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Encapsulation and *In vitro* Drug Release Studies of Anti Tuberculosis Drug Using Chitosan Nanoparticles

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Abstract: The findings of the present study demonstrate that molecular weight of chitosan, Rifampicin and concentration of Tripolyphosphate are important factors needed to be considered for development of chitosan nanoparticles by ionic gelation method with desirable physico-chemical and release characteristics. In future, cost effective drug will be developed based on the polymeric nano particles.

Key words: Nano Particle · Chitosan · Anti TB

INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* which most commonly affects the lungs but also can involve almost any organ of the body. Tuberculosis is still being the leading cause of death all over the world. The status of this infection has been increased to nearly 8.8– 9.9 million new cases emerging every year [1]. The major difficulties encountered in the treatment of tuberculosis are side effects and patients noncompliance due to the prolong treatment which is the common cause of drug resistance [2]. This problem can be eliminated by prescribing a single dose with a sustained release, which is one of the major advantages present in our prepared formulation. Rifampicin is an antituberculous drug which is bacteriostatic. These tablets are so bulky that some patients find it difficult to swallow. This size and the frequency of dosage can be reduced when the drug is loaded with a polymer and converted into nanoparticles form. The conventional drug delivery system has a disadvantage of fluctuating drug plasma level which will need frequent doses to maintain the drug level constant.

Nano-antibiotics are novel drug candidates of promise to combat both microbial infections and the increased bacterial resistance to antibiotics. Nano-antibiotics provide efficient anti-infective agents for biomedical and therapeutic applications. Nano-antibiotics are considered a potential treatment option to control infectious diseases and to reduce the overwhelming microbial resistance prevalent nowadays [3]. Nanoparticles (NPs) are a special group of materials with unique features and extensive applications in diverse fields [4]. In the recent years, Chitosan nanoparticles arious micro/nano carriers, particularly nanoparticles and nanofibers, have become available for enzyme immobilization [5]. Typically, smaller particles provide a larger surface area for the attachment of enzymes and a shorter diffusional path for the substrates. Thus, nano-structured carriers have been utilized as carriers for enzyme immobilization.

Recently, several pharmaceutical approaches were attempted to improve the bio availability of rifampicin. Controlled drug delivery systems, nanoparticles, liposomes and microspheres were developed for the sustained drug delivery of anti-TB drugs that have demonstrated better chemotherapeutic efficacy when investigated in animal models [6].

Various polymers have been recommended for preparation of nanoparticles. Biodegradable polymers are preferred as they are eliminated from the body and therefore polymer induced toxicity is unlikely. As TB requires long term treatment, naturally occurring bio degradable polymers are recommended for chronic

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infections [7]. Among various polymers chitosan has shown favorable biocompatibility and membrane permeability both in vitro and in vivo [8]. Chitosan has many advantages particularly for developing micro/nanoparticles. These include its ability to control the release of active agents; it avoids the use of hazardous organic solvents while fabricating particle since it is soluble in aqueous acidic solution.

MATERIALS AND METHODS

Collection of Sample: The chitin used in this study was purchased from Hi-media and anti-TB drugs such as Rifampicin, Isoniazid, pyrazinamide, Ethambutol were purchased from commercial shop collected from Medical shop nearby Kanchipuram.

Preparation of Chitosan: The chitin was converted to chitosan by deacetylation in hot NAOH solution (40 to 50% W/V), at 90 to 120°C for 4 to 5 hours. The crude chitosan was dissolved in aqueous 2% W/V acetic acid. Then the insoluble material was removed giving a clear supernatant solution, which was neutralized with NAOH solution resulting in a purified sample of chitosan as a white precipitate. Further purification may be necessary to prepare medical and pharmaceutical grade chitosan [9].

Purification of Chitosan: The obtained chitosan have to be purified to make it suitable for use. The purification process was designed in three steps – removal of insoluble materials by filtration, precipitation of chitosan with 1N sodium hydroxide, demetallization of retrieved chitosan [10].

Biosynthesis of Chitosan Nanoparticles: Chitosan nanoparticles were prepared according to the ionotropic process. Nanoparticles were obtained upon the addition of a sodium tripolyphosphate (50mg TPP/25ml) aqueous solution to a chitosan solution (250mg/50ml) stirred at room temperature. The formation of nanoparticles was a result of the interaction between the negative groups of TPP and the positively charged amino groups of chitosan. The ratio of chitosan/TPP was established according to the preliminary studies. Rifampicin loaded nanoparticle were obtained according to the same procedure and the ratio of chitosan/TPP remained unchanged. Variable amounts of drugs were incorporated to the chitosan solution prior to the formation of nanoparticles in order to investigate the effect of the anti-TB drugs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol) concentration on the nanoparticle characteristics and in-vitro release and encapsulation efficiency profiles. Nanoparticles were collected by centrifugation at 10,000 rpm for a period of 30 minutes and supernatant were discarded. Followed by drying, nanoparticles were collected [11].

Drug Encapsulation Efficiency: The encapsulation efficiency was analyzed according to the procedure reported by Urrusuno and his research team [12]. After drug loading, nanoparticles were separated from the suspension by ultracentrifugation at 15500 rpm and 4°C for 30 min. The amount of free drugs in the supernatant was measured by UV-Vis spectrophotometer (Cecil spectrophotometer) at 270 nm. The encapsulation efficiency (EE) was calculated by the equation 2: E% = ×100 (Equation 2) Where F is the free amount of drug in the supernatant and T is total amount of drug added into CS solution. A blank sample was made from nanoparticles without loaded drug but treated similarly as the drug-loaded nanoparticles.

***In-vitro* Drug Release Studies:** The in-vitro drug release tests were carried out on all formulations (3 and 6% drug loaded samples). Fifty milligrams of each sample were placed in dialysis membrane suspended in various pH 5.0, 6.8 and 7.4 of 100 ml of PBS buffer at 37°C and placed in a incubated shaker at 120 rpm. At predetermined time intervals, 3ml of aliquots was withdrawn and the concentration of drug released was monitored by UV spectrophotometer (Cecil spectrophotometer) at 270nm. The dissolution medium was replaced with fresh buffer to maintain the total volume. The drug release percent can be determined by the following formula.

$$\text{Drug release \%} = C(o)\ C(t) = Ct \times 100$$

where C (o) and C (t) represents the amount of drug loaded and amount of drug released at a time t, respectively. All the studies were done in a triplicate.

Anti Mycobacterial Screening Luciferase Reporter Phage Assay (LRP): Standard strain, *M. tuberculosis* H37Rv, a drug sensitive reference strain grown and maintained on Lowenstein Jensen medium in the Department of Bacteriology, Centre for drug discovery and development, Sathyabama University, Chennai, was used for the study.

About 100 µL of *M. tuberculosis* cell suspension was added to 400 µL of G7H9 with desired concentration of test compound (Rifampicin loaded chitosan nanoparticle)

500 µg/mL. For each sample, one reference control rifampicin (2 µg/mL), a drug free control plain media and a solvent control [dimethyl sulfoxide 1% (v/v)] were prepared. This set up was incubated for 72 h at 37 °C and after incubation 50 µL of the high titer LRP (phage 129) and 40 µL of 0.1 mol/L CaCl2 was added to all the vials and this setup was incubated at 37 °C for 4 h. After incubation 100 µL of the mixture was taken from each tube into a cuvette and 100 µL of working D-Luciferin solution was added. The relative light units (RLU) was measured in luminometer with integration time of 10 seconds. The percentage reduction in the RLU was calculated in test sample compared with control as follows**.**

RESULTS

Preparation of Rifampicin Loaded Chitosan Nanoparticles: The present study followed ionic gelation method for preparation of chitosan nanoparticles loaded with rifampicin drugs using the natural and biodegradable polymer of chitosan of different molecular weights.

Encapsulation Efficiency: The initial concentration of RIF plays an important role in the encapsulation efficiency. When the concentration of RIF is increased, the encapsulation efficiency of CS and CS-RIF polymer combination is increased. The CS-RIF nanoparticles encapsulation efficiency was slightly greater than the CS nanoparticles. However, the increased RIF concentration leads to an increase of both encapsulation efficiency of CS-RIF nanoparticles. It was found that the EE of CS-RIF nanoparticles are higher than that of CS nanoparticles. When the initial concentration of RIF is the same, which could be attributed to the fact that CS-RIF nanoparticles is a more electrostatic attraction than CS nanoparticles (Table 1).

***In-Vitro* Drug Release Studies:** In order to investigate the effect of pH on the swelling of composite and chitosan, we have measured the percentage cumulative release in both pH-7.

Anti Mycobacterial Screening Luciferase Reporter Phage Assay (LRP): Laboratory reference strain *M. tuberculosis* H37Rv was used for screening of chitosan nanoparticles loaded with Rifampicin for antimycobacterial activity. Among the chitosan nanoparticles, Rifampicin and chitosan nanoparticles loaded Rifampicin (CHD3) tested, *M. tuberculosis* H37Rv was found to show good bio- activity at 500µg/ml concentration. The result was showed at Table 2.

Luciferase Feporter Phage Assay [LRP] Result Interpretation: The test compound /extract showed inhibition against *M. tuberculosis* H37Rv strain µg/ml concentration.

DISCUSSION

In this present investigation, synthesize of chitosan nano particle though ionic gelation method. Chitosan, a cationic polysaccharide with a high molecular weight, linear polymer that comprises -1,4- linked glucosamine (GlcN) with various amounts of N- acetylated GlcN residues. It is typically obtained by the alkaline deactivation of chitin extracted from an abundant source of shellfish exoskeletons or the cell walls of some microorganisms and fungi [12]. Chitosan on its own have various pharmacological properties Burrows and his research team in 2007 [13] extracted and evaluated chitosan from crab exoskeleton as a seed fungicide and plant growth enhancer. In the present study,TB-loaded CS-NPs with good dimensional features were prepared,

Table 1: The encapsulation efficiency and loading capacity of RIF-CS.

Mass ratio (RIF:CS)	EE%
1:4	43
1:2	44
1:1	45

Table 2: Antimycobacterial Screning Luciferase Feporter Phage Assay [LRP] Result

			H37Rv		
SAMPLES NAME	CDDD lab NO:	Concentration Tested	INH-0.2µ/ml	RIF-2µ/ml	TEST COMPOUND
Rifampicin (D2)	ATBS0066	500µ/ml	S	S	Inhibition
Chitosan Nanoparticles loaded with drugs(CHD3)	ATBS0067	500µ/ml	S	S	Inhibition
Chitosan nanoparticles (CH1)	ATBS0068	500µ/ml	S	S	Inhibition

based on the ionotropic gelation between CS and sodium tripolyphosphate [14]. The encapsulation efficiency of TB in the formulation was up to 75%. Release kinetic studies highlighted a linear release of the peptide from the Nano carrier, in the adopted experimental conditions.

Biodegradable nanoparticles are frequently used to enhance the therapeutic value of various water soluble/insoluble medicinal drugs and bioactive molecules by improving bioavailability, solubility and retention time [15]. The CS-RIF nanoparticles encapsulation efficiency was slightly greater than the CS nanoparticles. However, the increased RIF concentration leads to an increase of both encapsulation efficiency of CS-RIF nanoparticles. It was found that the EE of CS-RIF nanoparticles are higher than that of CS nanoparticles. When the initial concentration of RIF is the same, which could be attributed to the fact that CS-RIF nanoparticles has more electrostatic attraction than CS nanoparticles. Rafeeq and his research team [16] reported, to load first line antitubercular drug, isoniazid in chitosan Nanoparticles in order to enhance bioavailability and to reduce dose frequency. Chitosan was dissolved in acetic acid aqueous solution at various concentrations; Drug was dispersed in above Chitosan solution kept over magnetic stirrer at room temperature for a period of 30 minute. The Tripolyphosphate aqueous solution with various concentrations added drop wise to the above solution. Followed by sonication for 5 min. The resulting Chitosan nanoparticles suspension was centrifuged at 16,000 rpm for 30 min. After freeze drying the Nanoparticles were collected. SEM studies show that formulation No: 2 having optimum nanonized particles. Zeta potential shows good positive potentials. It shows good encapsulation efficiency. And good release profile follows first order release kinetics. From all these results it concludes that formulation No 2 is the best formulation and which is recommended for future studies like nano dry powder preparation.

An efficient vaccine against TB is an urgent need. TB peptides are safe candidate but they are weak immunogens and needs to be potentiated by adjuvant/ delivery systems. The main purpose of the present study was to determine the potential of CHT based NPs containing ESAT-6 antigen of *M. tuberculosis* for inducing mucosal and systemic immune responses after intranasal and subcutaneous injection in mice model [17].

Chitosan and chitosan derived nanoparticles and Nano composites are very promising due to their versatile properties and have found applications in various areas such as biomedical, foods, food packaging and personal care. Many studies suggest that chitosan nanoparticles and their derivatives are one of the best barrier and film coating materials for preserving the quality of foods and for delivering skin care products, mainly due to their biodegradability and anti-microbial properties. Future studies shall address establishment of industrial scale up of chitosan nanoparticles, safety and quality aspects of these chitosan derived products for human use [18].

Reduction in the dosing frequency of antituberculosis drugs (ATDs) by applying drug delivery technology has the potential to improve the patient compliance in tuberculosis (TB). Alginate (a natural polymer) based Nano particulate delivery system was developed for frontline ATDs (rifampicin, isoniazid, pyrazinamide and ethambutol). Alginate nanoparticles were prepared by the controlled cation induced gelification method and administrated orally to mice. The drug levels were analyzed by high performance liquid chromatography (HPLC) in plasma/tissues. The therapeutic efficiency was evaluated in *M. tuberculosis* $H_{37}Rv$ infected mice. Alginate nanoparticles appear to have the potential for intermittent therapy [19].

REFERENCES

1. World Health Organization (WHO). 2014. Global tuberculosis report.
2. Yee, D., M.D. Valiquette, I.P.P. Rocher and D. Menzies, 2003. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. Am J Respir Crit Care Med., 167: 1472-77.
3. Ezzat, M., E. Zowalaty, A.S.H. Hussein, M.I. Hussein, B.M. Geilich, T.J. Webster and M.Z. Hussein, 2015. The ability of streptomycin-loaded chitosan coated magnetic nanocomposites to possess antimicrobial and antituberculosis activities. International Journal of Nanomedicine, 10: 3269-3274.
4. Matei, A., I. Cernica, O. Cadar, C. Roman and V. Schiopu, 2008. Synthesis and characterization of ZnO polymer nanocomposites. International Journal of Material Forming, pp: 33.
5. Jia, H.F., G.Y. Zhu, B. Vugrinovich, W. Kataphinan, D.H. Reneker and P. Wang, 2002. Enzymecarrying polymeric nanofibers prepared via electrospinning for use as unique biocatalysts. Biotechnol. Prog., 18: 1027-1032.
6. Falk, R., T.W. Randolph, J.D. Meyer, R.M. Kelly and M.C. Manning, 1997. Controlled release of ionic compounds from poly (L-lactide) microspheres produced by precipitation with a compressed antisolvent. J Contr Rel., 44(1): 77-85.

7. Lifeng, Q.I., X.U. Zirong., J. Xia., H. Caihong and Xiangfeiz 2004. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydrates Res., 339: 2693-2700.
8. Yang. T.W, H.O. Park, H.N. Jang, J.H. Yang, S.H. Kim, S.H. Moon, J.H. Byun, C.E. Lee, J.W. Kim and D.H. Kang, 2017. Side effects associated with the treatment of multidrug-resistant tuberculosis at a tuberculosis referral hospital in South Korea. Medicine (baltimore), 96(28): 7482.
9. Bhargavi K. Sailaja and R. Shyam Sunder, 2013. Formulation and Evaluation of Ranitidine Hydrochloride Floating Tablets using various Polymers. International Journal of Pharmacy and Integrated Life Sciences, 1(5): 150-161.
10. Qian, R.Q. and R.W. Glanville, 2005. .Methods for purifying chitosan providence health system. US Patent, pp: 6896809.
11. Rafeeq, M.P.E., V. Junise, R. Saraswathi, P.N. Krishnan and C. Dilip, 2010. Development and characterization of chitosan nanoparticles loaded with isoniazid for the treatment of Tuberculosis. RJPBCS, 1(4): 383.
12. Urrusuno, F.R.P., C. Calvo, R. Lopez, J.L. Vila-Jato and M.J. Alonso, 1999. Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm. Res., 16: 1576-1581.
13. Uztuzun, S.O., N. Tandogan, M. Turk, S. Karahan and Z. Suludere, 2015. The Apoptotic and Necrotic Effects of Cisplatin Loaded Chitosan Nanoparticles on Hela Cell Lines. Academic Journal of Cancer Research, 8(4): 58-68.
14. Rajalakshmi, N. Krithiga and A. Jayachitra, 2013. Antioxidant Activity of the Chitosan Extracted from Shrimp Exoskeleton. Middle-East Journal of Scientific Research, 16(10): 1446-1451.
15. Burrows F., C. Louime, M. Abazinge, Oghenekome Onokpise. 2007. Extraction and Evaluation of Chitosan from Crab Exoskeleton as a Seed Fungicide and Plant Growth Enhancer. American-Eurasian J. Agric. & Environ. Sci., 2 (2): 103-111.
16. Piras, A.M., G. Maisetta, S. Sandreschi, M. Gazzarri, C. Bartoli, L. Grass, S. Esin, F. Chiellini and G. Batoni 2015. Chitosan nanoparticles loaded with the antimicrobial peptide temporin B exert a long-term antibacterial activity in vitro against clinical isolates of *Staphylococcus epidermidis.* Front. Microbial., 6: 372.
17. Seyedeh, M.K., S. Mojtaba, K. Frazad and T. Mohsen, 2016. Chitosan (CHT) and trimethylchitosan (TMC) nanoparticles as adjuvant/delivery system for parenteral and nasal immunization against *Mycobacterium tuberculosis* (MTb) ESAT-6 antigen. Journal of Nano Med, 3(4): 223-229.
18. Radhakrishnan, Y., G. Gopal, C.C. Lakshmanan and K.S. Nandakumar, 2015. Chitosan nanoparticles for generating novel systems for better applications: A Review. Journal of Molecular and Genetic Medicine, 4: 5.
19. Ahmad, Z., R. Pandey., S. Sharma and G.K. Khuller, 2006. Alginate nanoparticles as antituberculosis drug carriers: Formulation, development, pharmacokinetics and therapeutic potential. The Indian Journal of Chest Diseases & Allied Sciences, 48: 171-176.