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Amelioration Studies on Optimization of Low Molecular Weight Chitosan Nanoparticle Preparation, Characterization With Potassium Per Sulphate and Silver Nitrate Combined Action With Aid of Drug Delivery to Tetracycline Resistant Bacteria

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ABSTRACT
Low molecular weight chitosan nanoparticles were prepared by ionotropic gelation of depolymerised chitosan for effective drug delivery in drug resistant bacteria. Depolymerisation reaction was performed by potassium persulfate oxidation at an optimized condition in presence of silver nitrate. Optimized condition for depolymerisation was anticipated at 37°C, pH 4, 2 days reaction time and 0.05 M concentration of potassium per sulphate with 1 mM silver nitrate in final reaction mixture. Chemical characteristics of depolymerised chitosan and low molecular weight chitosan nanoparticles were analyzed by gel permeation chromatography, nuclear magnetic resonance, scanning electron microscopy, X-ray diffraction, and transmission electron microscopy. Drug loading efficiency and drug releasing efficiency were also studied. Finally, antimicrobial activity of tetracycline loaded low molecular chitosan nanoparticles was determined in terms of minimal inhibitory concentration and putative mode of action on tetracycline resistant bacteria *Escherichia coli XL-1 Blue*.

Keywords: Low molecular weight, Chitosan, Drug delivery, Minimal inhibitory concentration, Drug resistance bacteria.

INTRODUCTION

Chitosan is a polycationic polysaccharide based biodegradable polymer. It is the partially N- deacetylated product of chitin. Presently, chitosan has dragged an increasing attention due its superior solubility and reactivity. Chitosan has comparatively better anti-microbial, anti-tumor and immune-stimulation biofunctionalities. [1-2] Anti-microbial activity of chitosan is correlated with its molecular weight. [3] Instead of that, chitosan anti-microbial property has also been influenced by several other factors. These factors include type of microbial species, [4] degree of polymerisation, [5] and degree of deacetylation. [6] It has been reported that low molecular weight chitosan (LMWC) is more amenable for a wide variety of biomedical applications due to its higher solubility in water. It has also been shown that LMWCs (5-10 kDa) have highest bactericidal activity against pathogenic bacteria. [7] LMWCs have been prepared by physical, chemical or enzymatic methods based on molecular depolymerisation of high molecular weight chitosan (HMWC). But these approaches still endure from various limitations. Physical methods (shearing, sonication) require special equipment, while reactions from chemical hydrolysis using HCl, H_2SO_4, H_2O_2 and HNO_2 are difficult to control, often resulting inconsistent and over depolymerised products. On the other hand, the enzymatic hydrolysis (chitinase and chitosanase) has been limited due to its expensiveness, non-specific depolymerisation. [8-9]

Microbiological perspective of this present work deals with rapid emergence of multi drug resistance has become a burning issue, especially for tetracycline resistance. Prior to mid 1950s tetracycline was the most widely used antibiotics in human medicine and animal agriculture. It was relatively inexpensive. It can be administered orally, and have relative few side effects. [10] But extensive usage of this antibiotic since 30 years has emerged a burning issue of tetracycline resistance in 1980s. Genetic and biochemical mechanism of tetracycline resistance is mainly associated with the efflux gene transfer by resistant-plasmid, transposons and ribosomal

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protection. Efflux gene encoded proteins block the uptake of tetracycline. Ribosomal protection is associated with some mutated cytoplasmic proteins that protect the ribosome from tetracycline binding in resistant bacteria. [11]

Emergence of this tetracycline resistance has adversely impacted the two major fields. The two major fields are therapeutics and environment. Excess use of tetracycline in human has developed potent tetracycline resistance in various pathogenic bacteria and increased the difficulties in disease treatments. [12] Even a toxic compound anhydrotetracycline has been formed during a high-temperature treatment of animal-derived feed contaminated with excess tetracycline. [13] On the other hand, various veterinary farms have been still using tetracycline in non therapeutic level or excess since last 50 years for animal medications. [14] Moreover, effluents of these farms and public hospitals have been entered in to the water streams [15] and soil [16] through municipal sewage systems. As a result, it increases the risk of further spread of tetracycline resistance in the ecosystem.

Recently, medicinal chemistry has incorporated various new remedies for drug resistance problem in human and animals. These new techniques include use of chemically modified drug, [17] combinatorial therapy, [18] use of helper drug or efflux pump inhibitors, [19] phage application, [20] and plasmid curing technology. [21] Still these mentioned technologies are not potentially effective and produced side effects likely chemically modified tetracycline has induced apoptotic pathways [22] and depression of skin collagen synthesis [23]. Even more recently some research group prepared the tetracycline encapsulated microspheres of chitosan. [24] Moreover, hyaluronic acid, alginic acid were also used for microencapsulation of drug. Even several research groups have been working with chitosan based biopolymer to prepare the nanoparticles because chitosan is biocompatible, non-toxic, biodegradable, and cheap chemical compound. [25] But major problems have been still associated with its comparatively larger size, high molecular weight. It causes inefficient transport of drug and induces immunogenic clearance. [26] Therefore; current trend of drug delivery system has been shifted towards low molecular weight nanoparticle based drug delivery system. [27]

Here, we prepared LMWC based nanoparticles through depolymerisation of HMWC by potassium per sulphate treatment. We also standardized the preparation protocol on the basic of temperature, pH and incubation reaction time. After that we did the physico-chemical characterizations on LMWC based nanoparticles and tetracycline drug was loaded. Furthermore, loading and release efficiency of tetracycline was monitored. Then tetracycline loaded LWMC based nanoparticles were applied on tetracycline resistant bacteria *Escherichia coli XL-1 Blue*. Finally, Minimal inhibitory concentration (MIC) was determined.

MATERIALS AND METHODS

Initially, 1% (w/v) of 100 ml High molecular weight (HMW) native chitosan (Mw 250 kDa) (Sigma, India) suspension was prepared in phosphate buffer (pH 4.5) in presence of 600µl glacial acetic acid (v/v). Then 15 ml of potassium per sulphate ($K_2S_2O_8$) (Sigma, India) stock solution was mixed with 15 ml of chitosan 1% (w/v) suspension to make final $K_2S_2O_8$ concentration of 0.05 M. 1 ml silver nitrate (CDH, India) was added from stock solution to make final concentration of 1 mM in the reaction mixture. Finally, 100µl of pyridine was added in reaction mixture. Then the reaction mixture was allowed for 48 h (2 days) at 37°C in a shaking mode of 250 rpm. The reaction mixture was mixed with 10 ml of 0.006% (w/v) NaCl-100% (v/v) ethanol solution and kept at 4°C to facilitate precipitation for overnight (18 h). Then the visible white precipitate was subjected to centrifugation at 14,000 rpm for 30 min and pellet was vacuum dried. The dried pellet was dispersed in 5 ml of deionized distilled water by sonication (Sigma Ultrasonic 103 Processor, Sonix-600, having power output 12W) and finally subjected to lyophilisation (Perkin Elmer, USA). Finally Lyophilized pellet was kept at 4°C for further experiments.

Effect of $K_2S_2O_8$ concentration (range 0.025-0.125 M), pH (4-8) and reaction time (1-5 days) were determined on depolymerised chitosan preparation. The hydrodynamic diameter (HD) of depolymerised chitosan was detected by laser light scattering using a Brookhaven 90 Plus particle size analyzer. HD measurements were performed at 20°C and 90° angle, after dilution [1:1000 (v/v)] of the depolymerised chitosan sample in deionised water.

In vitro depolymerisation of HMW native chitosan was determined for emphasizing potassium per sulphate is effectively depolymerise the HMW native chitosan in a time dependent manner. Depolymerisation was continued for consecutively for 5 days. Samples (prepared by generalized protocol) were precipitated by absolute ethanol (ice cold), centrifuged at 14,000 rpm for 30 min and filtered by 0.22µm filter. Finally, the supernatant was used for dinitro salicylic acid (DNS) assay [28] and pellet was vacuum dried for depolymerisation ratio (DM) measurement. *In vitro* depolymerisation was detected by measuring two major parameters namely DM and reducing sugar by (DNS) assay. DM was calculated as equation [1]

$$DM = (D_i - D_f)/D_i \quad [1]$$

Where, D_i and D_f are initial dry weight and final dry weight, respectively. On the other hand, DNS assay was also performed for the measurement of reducing sugar (O.D at 540 nm) to cross check the time dependent depolymerisation fact by potassium persulfate treatment on HMW native chitosan.

After potassium persulfate treated depolymerised chitosan was used without further purification for nanoparticle formation via ionotropic gelation with sodium salt of tri polyphosphate (TPP). LMWC nanoparticles were synthesized according to the method described by Ferna´ndez-Urrusuno *et al*. [29] Briefly, 400µl of 0.084% TPP was added to 1 mL of 0.2% depolymerised chitosan in sterilized distilled water under magnetic stirring (150 rpm for overnight) for a final depolymerised chitosan/TPP ratio of 6/1 by weight. Newly synthesized LMWC nanoparticles were retrieved by centrifugation at 14,000 rpm for 30 min and washed twice with autoclaved distilled water.

LMWC nanoparticles were dispersed in deionized distilled water and tetracycline (Merck, USA) (15mg/ml) was dissolved in 50% (w/v) ethanol. LMWC nanoparticles dispersed solution and tetracycline solution were mixed together in equal volumetric ratio (1:1) and incubated for overnight (18 h) at 37°C temperature in orbital shaker (250 rpm). Mixture was subjected to ice cold 100% ethanol and kept at 4°C to facilitate precipitation for overnight (18 h). Then the visible precipitate was subjected to centrifugation at

14,000 rpm for 30 min and pellet was vacuum dried. The dried pellet was dispersed in 5 ml of deionized distilled water by sonication and finally subjected to lyophilisation. Lyophilized pellet was kept at 4°C for physico-chemical characterization and minimal inhibitory concentration (MIC) assay.

^{1}H-NMR (400 MHz) spectra was recorded on a Bruker ARX 300 spectrophotometer at 80^{o}C, with more than 2000 scans numbers for native and LMWC nanoparticles. Range of ^{1}H-NMR was 1 to 16 ppm for each run. Depolymerised chitosan samples were dispersed in D_2O in the concentration of 1 mg per ml (w/v). ^{1}H-NMR spectral analysis was performed to show the similar spectral pattern of both native and depolymerised chitosan.

FTIR spectra (4000-500 cm^{-1}) were recorded from a Perkin-Elmer spectrum RX-1 IR spectrophotometer using 5 mg of dry polymer samples and KBr discs under dry air at room temperature. Each FTIR spectrum represents 16 scan with 4 cm^{-1} spectral resolution.

HR-TEM was performed by using Phillips CM 200 and operated at an acceleration voltage of 200 kV to determine the exact particle size of prepared LMW chitosan nanoparticle. Polymer samples were diluted in deionized water, placed on special copper grid and finally dried under vacuum. Polymer containing copper grid was natively stained by phosphotungstic acid before HR-TEM observation for better resolution.

The SEM provides useful analysis of surface structures and morphology. The powdered polymer sample was spread on a double-sided conducting adhesive tape pasted on a metallic stub, were coated with gold (100µg) in a sputter coating unit for 5 min and observed under SEM machine (Olympus) at 20 kV. Bacterial samples were prepared on grease free vacuum dried glass slides and then followed the same protocol for morphological analysis.

Average molecular weight of the depolymerised LMWC and native chitosan was determined by gel permeation chromatography (GPC) by a Viscotek Gel Permeation Instrument; Model 350 High Temperature Triple Detector Array (HT-TDA) (RI, viscometer and light scattering) instrument. Average molecular weight value was calculated using the OmniSEC 4.2 software. Tetrahydrofuran (THF) was used as the eluant at a flow rate of 1.0 ml/min. The polymer samples were dissolved in THF to a final concentration of 2 mg/ml (solubility of chitosan polymer in THF is approximately 1-5 mg/ml). GPC was calibrated with standard marker dextran (2mg/ml).

Zetapotential of LMW chitosan was measured by using zeta potentiometer (Zetasizer 4, Malvern Instruments, UK) to confirm the polydispersity and surface charge for better interaction to bacterial cells. LMW chitosan nanoparticles in deionized water were used to assess the zetapotential using DTS1060C type clear possible zeta cell at 25^{o}C temperature. The Smoluchowsky approximation was applied in the calculation of the zetapotential.

Physical loading efficiency (PLE %) was estimated based on the weight ratio of the amount of incorporated tetracycline to that of total tetracycline used for tetracycline loaded LMWC nanoparticles formation. Briefly, the tetracycline loaded LMWC nanoparticle was precipitated by absolute ethanol (ice cold) to extract the tetracycline in supernatant (dilution factor was taken into account for each set). The supernatant was centrifuged at 14,000 rpm for 30 min and finally was filtered through 0.22µm membrane filter. Free tetracycline in supernatant suspension was the drug amount not encapsulated into LMWC nanoparticles; its concentration was assayed by spectrophotometry at 423 nm. [30] The PLE % was calculated from the equation [2]

$$PLE\ \% = [(Wtotal - Wfree) / Wtotal] \times 100\ \% \qquad [2]$$

Where Wfree is the free tetracycline amount and Wtotal the total tetracycline amount used for tetracycline loaded LMWC nanoparticle formation.

In vitro release of tetracycline from tetracycline loaded LMWC and unloaded LMWC nanoparticle was determined using shaking water test at 37°C. The shaking speed was placed set at 100 rpm. About 100 mg of tetracycline loaded LMWC sample was placed in a dialysis bag and 5 ml of preheated phosphate buffer saline (PBS) was subsequently added. The dialysis bag was then sealed and put into a glass bottle. Another 95 ml of PBS solution was added in the glass bottle. At each time point, 2 ml of PBS was taken out for analysis and another 2 ml of fresh PBS was added to keep total volume constant throughout experimental assay. The amount of released tetracycline was assayed spectrophotometrically at 423 nm.

Antimicrobial effect of the tetracycline loaded LMWC nanoparticles was evaluated by MIC assay. MIC was ultimately defined as the lowest concentration of the drug or drug loaded nanoparticle required to inhibit bacterial growth that of control uninoculated growth medium (nutrient broth) after 48 h. In brief, Bacteria were incubated at 37°C, shaken at 250 rpm aerobically. At the exponential phase, bacteria were harvested by centrifuge at 6,000 rpm for10 min at 4°C, then washed twice with10mM phosphate buffer saline (PBS, pH 7.2). The bacteria were suspended in PBS and adjusted to ~1 × 10^7 CFU per ml for further use. Then the tetracycline loaded LMWC nanoparticles was gradually diluted for final reaction concentration range of 100 to 900µg/ml in nutrient broth. Bacteria were inoculated to achieve a bacterial concentration of 1-2 × 10^5 colony forming unit (CFU) per ml. The MIC was read after 48 hours of incubation at 37°C, equivalent to the concentration of the tube without visible growth. MIC of tetracycline loaded LMWC nanoparticles was determined to inhibit the growth of tetracycline resistant (tetR) bacteria *Escherichia coli XL-1 Blue* (Stratagene, India). Dilution of nutrient broth containing the bacteria and test sample (at MIC) was allowed for SEM after 12 h incubation to confirm the morphological changes by lytic effect, wherein complete disappearance of the cells was observed after 48 h.

Mean values and standard deviations were calculated from the data of tests performed three times per sample. Results were compared by least significant difference test and multiple regressions (R^2) analysis using Minitab Version 15 statistical software.

RESULTS

Effects of pH, incubation time, concentration of potassium persulfate were analysed for depolymerised chitosan preparation in terms of hydrodynamic size measurements. Profiles of hydrodynamic size (Fig. 1) clearly showed that concentration of potassium persulfate has a comparatively immense effect on LMWC nanoparticle preparation. Depolymerisation of HMWC by potassium persulfate treatment was represented as degradation ratio and optical density at 590 nm for reducing sugars (Fig. 2). It was clearly

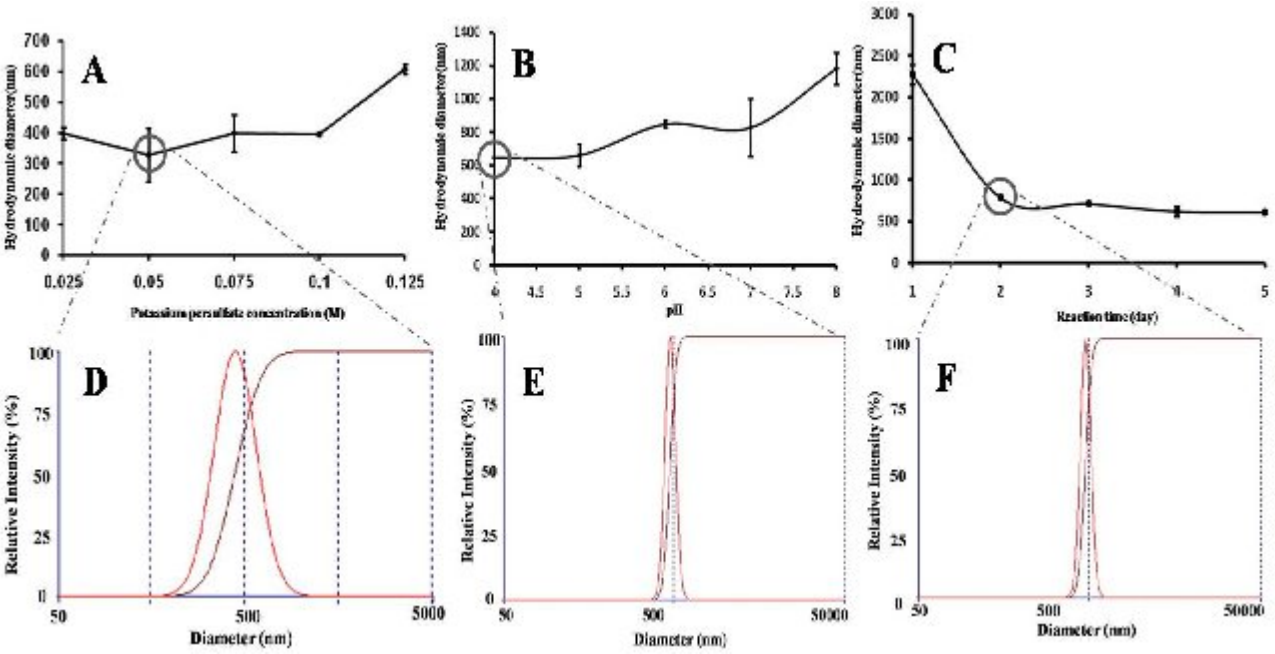

Fig. 1: Effect of $K_2S_2O_8$ concentration, pH and reaction time on *in vitro* depolymerisation of native chitosan. (A) HD profile on $K_2S_2O_8$ concentration, (B) HD profile on pH, (C) HD profile on reaction time, (D) HD distribution of depolymerised chitosan at optimized 0.05 M potassium persulfate concentration, (E) HD distribution of depolymerised chitosan at optimized pH 4, (F) HD distribution of depolymerised chitosan at optimized reaction time 2 days

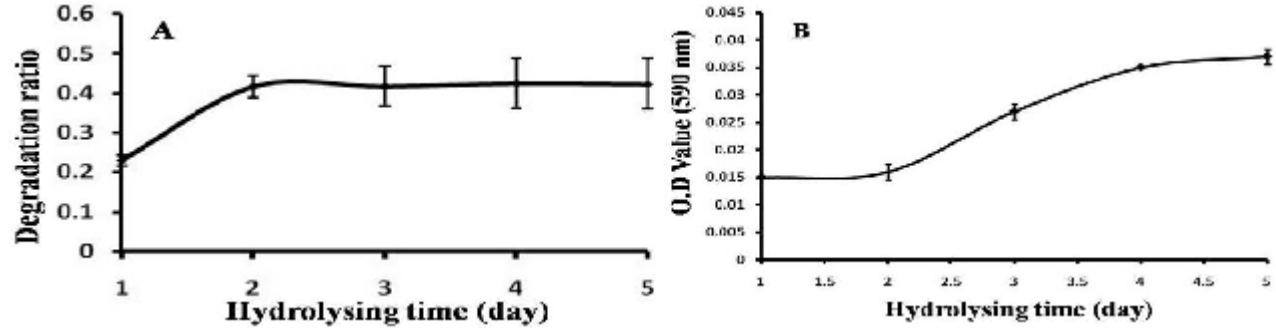

Fig. 2: Determination of *In vitro* depolymerisation measurement of HMWC. (A) Profile of degradation ratio with hydrolysing time (B) O.D (590 nm) profile with hydrolysing time

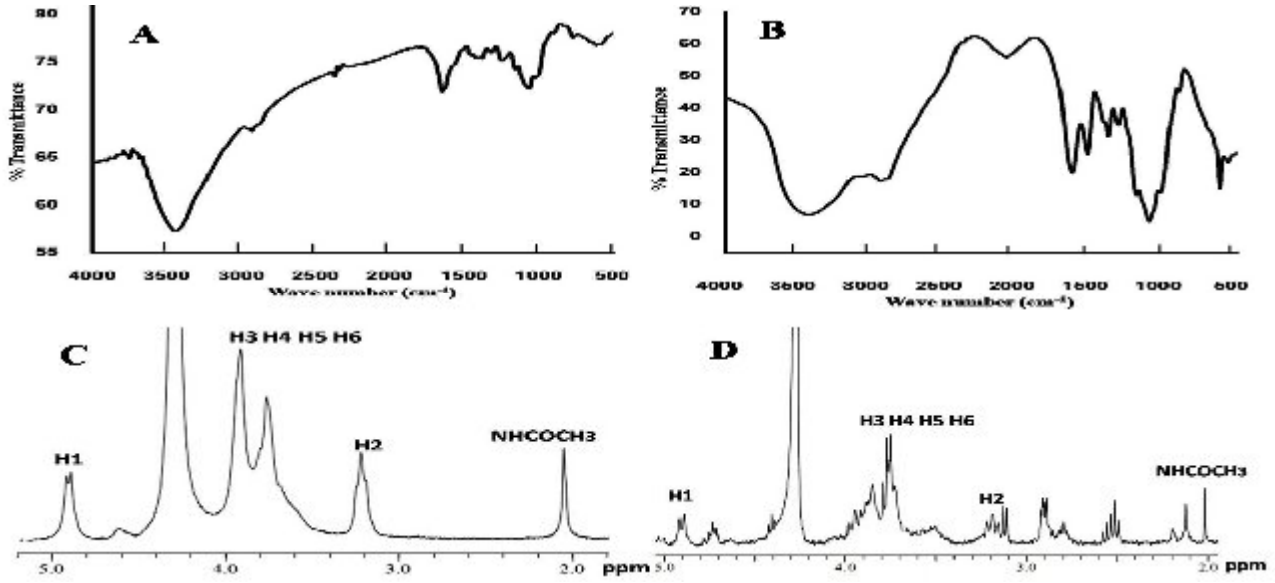

Fig. 3: Characterization of native and depolymerised chitosan after $K_2S_2O_8$ treatment. (A) FTIR of native chitosan, (B) FTIR of depolymerised chitosan, (C) ^{1}H NMR of native chitosan, (D) ^{1}H NMR of depolymerised chitosan

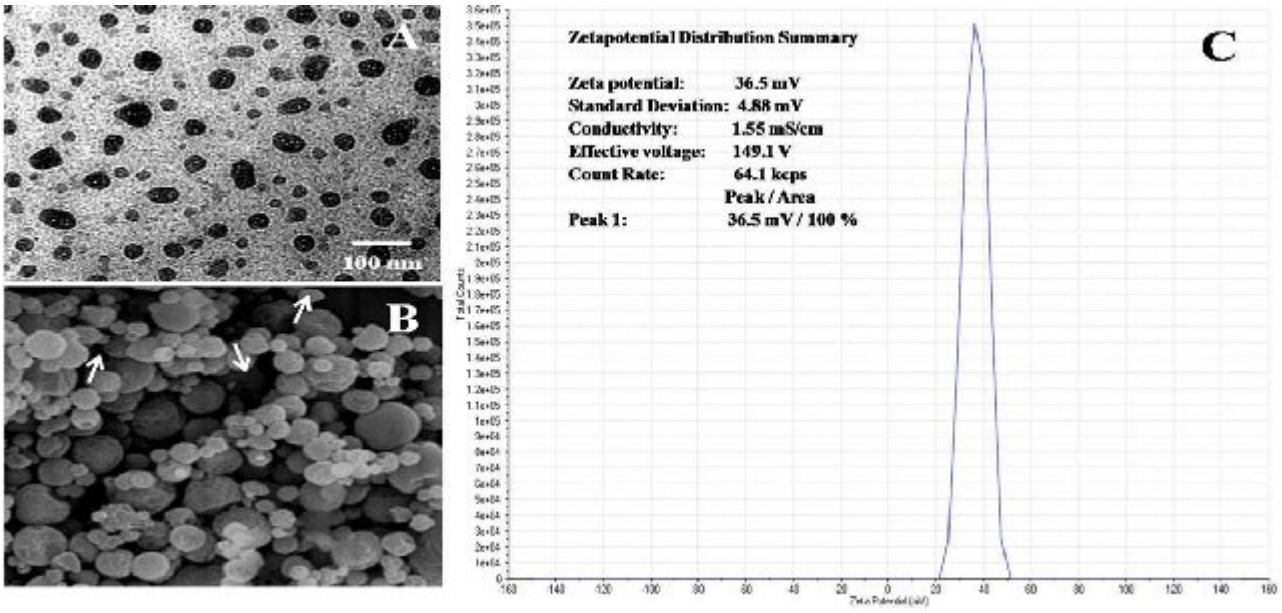

Fig. 4: Characterization of LMWC nanoparticles after $K_2S_2O_8$ treatment. (A) HR TEM image of LMWC nanoparticle at 20,000 X magnification (B) SEM image of LMWC nanoparticle at 10,000 X magnification, (C) Zetapotential distribution summary of LMWC nanoparticle at 25°C using deionised distilled water

shown that the depolymerisation efficiency of potassium persulfate was directly proportional to hydrolysis time. But we chose the chitosan hydrolysis time 2 days as more exposure to potassium persulfate was highly effecting the stable LWMC nanoparticle formation.

FTIR and ^{1}H-NMR spectra were recorded to investigate structural changes in depolymerised chitosan during the depolymerisation process. FTIR spectra of native and depolymerised chitosan were measured at wave number ranging from 4000 to 500 cm^{-1}. The FTIR spectra (Fig. 3A and 3B) present the following bands: axial stretching of the OH group at 3274, 3273 cm^{-1}, amide I band (N-acetyl glucosamine units) at 1649, 1650, cm^{-1}, N–H angular deformation at 1565, 1565 cm^{-1} (N-acetyl glucosamine amide II), CH_3 symmetrical angular deformation at 1386, 1386 cm^{-1} , C–N axial deformation of the amino groups at 1411, 1411 cm^{-1} and C–N axial deformation of the amino groups at 1324, 1324 cm^{-1}, respectively for native and depolymerised chitosan. Besides the three characteristic polysaccharide bands at 1153, 1153 cm^{-1}; 1097, 1097 cm^{-1}; 1031, 1031 cm^{-1} have evidenced from FTIR spectrum for both native and depolymerised chitosan. [31] The ^{1}H NMR spectrum (Fig. 3C and 3D) of chitosan has shown signal at 4.89 ppm related to the H1 from unsubstituted D-glucosamine unit (characteristic of chitosan) for both native and depolymerised chitosan. Even other peaks for H2, H3, H4, H5, H6 and $NHCOCH_3$ have shown same pattern in ^{1}H NMR profiles for both native and depolymerised chitosan. Thus, FTIR and ^{1}H NMR spectra indicate that potassium persulfate mediated depolymerisation has no significant influence on basic structure of chitosan.

Scanning electron microscopy (SEM) showed that LMWC nanoparticles were morphological mostly spherical in nature (Fig. 4B). Particular size of LMWC nanoparticles were determined by TEM analysis. TEM result shows particular size of LMWC nanoparticles are in the range of 30-50 nm (Fig. 4A). Smaller size of LMWC nanoparticles indicates higher water solubility. Moreover, observed LMWC nanoparticle size has approximately smaller than observed hydrodynamic diameter obtained from the DLS experiment. TEM analysis has described the size in the dried state of the sample, whereas DLS depicts the size in the hydrated state of the sample. Therefore, measured size in DLS represents a hydrodynamic diameter. It has a larger particle size. However, one has to bear in mind that by TEM we image single particle, while DLS gives an average size estimation, which is biased towards the larger-size end of the population distribution.

Average molecular weight of LMWC nanoparticle has been determined by GPC using THF as solvent. GPC result has showed that average molecular weight of depolymerised LMWC is 19.3 (± 0.56) kDa (Table 1). The maximum zetapotential has found to be + 36.5 (± 4.88) mV for LMWC nanoparticles (Fig. 4C). These results clearly indicate that potassium persulfate treatment on LMWC nanoparticles preparation has profound effect for increasing the surface charges. This higher zeta potential of LMWC nanoparticles has surely enhanced the efficiency of drug delivery in tetracycline resistant bacteria *Escherichia coli XL-1 Blue*.

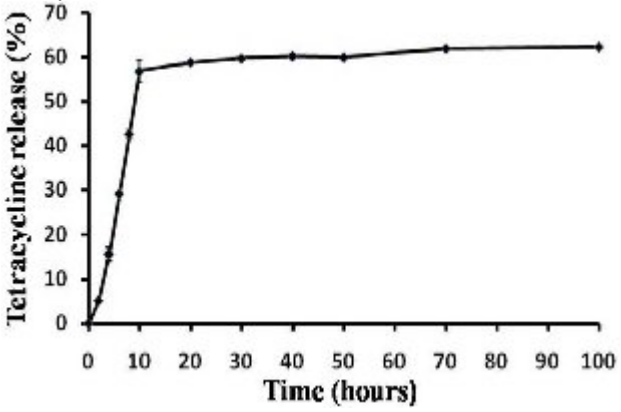

Fig. 5: *In vitro* release profile of tetracycline from tetracycline loaded LMWC nanoparticles. Each data point is the mean of three sample and error bars are standard deviation.

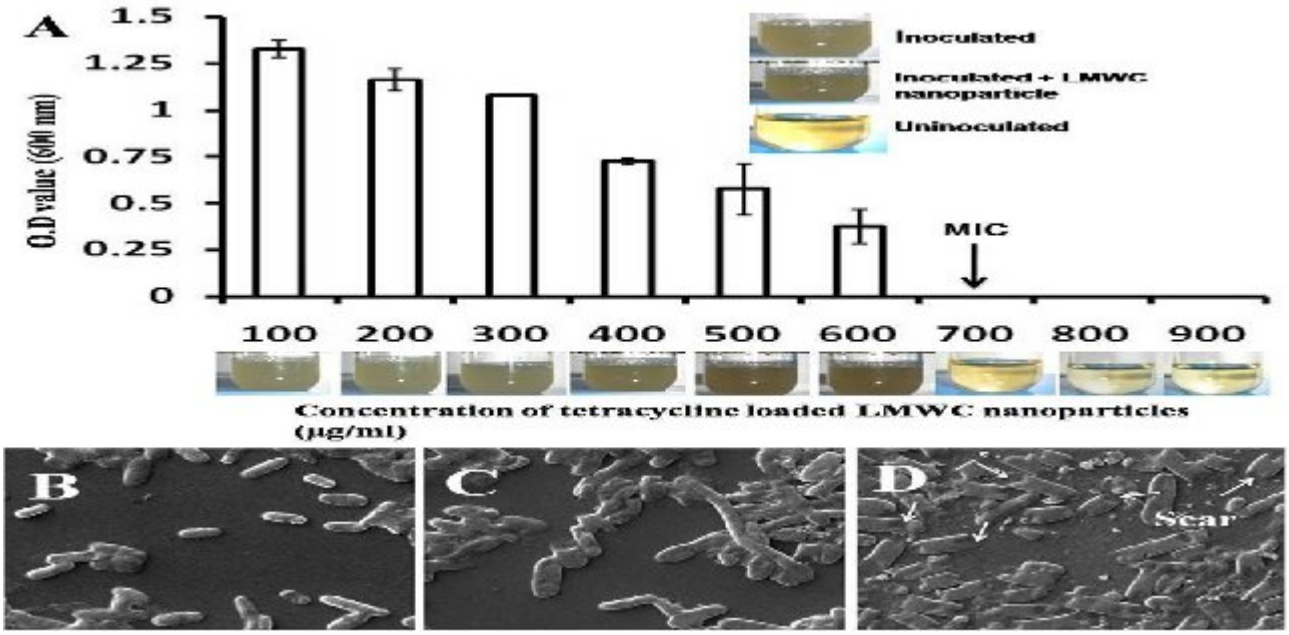

Fig. 6: Evaluation of MIC and putative mode of action of tetracycline loaded LMWC nanoparticles on tetracycline resistant bacteria *Escherichia coli XL-1 Blue.* (A) Uninoculated represents the only autoclaved nutrient broth (negative control), Inoculated represents the autoclaved nutrient with tetracycline resistant bacteria *Escherichia coli XL-1 Blue*, Inoculated + LWMC nanoparticle designates that LMWC nanoparticle without tetracycline loading in drug resistant bacterial culture (positive control), (B) Untreated bacterial cells, (C) Bacterial cells treated with LMWC nanoparticles without tetracycline loading, (D) Deformed bacterial cells after tetracycline loaded LMWC nanoparticles treatment after 12 h incubation time.

Drug can be loaded into a nanoparticulate system in two ways: during the preparation of the particles or following their formation. In such systems, the drug molecules are embedded physically in to the matrix or absorbed onto the surface. Drug loading can be maximized by incorporating the drug during the formation of the nanoparticulate system. In the present study the tetracycline physical loading efficiency in LMWC nanoparticulate system was 66.56 (± 1.88) %.

Due to the mutation in the tetracycline drug transporter protein in tetracycline resistant bacteria, effective release of tetracycline from LMWC nanoparticles is desired. Tetracycline *in vitro* release profile is shown in (Fig. 5). The profile shows two stages. First stage belongs to fast release till first 10 hours. The second stage belongs to after 20 hours to 100 hours with much more slower and gradual release. Within first 10 hours, 56.89 (± 2.34) % of tetracycline was released and after 100 hours a total of 62.4 (±0.14) % of tetracycline was released. The initial burst of tetracycline may be attributed to tetracycline existing at or near the surface of LMWC nanoparticles. However, the gradual release of tetracycline at second stage was probably caused by slow diffusion of tetracycline from the LMWC nanoparticles. In addition even after 100 hours, the release rate of tetracycline is still constant and there is no tail off or burst. Antibacterial effect of tetracycline loaded LMWC was evaluated by MIC assay using drug resistant bacteria *Escherichia coli XL-1 Blue*. The minimal inhibitory concentration of tetracycline loaded LMWC nanoparticle was found to be 700µg/ ml (w/v) for *Escherichia coli XL-1 Blue* (Fig. 6A).

A further SEM study was performed to find out the putative action of antibacterial activity of tetracycline loaded LMWC nanoparticles on drug resistant bacteria. LMWC nanoparticles with higher positive charge (at below pH 6.2) and HMWC nanoparticles cannot pass through bacterial membrane and hence stack to the cell surface, which blocks nutrient transport or permeablilizes the bacterial cell membrane, resulting cell lysis. Blockage of the transport system looks more practical because, when the chitosan nanoparticles were added to the test cultures in the nutrient broth, there was the formation of turbidity, which could be due to the binding of positively, charged chitosan onto the negatively charged cell surface thus causing bacterial micro-aggregation. [32]

While LMWC show a similar bactericidal action, owing to their small size and being higher water dispersion, they can transverse the bacterial membrane and may bind and regulate DNA transcription. Even this kind of LMWC could also bind to trace metals, distorting the membrane structure or its water binding capability may deprive water availability for bacteria causing cellular death. [33]

In the present study, there was no turbidity appeared after the tetracycline loaded LMWC nanoparticles addition into the test culture media. It clearly pointed out that the aggregation did not occur due to comparatively smaller molecular size, and higher solubility of tetracycline loaded LMWC nanoparticles. Further, SEM studies indicated scar formation on the bacterial cell surface especially in the bacterial polar ends after 12 h treatment with tetracycline loaded LMWC nanoparticles (Fig. 6B, 6C, and 6D). Scar formation might be happened due to erroneous proteins synthesis and membrane assembly after tetracycline entry inside the bacterial cell through LMWC nanoparticle.

Table 1: Average molecular weight determination of depolymerised chitosan by GPC

Sample	Average molecular weight (kDa)	Standard deviation
Native chitosan	250.0**	±3.15
Depolymerised chitosan*	19.3	±0.56

* Molecular weight was determined by taking the sample which are collected and purified from optimized depolymerised condition 37oC, pH-4, and potassium persulfate concentration 0.05 M in the reaction mixture

** Degree of deacetylation 90.2% as per commercial product catalog information

DISCUSSION

In summary, LMWC nanoparticles can be prepared with a starting material depolymerised native chitosan through potassium persulfate and silver nitrate composite actions in an optimized condition. The depolymerisation of native chitosan is highly influenced by the potassium persulfate concentration, silver nitrate concentration, pH and reaction time. The hydrodynamic diameter decreases rapidly with degree of oxidation especially with increase in potassium persulfate concentration. Oxidation of native chitosan produces a decreasing in the stiffness of the polymer by breaking C2-C3 bond with a chain scission as a simultaneous reaction. In the present study, LMWC nanoparticles were developed by ionotropic gelation with salt of TPP using depolymerised chitosan. Average molecular weight of depolymerised LWMC was determined by GPC analysis. Even physico-chemical parameters of newly developed LMWC nanoparticles were clearly shown that these nanoparticles are highly stable and most effective as drug delivery vehicle. Finally, the antimicrobial activity was determined by following MIC assay using tetracycline loaded LWMC nanoparticle on tetracycline resistant bacteria *Escherichia coli XL-1 Blue*. Even we tried to provide a tentative idea about the molecular action of this drug loaded nanoparticle on drug resistant bacteria by a special time scale SEM analysis. However, there are significant remaining issues to practical implementation, such as large scale production, interaction of these drug loaded LMWC nanoparticles with human tissue cells and metabolic fates. Hence further deep research for solving these practical barriers may be pioneered a new avenue in near future.

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