 before and between 1 - 2.5 after centrifugation. PI of population of molecules and macromolecules with $M_w$ $10^3$-$10^6$ g/mol narrows to 1-1.5 because of centrifugation (fig. 6b). DMW is mono-modal before and becomes bimodal after centrifugation when the two main populations are those of molecules with $M_w$ between $10^2$ – $10^4$ g/mol which is of about 45% and the other one with $M_w$ between $5\cdot10^4$ g/mol and $2\cdot10^5$ g/mol by approx. 25% (fig 7a). Separation time is shorter from high molecules and longer for smaller ones which remain more time into the pores of chromatographic column (fig 7b).

![Fig.6 Dependency of the proportion of populations (a) and of polydispersity index (b) by Mw for chitosan solubilised in 0.1 M acetic acid aqueous solution, before and after centrifugation](image1)

![Fig.7 The distribution of molecular weights (a) and the elution diagram (b) for chitosan solubilised in water with 0.1 M acetic acid before and after centrifugation](image2)

The 1.5 % chitosan aqueous solution with 0.15M acetic acid has 4.36 pH before and 4.28 pH after centrifugation. After centrifugation of this solution, the proportion of populations of macromolecules diminishes in favour of those of molecules and oligomers. The population of molecules with $M_w$ up to 100 g/mol grows from 6% to 15%, those of molecules with $M_w$ $10^2$-$10^3$ g/mol increases from 15% to 18% and those of oligomers with $M_w$ $10^3$-$10^4$ g/mol rises from 28% to 31%. All the other populations decrease, namely: those of chains with $M_w$ between $10^4$ g/mol and $10^5$ g/mol from 31% to 25%, for chains population with $M_w$ $10^5$-$3\cdot10^5$ g/mol from 13% to 10%, for chains fraction with $M_w$ $3\cdot10^5$-$10^6$ g/mol from 6% to 4% and for chains fraction with $M_w$ higher than $10^6$ g/mol from 3% to 1% (fig 8a). Due to centrifugation the chains population with $M_w$ bigger than $10^4$ g/mol has PI at around 1 (fig. 8b), DMW is changing from mono-modal before to bimodal after centrifugation (fig 9a) and the chains with high $M_w$ are separated first (fig 9b). After centrifugation the main population by approx. 45 % is those of chains with $M_w$ between $2\cdot10^2$ g/mol and $2\cdot10^4$ g/mol and the second one in proportion of 35% contains chains with $M_w$ $2\cdot10^4$ g/mol to $2\cdot10^5$ g/mol (fig 9a).
Fig. 8 Dependency of the proportion of populations (a) and of polydispersity index (b) by $M_w$ for chitosan solubilised in 0.15 M acetic acid aqueous solution, before and after centrifugation.

Fig. 9 The distribution of molecular weights (a) and the elution diagram (b) for chitosan solubilised in water with 0.1 M acetic acid before and after centrifugation.

Regardless the acid concentration, the non-centrifugal solutions conductivity is lower than those of centrifuged solutions (Fig. 10). The conductivity of non-centrifugal and centrifuged polymeric solutions is 3-4 times higher than those of acidic solutions without polymer. In all cases the conductivity are smaller for solutions with 0.05M acidity and growths with solution acidity, more at acid concentration increasing from 0.05M to 0.075M and less for acidity ranged as 0.075 M - 0.15M.

Fig. 10 Dependence of 1.5 % chitosan solution conductivity on the acetic acid concentration.
4. Discussion

If chitosan is converted by stoichiometric protonation of amino groups in stable form RNH$^+$ it can be solubilised in acidic solution because the presence of positive charges on its skeleton increases the repulsion between the different polymer chains, facilitating their solubilisation [31, 32]. Chitosan is soluble only in acidic solutions of pH below 6.5 (value is approximately the pKa of amino group) required to ensure the protonation of the primary amine value at which the repulsion between different polymer chains overcome the associative forces between chains. Chitosan solubilisation depends on the DD and $M_w$ of polymer and also on the acid type and concentration [33 - 35]. Chitosan can be dissolve in certain inorganic and organic acids such as hydrochloric acid, phosphoric acid, lactic acid, propionic acid, succinic acid, acetic acid, tartaric acid, citric acid and formic acid in mentioned pH conditions, after prolonged stirring [36, 37].

Chitosan macromolecules have in solution a semi-flexible road conformation or a rigid coil, depending on several parameters: protonation degree, value and molecular weight distribution, N-acetylglucosamine groups content, pH value [38-42]. Chitosan small macromolecules are linear, flexible and they change their conformation depending by the temperature value. High macromolecules are rigid asymmetric structures which do not change their conformation by increasing the temperature [42].

The chitosan chain behaviour in solution depends on polymer obtaining procedure and its DD. For ex. chitosan produced by heterogeneous deacetylation, with a block arrangement of acetylated and deacetylated units, have a tendency to form aggregates in aqueous [41 - 43]. Extensive aggregation and intermolecular interactions may reduce available sites on the chitosan molecule. DD > 50%, where associations of chitosan chains lead to the formation of stable aggregates [41- 43].

The behaviour of solubilised chitosan depends also on the solution pH. At pH below 4, most of the amino groups of chitosan are supposed to be protonated, and since this effect promotes electrostatic repelling between charged groups of the same sign, it leads to enhanced swelling of the polymer network [3, 38]. At pH 5.2, an unstable structure is generated. The free amino groups form intermolecular hydrogen bonds with the oxygen of the adjacent chains [39]. At pH value greater than 6.5 the size of the aggregates increases and phase separation occurs. The polymer coagulates and can be recovered as an amorphous solid [38, 40].

The intra- and inter-molecular hydrogen bonds which generate a stable and rigid semi-crystalline structure makes chitosan also degradable before melting because of the melt high viscosity, which is typical for polysaccharides with extensive hydrogen bonding. For getting chitosan with low molecular weight generally chitosan is degraded considering chemically, enzymatically or physically procedures [2].

Chemical degradation is mainly carried out by a scission mechanism of acid hydrolysis in which the acid of moderate strength (e.g., HCl, H$_3$PO$_4$, HNO$_2$), and to a lesser extent by bases, attacks the amino group from D-glucosamine units or the acetamido group from N-acetylglucosamine units, with subsequent cleavage of the adjacent glycosidic linkage [44]. The rate of degradation depends on the type and the concentration of the acid and on the temperature [45, 46]. The molecular weight of the degraded chitosan was influenced by the initial concentration and the source of chitosan. Chitosan with larger molecular weight was more sensitive to degradation the degradation rate being proportional with $M_w$ [47].

If the chemical attack is associated with mechanically stress than chemical attack weaken the glycosidic linkages and sufficient mechanical agitation break the weakened linkages yielding a polymer of low $M_w$ [47 – 48]. The known physical methods to mechanically degrade chitosan include: sonication [48], high pressure homogenization [49], shearing [50].

The centrifugation technique clearly influences the molecular weights distribution of different chitosan chains by dissociating what initially appears like one chitosan population in another two populations. The acidity of the samples influences also the molecular weights of chitosan samples, the molecular weight decreasing with the increasing of solution acidity from 0.05 M to 0.15 M.
However the obtained results can be understood as a possible small scale mechanical degradation of chitosan in conditions of centrifugation favoured by the acid concentration which decreases the proportion of population with high M_w in favour of those of oligomers and small molecules.

The studied chitosan contains low molecular species with M_w up to 10^4 g/mol, oligomers with M_w ranged from 10^4 g/mol to 5·10^4 g/mol and macromolecules with low, medium very high up to 10^6 g/mol macromolecules. In the 4 solutions in which was solubilised, chitosan has different protonation degrees, which correspond to the medium acidity, lower for 0.05 M solution and increasing for those with 0.075 M - 0.15 M acidity.

At the same N-acetylglucosamine (18.24%) content, polymer concentration and environment temperature, depending on Mw and protonation degree, the macromolecules will have in solution, different shapes. First of all if small molecules will be flexible, the macromolecules those will be either rigid if the prolongation degree is small as in the case of the 0.05 M solution which has pH 5.59, either as coil if the protonation degree is high as in case of 0.1M – 0.15 M solutions which have a pH of 4.6 - 4.36. It is obvious that, depending on the Mw value and protonation degree, the molecules have in solutions smaller or larger kinetics independency, lower for large macromolecules and smaller protonation degree as in solution with 0.05 M which means less acidic pH.

The effect of centrifugation in the same conditions on the chitosan molecules will depend on the kinetic independency of each molecular species from solution. In case of 0.05M acidic which has a pH by 5.57, the chitosan macromolecules are rigid because the amino groups which were not protonated forms inter-molecular bonds with the oxygen from adjacent chains generating certain structures with lower stability [39]. Also can be possible other inter-molecular interactions with effect on macromolecules conformation. It is possible that the mechanical stresses during centrifugation, helped by the acid from the solution, to weaken and ultimately to break the glycoside bond near the protonated amino group and generating in this way the destruction of high macromolecules and increasing the number of oligomers and low molecules [47 – 50]. Will be affected in a lesser extent by this mechanism, the macromolecules which were not protonated and which belong to rigid structures. The evidence that this finding can be true is the reality that only in case of the solution with 0.05M acidity, after centrifugation, 62% is represented by the population of macromolecules with M_w = 2·10^4 – (2-4)·10^5 g/mol population which represents only 28 – 35 % in case of solution with higher acidity of 0.075 M –0.15 M.

If chitosan was solubilised in solution with 0.15M acid (solution pH 4.36 before centrifugation) the protonation degree of amino group from D-glucosamine units is higher which means that a large number of macromolecules are kinetic independent. In the same centrifugation conditions, with the help of high acid quantity from solution, a bigger number of glycoside bound can be weakened and broken. In a solution with 0.15 M acid and pH near 4, the polymer macromolecules are more flexible and can take the coil conformation. The existence after centrifugation as representative populations those of molecules and oligomers with Mw up to (1-2)·10^5 - (1-2)·10^4 g/mol can be a proof that the destruction of chitosan coils occurs from their outside to their inside and also a explanation of small proportion of only 26 % of the macromolecules population with Mw bigger than 10^5.

The obtained results demonstrate that the destruction mechanism of chitosan solubilised in weak acid aqueous solution because of centrifugation depends by the solution acidity. The destruction of chitosan solubilised in 0.075 M solution occurs after a mixed mechanism which combine the features of the above described those, one for destruction in 0.05 M solutions and the other for solutions with 0.1 M - 0.15 M acidity. The proof is provided by the approximately equal two populations retrieved as majority after centrifugation of 0.075 M solution, one represented by the population of macromolecular chains as in case of 0.05 M solution and the others by the population of oligomers and low molecular species as in case of 0.1 – 0.15 M solutions.

It should be noted that the observed phenomenon is not an extremely strong one, the modification of chitosan Mw because of centrifugation being by 3 – 17 % in case of 0.05 M solution, 3-12 % for those with 0.075 M, 2-5 % for solutions with 0.1 M acetic acid and 2-11 % for the solution with 0.15 M acetic acid.
Because pH refers to the solution activity of hydrogen ion, the lower pH lower values after centrifugation can be explained by increasing of the small molecular species which hinder the migration capacity if the hydrogen ions in proportion with number of the new appeared small molecules.

Lower conductivity values in the case of less acidic solutions (0.05 M) is determined by the formation in this condition, as main population in proportion of 62% macromolecular chains which have less mobility. High conductivity of solution with 0.15 M is a consequence of appearance because of centrifugation of small molecules and oligomers which have high mobility. After centrifugation the molecules with Mw up to $10^2$ g/mol is 4% for 0.05 M solution, 8% in 0.075 M solutions, 9% for chitosan dissolved in 0.1% and 14% if the solution is 0.15 M acid.

5. Conclusions

Centrifugation changes the molecular weight distribution from single-modal to bimodal. The proportion of the two main populations which appear after centrifugation and Mw of molecular species characteristic of each population depends on the solution acidity. Population of macromolecular chains with Mw greater than $2 \cdot 10^4$ g/mol and less than $2 \cdot 10^4 \cdot 10^5$ g/mol is 62% for 0.05 M solution, by 35% in case of 0.15 M solution, near 25% for 0.1 M solution and by 28% for 0.075 M solution. A second representative population appeared because of centrifugation is those of molecules and oligomers with Mw (1-2) $\cdot 10^2$ - (1-2) $\cdot 10^4$ g/mol which appears in followings proportions: 45% for 0.1 - 0.15 M, 26% for 0.075 M and less than 17% for 0.05 M.

Centrifugation narrow the polydispersity index of molecular species with Mw = $10^2$-$10^6$ g/mol which becomes approx. 1 regardless of acid concentration and widens polydispersity index of molecules with lower Mw of $10^2$-$10^4$ g/mol which reaches till 7 value.

The obtained results can be understood as a possible small scale mechanical degradation of chitosan in conditions of centrifugation favoured by the acid concentration which decreases the proportion of population with high Mw in favour of those of oligomers and small molecules. Mechanism of destruction is dependent on the solution acid concentration because the protonation degree controls the conformation of macromolecules.

Acknowledgement

This study was conducted within the framework of the 248 / 2010 project from the Executive Unit for Higher Education, Research, Development and Innovation Funding (UEFISCDI) of Romanian National Ministry of Education (MEC). The help of our colleague Inna Trandafir which had prepared the chitosan solution is gratefully acknowledged.

References

[29] D. Dimonie, M. Petrache, R. Gabor, I. Trandafir, M. Dimonie, E. Vasile, S. Dinescu, New smart chitosan hydrogels designed for tissue engineering, presented at Romanian International Conference on Chemistry and Chemical Engineering XVIII, 4-7 September 2013, Sinaia, Romania.
[37] A. N. Sonina, G. A. Vikhoreva, G. K. Morgunov, and L. S. Gal’braikh, Chitosan acetic-acid forming solutions and control of the properties determining their capability for electroforming,
[44] H. Wilczura-Wachnik, Depolymerization of natural and synthetic polymers, thesys, University of Warshaw
[50] Acharya B. Vishu Kumar, Mandyam C. Varadaraj, Lalitha R. Gowda,
Rudrapatnam N. Tharanath, Low molecular weight chitosans —Preparation with the aid of pronase, characterization and their bactericidal activity towards Bacillus cereus and Escherichia coli, Biochimica et Biophysica Acta, 1770(4), 495 (2007).