Research Journal of Engineering Sciences ISSN 2278 – 9472
Vol. **1(4),** 9-15, October **(2012)** *Res. J. Engineering Sci.*

Studies on Synthesis, Characterization and Viscosity Behaviour of Nano Chitosan

Chattopadhyay D.P.[1]* and Inamdar M.S.[2]
[1]Department of Textile Chemistry, Faculty of Technology and Engineering, The M.S.University of Baroda, Vadodara-390001, INDIA
[2]Faculty of Textile Processing, Sarvajanik College of Engineering and Technology, Surat-395001, INDIA

Available online at: www.isca.in
Received 25th September 2012, revised 1st October 2012 , accepted 4th October 2012

Abstract

The enhanced performance of chitosan (CHT) treated cotton fabric is anticipated tobe achieved by scaling down the particle size of former to nano level for its greater penetration into the fabric structure. Nano-chitosan (CHTN) was synthesized by ionic gelation of CHT and sodium tripolyphosphate (TPP). The sample was characterized by the determination of particle size and polydispersity index (pdi) on particle size analyzer. Effect of various parameters such as molecular weight and concentration of CHT, concentrations of TPP on particle size were studied. Low molecular weight chitosans were prepared by nitrous acid hydrolysis method and the molecular weights were determined viscometrically. Attempts were made to correlate the viscosity behaviour with particle size of chitosan. The storage stability of CHTN dispersions was studied by periodic evaluation of their viscosity.

Keywords: Chitosan, nano-chitosan, characterization, viscosity behaviour, storage stability.

Introduction

The biopolymer based cationic polysaccharide, chitosan, is obtained by alkaline deacetylation of chitin which is widely distributed in shells of crustacean like lobsters, shrimps, crabs etc. Chemically, chitosan is a linear (1-4) linked 2- amino-2-deoxy-β- d- glucan (i.e. β- d-glucosamine) having the structure very much close to that of cellulose except the hydroxyl group in C (2) of cellulose is being replaced by amino group in chitosan. Indeed, it is a copolymer of N-acetyl-glucosamine and glucosamine units[1-3].

The potential use of chitosan in textiles and various allied fields has attracted strong interest for the development of its several derivatives and colloidal particles of nano level[4-8]. This has been attributed to its valuable inherent properties such as antibacterial, antifungal, antiviral, antacid, non toxic, total biodegradable, biocompatible with animal and plant tissues as well as film formation, fiber formation, hydrogel formation. It is totally eco friendly and renewable[2,9]. Macromolecular chitosan, however, encounters several challenges while its applications to textiles, in particular. Investigations have shown that the inherent properties of cotton fabric such as appearance and handle and other essential properties like fastness of dyes to various agencies due normal chitosan treatment were found tobe affected[10]. These detrimental effects of chitosan are mainly attributed to its lack of penetration into fabric structure causing the surface deposition of film [11,12]. One possible way to enhance its effectiveness is to reduce the particle size closer to nano level, which facilitates the greater penetration of CHT into fabric structure.

The applications of nano chitosan are well demonstrated in medical field particularly as controlled drug delivery systems[13-16]and water treatment for removal of heavy metals[17] and highly pollutant organic compounds like alkyl phenols[18]. However, very few applications nano chitosan in textiles are addressed. Lee et al[19] obtained chitosan and PVA-Chitosan blended submicroscopic fibres by electro spinning method having the average diameter of 200-400 nm. Nano chitosan can be loaded with metal ions such as Ag, Cu, Zn etc to enhance its antibacterial property, as reported by Du et al[20]. The practical applications of nano chitosan to textiles at shop floor level needs suitable technology for its productions, characterization and the analysis of its stability in standing baths. The present work was, therefore, aimed at finding out a simple method of synthesising nano chitosan by ionotropic gelation with sodium tripolyphosphate (TPP) and with the objective to its applications to cotton textiles. The characterization was done by the determination of particle size and polydispersity index (pdi) on particle size analyzer. Chitosans of different molecular weights, obtained by controlled depolymerisation of parent chitosan with nitrous acid hydrolysis, were used for the synthesis of nano particles. The effects of particle size on the viscosity behaviour and the storage stability of their sols are reported.

Material and Methods

Materials: Chitosan (CHT1), having DAC value 90% and viscosity 22cPs, was kindly supplied by M/s Mahtani Chitosan Pvt. Ltd., Gujarat State, India. Other chemicals used such as sodium tripolyphosphate (TPP), acetic acid, sodium acetate (anhydrous), methanol, sodium hydroxide etc were of analytical grade of reputed brands.

Synthesis of low molecular weight chitosan: Different molecular weight grades chitosans were obtained by depolymerization of CHT1 by nitrous acid hydrolysis method as described elsewhere[21]. In general, a 2 % solution of chitosan in acetic acid was prepared. Predissolved dilute solution of sodium nitrite was then added gradually to chitosan solution and stirred for two hrs at 30°C to get desired viscosity level. The depolymerised chitosan was then precipitated out by caustic solution and washed to neutral pH. The precipitates of chitosan was then washed thrice with methanol and dried at 60°C. The molecular weights of these samples were determined viscometrically[22].

Synthesis of nano-chitosan and its characterization: Nano-chitosan dispersions were obtained as described elsewhere[21]. However in general, chitosan (CHT1) was dissolved in acetic acid solution and optimized quantity of TPP was added drop wise with rapid stirring (about 400 rpm) to obtain an opalescent solution. The sample was allowed to stand overnight and filtered through sintered glass filter of porosity grade G3 and preserved in refrigerator. The prepared nano-chitosan was nomenclatured as CHT1N.

The particle size and size distribution of the chitosan were analyzed on the particle size analyzer (Model: Zetasizer Nano ZS90, Make: Malvern Instruments Ltd, UK).

Determination of viscosity: The viscosity behaviour of chitosan solution and nano-chitosan sols were studied using Ubbelohde capillary viscometer (No 1A) at 30°C having flow time for distilled water, $T_{0=}$15.57 seconds**.**

Results and discussion

Synthesis and characterization of nano chitosan: Chitosan has fairly long linear structure with rigid conformation. These long molecules in solid state are, mostly, in the form of tightly folded random coils. Individual molecular coils are also not discrete and separate but are interpenetrating and entangled with each other. In solution, the solvent gradually diffuse into the polymer aggregates resulting into the swelling of the polymer. As swelling continues, the segments of the polymer are solvated and loosened out as bunches of entangled molecules, known as 'hydrodynamic' sphere or ellipsoid[23-25]. The characteristic size of CHT1 hydrodynamic sphere, in our case, at 1 gpl concentration was determined to be 4014 nm. Such higher particle size offer higher viscosity to the solution. It is possible, for a given molecular size chitosan, to reduce the particle size to nano level by 'bottom-up' approach as a result of a self assembling or cross linking processes in which the molecules arrange themselves in to ordered nano scale structure either by physical or covalent inter- or intramolecular interactions[26]. By virtue of primary amino groups, chitosan under goes Schiff's base formation with aldehydes such as glutaraldehyde, salicylaldehyde etc giving chemically cross linked leading to a quite stable matrixes of nano chitosan[14,26,27]. In another kind of reaction, chitosan hydrogels are obtained by ionic gelation, where nano particles are formed by means of electrostatic interactions with polyanions such as pentasodium tripolyphosphate (TPP), ethylene diamine tetra acetic acid (EDTA) etc. Such particles are stabilized by electrostatic hindrance due to coulombic repulsion between particles of same ionic charges[29-31]. Several other methods of synthesis of nano chitosan such as desolvation method, emulsion-droplet coalescence method, reverse micellar method, self-assembly via chemical modification, spray drying[13,14], and nonaqueous electrochemical method[32] are described in liturature. Owing to faster ionic reactions between chitosan and TPP, non toxic nature of these components[26] and ease of operation, we adopted the gel ionization technique for the synthesis of nano chitosan particles. The intramolecular cross linking in chitosan molecule by gel ionization is schematically illustrated in figure 1. The particle size distributions of various samples of CHTN are presented in figure 3.

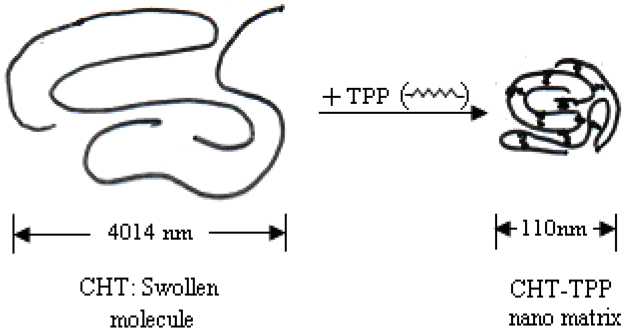

Figure-1
Ionic cross linking of chitosan

Effect of molecular weight of chitosan on particle size: Various grades of low molecular chitosan were produced by controlled depolymerization of high molecular weight one, namely CHT1. When chitosan solution is treated with nitrous acid, produced from acidic solution of sodium nitrite, it undergoes deamination reaction with subsequent cleavage of β-glycosidic linkages[33]. The IR spectra of chitosan and depolymerised chitosan of different molecular weights determined in earlier study[21] were found tobe almost similar indicating that the process of depolymerisation caused no significant chemical changes in their structures. These low molecular weight chitosan derivatives were employed for the synthesis of nano chitosan dispersions.

The influence of molecular weight of chitosan on particle size is presented in table 1 and the graphical correlation between intrinsic viscosity, a function of molecular weight, and particle size of nano chitosan is shown in figure 2. These data illustrate that, under a given condition of concentrations of CHT and TPP, with decrease in molecular weight, the particle size also decreased progressively and followed a curvilinear relation. It is well documented[23, 29] that the higher the molecular weight or the larger the molecular size, comparatively larger will be the nanomatrix and vise-versa. This relation of particle size with molecular weight in a definite condition of parameters may be useful for synthesis of nano chitosan of desired particle size.

Table-1
Effect of molecular weight on particle size

Parent Chitosan		Synthesized nano chitosan	
Sample Code	**Molecular Weight, Mv**	**Sample Code**	**Particle size (nm)**
CHT1	135,839	CHT1N	319.4
CHT2	71,676	CHT2N	271.6
CHT3	38,733	CHT3N	231
CHT4	20,698	CHT4N	195.2
CHT5	11,986	CHT5N	110.74

CHT:TPP:: 1:0.15

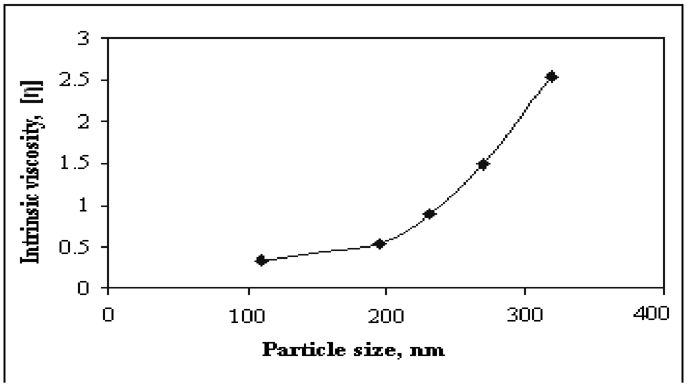

Figure-2
Particle size of chitosan as a function of intrinsic viscosity

Effect of concentration of chitosan on particle size: Two methods were employed for the synthesis of different concentrations of CHTN dispersions namely direct preparation method and dilution method. In first i.e. direct preparation method, the dispersions of nano chitosan from CHT1 of different concentrations such as 0.25, 0.50, 1.0 gpl etc were prepared separately and in the latter, higher concentration nano chitosan dispersion (2 gpl) was prepared first and then diluted to the required concentration with rapid stirring. The effects of these two methods on particle size are presented in table 2. It was observed that at higher concentration, in both the cases; the particle size of CHTN was comparatively large and progressively reduced with the lowering of concentration. The former method, however, was more effective in scaling down the particle size. The larger size of CHTN at higher concentrations may be due overlapping and intermolecular cross linking through TPP bridging resulting into aggregation of polymer molecules. Intramolecular cross linkages in polymer molecule due to TPP at low concentrations, on the other hand, are likely tobe favored for lower particle size. This table also manifests that the poly dispersity indices (pdi) of direct prepared samples were comparatively lower and independent while in dilution method the values were higher and seemed to follow the starting material. The particle size distribution curves, as illustrated in figure 3, were broader for higher concentration samples and became narrow for lower concentration samples. Further, these bands were comparatively narrow for directly prepared samples indicating the uniform size distribution and are close agreement with their lower pdi values.

Effect of TPP concentration on particle size: In ionic gelation reaction, TPP a major ingredient for cross linking has a pronounced effect on the properties of CHTN dispersion. It was observed that with increase in the concentration of TPP the appearance of the system changed from clear viscous liquid to opalescent fluid and then precipitated. The effect of TPP concentration on the particle size is demontrated in figure 4. At concentration of TPP below 0.05 g, very few phosphate ions were present to produce effective ionic linkages with chitosan amino groups; hence, the solution was clear. As the concentration of TPP was increased gradually, the solution became opalescent indicating the formation of nano chitosan. It was revealed from the same figure that with increase in concentration of TPP, the particle size of CHT1-TPP nanomatrix decreased, reached to minimum at about 0.25 g of TPP and then increased. Concentration of TPP above 0.30 g resulted precipitation. The precipitation at excessively higher concentration of TPP may be attributed to the aggregation of chitosan molecules due to excessive cross linking through TPP bridging. Similar trend in terms of viscosity was noticed when the relative viscosity was plotted against TPP concentration, figure 5.

Table-2
Effect of preparation method and concentration of chitosan on particle size

Direct preparation method			Dilution method		
CHT1 (gpl)	**Particle size (nm)**	**Poly dispersity index (pdi)**	**CHT1N (gpl)**	**Particle size (nm)**	**Poly dispersity index (pdi)**
0.25	304	0.421	0.25	347.3	0.465
0.50	313.5	0.441	0.50	354.9	0.471
1.00	319.4	0.422	1.00	468.1	0.515
1.50	408.73	0.441	1.50	516.43	0.515
2.00	534.2	0.515	-	-	-

(CHT1: TPP :: 1:0.15)

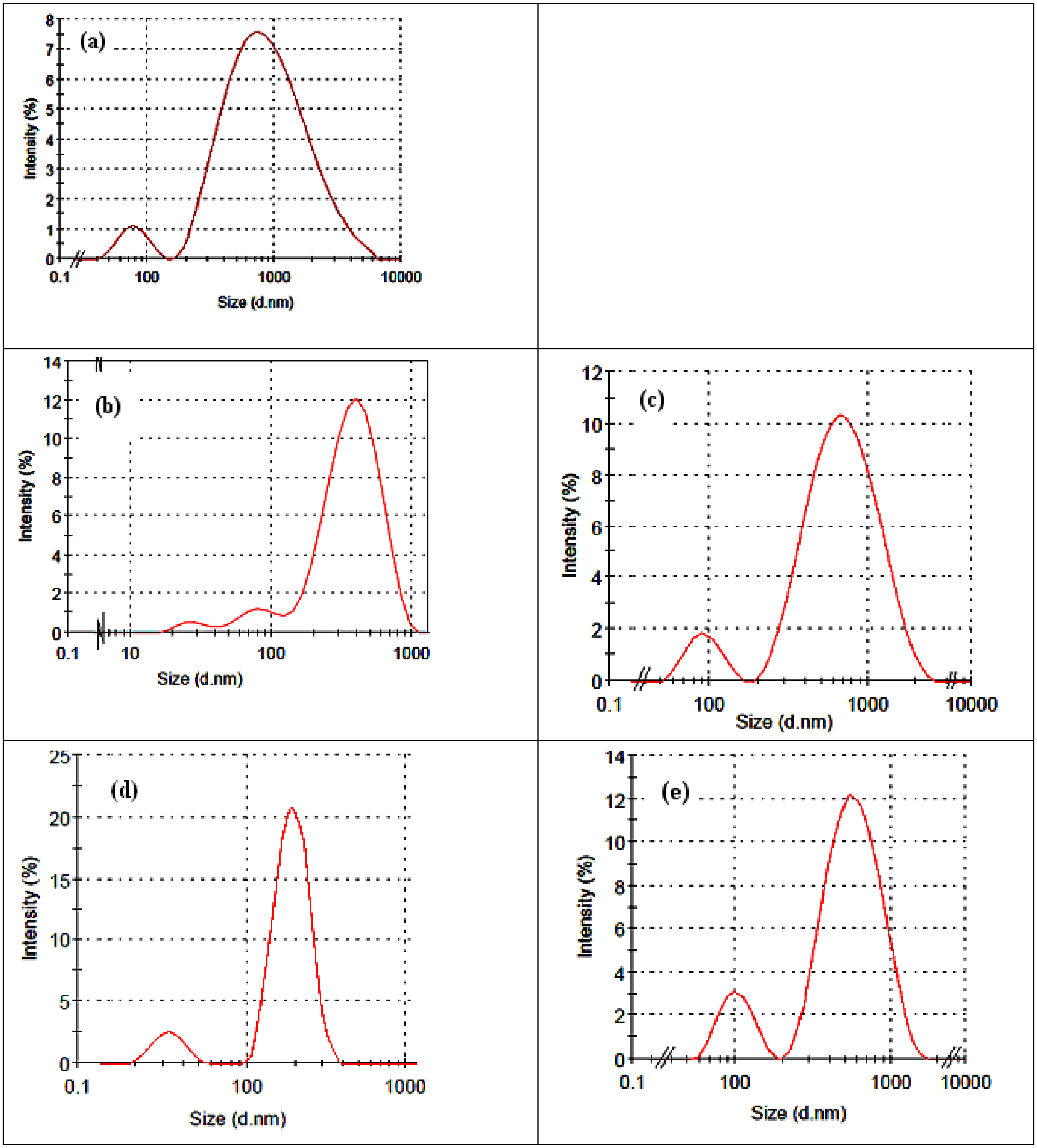

Figure-3

Particle size distribution of nano chitosan as a function of preparation methods: (a)Direct Method: 2gpl (534.2nm), (b) Direct Method: 1gpl (319.4nm), (c) Dilution Method: 1gpl (468.1 nm), (d) Direct Method: 0.5gpl (313.5nm), (e) Dilution Method: 0.5gpl (354.9 nm)

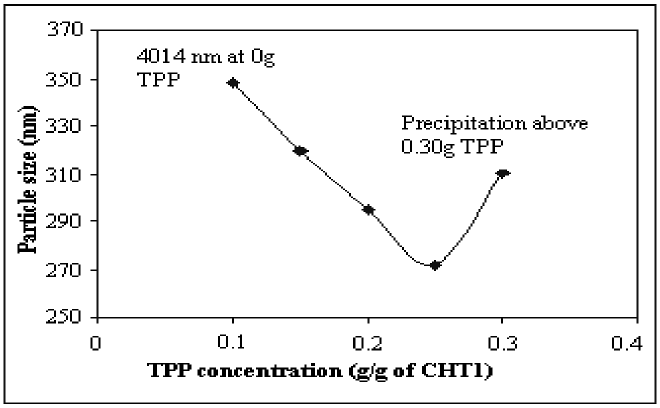

Figure-4
Effect of TPP concentration on particle size of CHT1

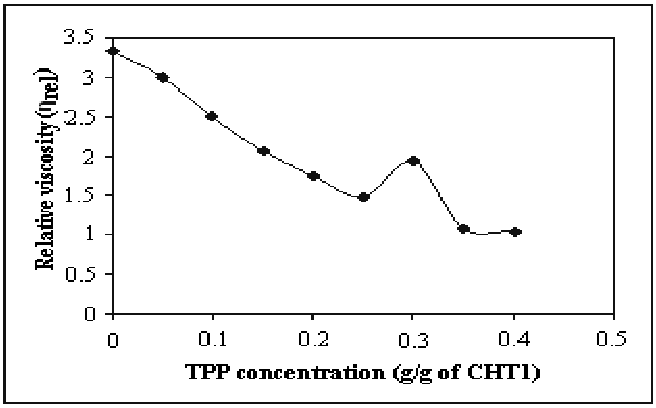

Figure-5
Relative viscosity CHT1 nano solution as a function of TPP concentration

Viscosity behaviour of nano chitosan: The viscosity of polymer solution, at the molecular level, is a direct measure of the hydrodynamic volume of the polymer molecules which in turn is governed by the molecular size or the chain length and hence the molecular weight[25]. Relative viscosity as a function of particle size and the extent to which a parent chitosan scales down to nano level at a given concentration of CHT and TPP are presented graphically in figure 6 and figure 7 respectively.

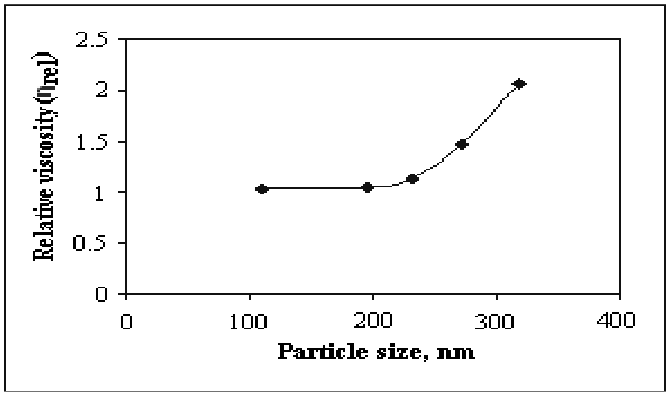

Figure-6
Viscosity as a function of particle size of CHTN

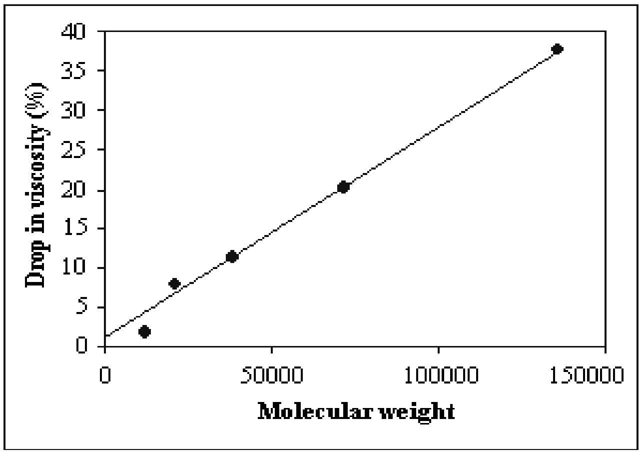

Figure-7
Drop in viscosity from parent (CHT) to nano chitosan (CHTN) solution as a function of molecular weight

It was revealed from figure 6 that the viscosity of CHTN dispersion decreased with reduction in particle size. However, the effect was more significant for larger particles than the smaller one. Obviously, the larger the particle size the higher will be the resistance offered for the flow of liquid and hence the higher will be the viscosity and vise versa. Comparatively slower fall in viscosity for small CHTN particles may be attributed to the low molecular weights of parent chitosan. It is demonstrated in figure 7 that the percentage drop in viscosity from molecular (CHT) solution to corresponding nano chitosan (CHTN) dispersion follows a straight line. It means higher molecular weight chitosan scales down to nano size to greater extent than the lower molecular weight chitosan. This can be explained as follows; large size chitosan molecules in solution accommodate comparatively more amount of solvent and occupy large 'hydrodynamic' volume. These swollen molecules compress to greater extent by ionotropic intramolecular cross linking with TPP by displacing the solvent, as demonstrated in figure 1. On the other hand, the extent of swelling of low molecular weight chitosan is comparatively less[24] and hence lesser will be its tendency to compress.

Stability of nano chitosan dispersion: The biodegradability of chitosan is anticipated to be influenced by its particle size. Therefore the stability behaviour of standing baths of nano chitosan dispersion should be taken into consideration during its applications particularly to textile fabrics. The stability of nano chitosan dispersions for 24 hrs were analysed by viscosity measurements as shown in table 3. The data illustrate that the stability in terms of change in viscosity of parent chitosan (CHT) solution is governed by its molecular weight, which is improved with decrease in molecular weight. The stability behaviour of nano chitosan (CHTN) dispersion, on the other hand, was found to be different from that of parent chitosan solutions. Nano chitosan dispersions obtained from higher molecular weight chitosans are found tobe more stable than the corresponding parent chitosan solutions. Where as the nano dispersions obtained from low molecular weight chitosans are seen tobe more susceptible to degradation. Complete biodegradation of nano chitosan dispersion, in general, was resulted in 3-4 days showing the formation of white globules as shown in figure 8. Therefore, utilization of nano chitosan dispersions in textile applications within the 24 hrs is advisable. Viscosity analysis and visual observations may be the useful tools for stability inspections.

Table-3
Stability of nano chitosan solution as a function of particle size

Parent chitosan(CHT) solution (1gpl)			**Nano chitosan solution(CHTN) (1gpl)**		
Sample Code	**Molecular Weight, Mv**	**Change in viscosity (%) after 24 hrs**	**Sample Code**	**Particle size (nm)**	**Change in viscosity (%) after 24 hrs**
CHT1	135,839	-10.27	CHT1N	319.4	-3.68
CHT2	71,676	-4.1	CHT2N	271.6	-1.05
CHT3	38,733	-2.6	CHT3N	231.01	-1.18
CHT4	20,698	-2.15	CHT4N	195.2	-3.6
CHT5	11,986	-1.73	CHT5N	110.74	-2.27

Figure-8
Stability study: white globular residue formed by microbial attack on CHT1N

Conclusion

Ionotropic gelation method for the preparation of nano chitosan dispersion using TPP is faster, convenient and non toxic. The concentration of chitosan in the formulation bath has the influence on the particle size. Particle size reduces with decrease in concentration. Direct preparation method gives much reduced size than that of dilution method. With decrease in intrinsic viscosity and hence the molecular weight, the particle size also decreases progressively and shows a curvilinear dependence on particle size. This relation may be useful in preparation nano chitosan dispersion of desired size. TPP concentration determines the particle size. With increase in concentration of TPP, the particle size reduces, reaches to minimum and again increase. Excessive TPP in system leads to precipitation. By reducing the particle size to nano level, the viscosity of chitosan solution is lowered significantly, but the storage stability was affected adversely. Use of freshly prepared nano-chitosan dispersions prior to applications may be the remedy.

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