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Research Article

ENCAPSULATION, CHARACTERISATION AND IN-VITRO RELEASE OF ANTI-TUBERCULOSIS DRUG USING CHITOSAN – POLY ETHYLENE GLYCOL NANOPARTICLES

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ABSTRACT

An attempt has been made to encapsulate anti-tuberculosis drug, Rifampicin (RIF) with a model drug delivery system. The designed carrier Chitosan (CS) and polyethylene glycol 600 (PEG) nanoparticles were prepared by Ionic gelation technology, and then used for entrapping RIF. The PEG binding with CS-RIF changed the character and the surface of the nanoparticles and slightly increased its particle size, while the drug encapsulation was also increased. PEG bind with CS-RIF achieved a significantly prolonged retention compared with non-coated CS-RIF. Various parameters and methodologies such as loading capacity, encapsulation efficiency, SEM, FTIR and in vitro release have been utilized for characterization of nanoparticles. The release of drugs was influenced by their initial drug concentration, indicating that the release of drugs could be controlled by varying the initial drug concentration. All results suggested that CS and CS-PEG nanoparticles are promising system for delivering RIF in treatment of tuberculosis.

Keywords: Chitosan; drug delivery, Nanoparticles, Polyethylene glycol, Rifampicin.

INTRODUCTION

The drug delivery technology landscape is highly competitive and rapidly evolving. Areas that are being targeted for improvements through device development include improved efficacy, reduced side effects, continuous dosing, reduced pain from administration, increased ease of use and improved mobility[1]. Modern drug carrier systems play an important role in controlled release of a pharmaceutical agent to the target at a therapeutically optimal rate and dose[2]. Among the developed drug carriers which include liposomes 3, polymeric micelles[4], dendrimers[5], polyelectrolyte capsules[6], ceramic nanoparticles[7], protein cage architectures[8], virus-derived capsid nanoparticles[9] and polyplexes[10], liposomes are the first carrier to reach clinics[11]. Hydrophobic drugs can be loaded into the lipid bilayer of liposomes and delivered in circulation. The limitations for liposomes to encapsulate hydrophobic drugs are the efficacy of drug loading procedures currently available and the difficulty in controlled release. Therefore, efficient encapsulation of hydrophobic drugs into a biocarrier and release drugs under control is still a challenge. In this connection, some biodegradable polymers such as chitosan, PLA, PCL etc., are commonly used as these polymers can be prepared in the moderate conditions, has a similar stiffness of the body and has an appropriate biodegradability and low crystallinity enough to be mixed well with many kinds of drugs[12]. There are some formulations for the drug delivery systems, for example, films, gels, porous matrices, microcapsules, micro spheres, nanoparticles, polymeric micelles and polymer linked drugs [13-15].

Tuberculosis (TB) is a common and in many cases lethal infectious disease caused by various strains of mycobacteria, usually mycobacterium tuberculosis. Tuberculosis usually attacks the lung, so improving drug pulmonary concentration has been one of the goals in anti-tuberculosis study areas. There are reported studies that focus on improving drug concentration in the lung, such as lung-targeting microspheres [16, 17] nanoparticles[18, 19] aerosolized liposome[20] and aerosols for inhalation administration[21-23]. Tuberculosis (TB) is an example of such disease for which limited number of antibiotics, for example, rifampicin, isoniazid, and ethambutol, are the essential choices for the clinical disease management[24-28]. Among others, Rifampicin (RIF) is a potent antituberculosis drug and has enormous pharmacological significance. It is a very effective antibiotic, particularly active against Mycobacterium tuberculosis, M. bovis, M. avium complex, and M. Intracellular[29-33], and hence it is highly effective in the treatment of TB. It is known that RIF is a specific inhibitor of bacterial RNA polymerase, which acts by direct interaction with the enzyme[34], probably by inhibiting the process of transcription at the level of initiation[35]. Although, RIF is poorly soluble in aqueous media, it is a clinically effective drug. The inescapable necessity and poor solubility of RIF automatically necessitates an alternate drug delivery/carrier system[36, 37]. Commonly RIF was taken on an empty stomach with a full glass of water. So it leads to side effect on stomach, heartburn, nausea, menstrual changes, headache and dizziness. Anti-tuberculosis drug administered in these ways could distribute to other non-targeted organs and may cause adverse and side effects. Hence, it is of importance to seek a substituted administration method. Interventional technology has been applied commonly in clinical treatment of tuberculosis because the drug can be delivered directly to the lung instead of through the blood vessel system. But the retention property of liquid or suspension, two commonly used formulations in interventional technology, is not satisfying because they cannot persistently stay in the lung[38]. Owing to this limitation, tuberculosis patients have to receive interventional operation frequently in order to maintain a therapeutic drug concentration. The objective of the present work was to improve patient compliance by developing a new drug delivery system that can be successfully used in interventional technology. In this regard, the interaction of RIF with various potential drug carrier systems is essential.

In these studies, the improvement and need of poorly soluble drug RIF was coated with biodegradable polymers. Rifampicin has a low solubility in water and varies with pH and type of solution. At 37 °C, 200 mg mL−1 is soluble in 0.1 M HCl while 9.9 mg mL−1 is soluble in phosphate buffer at pH 7.4. It is freely soluble in many organic solvents. The poorly soluble of RIF in aqueous media was improved by the surface coating by hydrophilic polymers such as chitosan (CS) and polyethylene glycol (PEG). Chitosan is a biodegradable, biocompatible cationic polymer with low toxicity, mucoadhesive properties, biodegradability and ability to enhance the penetration of large molecules across mucosal surfaces[39]. Hydrophilic polyethylene glycol (PEG) was introduced as an additional coating polymer and it has improved the dissolution characteristics of poorly soluble materials[40, 41]. PEG is an ideal material in biomedical applications. As a biocompatible, non-toxic, non-immunogenic and water soluble polymer, it is often used in cryoprotection, pharmaceutical preparation, tissue culture and organ protection[42,43]. Thus, we speculate that a combinatorial coating of chitosan and PEG of drug nanoparticles, due of their unique properties could serve as an ideal carrier system for the delivery.

MATERIALS AND METHODS

Materials

Chitosan (CS) derived from crab shell, was purchased from Otta Chemika-Biochemika reagents. Acetic acid, sodium tri polyphosphate

(TPP) and poly lactic acid were sourced from Merck Chemical Company Inc. (Mumbai, India). All other chemicals were of analytical grade and obtained from the chemical store of Periyar University (Salem, India).

Preparation of CS nanoparticles

The preparation of CS nanoparticles is based on an ionic interaction between positively charged CS solution and negatively charged TPP solution[44]. The chitosan nanoparticles were prepared by following the reported method[45] with is slightly modified. CS was firstly dissolved in 1% aqueous acetic acid solution at a concentration of 4 mg/ml, and TPP was dissolved in distilled water with concentration of 2 mg/ml. RIF was dissolved directly in TPP solution and prepared different (10, 20, 30, 40 and 50 %) percentage viz., 4, 8, 12, 16 and 20 mg of drug in 4 ml TPP before the synthesis of CS nanoparticles. Then, 4mL TPP solution (2 mg/ml) containing the combination drugs was dropped into 10mL CS solution under magnetic stirring (1000 rpm) at room temperature. CS nanoparticles were formed instantaneously. The CS nanoparticles suspension was kept stirring at 25℃ for 90 min for further cross linking of nanoparticles. Finally, CS nanoparticles were collected by centrifugation at 12, 500 rpm and freeze-drying at -40ºC for 24 h.

Coating of poly etheleneglycol (PEG)

The different percentage of encapsulated CS-RIF with PEG solution was prepared as follows. Firstly PEG was dissolved in water to form a 10 % solution. Then the above mentioned PEG was slowly added to an appropriate portion of different percentage encapsulated CS-RIF, followed by magnetic agitation at 25 ℃ for 90 min. Finally, different percentages of encapsulated CS-RIF-PEG nanoparticles were collected by centrifugation at 12,500 rpm and freeze-drying at -40 for 24 h.

Particle size

The particle size, size distribution and zeta potential of drug loaded nanoparticles were measured in a Zetasizer (Malvern instruments DTS Ver 4.10).

Morphological and FTIR analysis of CS nanoparticles

Scanning electron microscopy (SEM) was used in the morphological analysis of RIF, CS-RIF and PEG bind CS-RIF-PEG nanoparticles. The FTIR spectra of samples were measured using a Fourier transform infrared spectrometer (Spectrum GX-1, PerkinElmer, USA). Briefly, a small quantity of CS nanoparticles was mixed with 200 mg KBr and compressed to form tablets. These tablets were scanned, in transmission model, in the spectral region of 4000–400cm^{-1}, using a resolution of 4 cm^{-1} and 32 co-added scans.

Evaluation of drug loading capacity and encapsulation

CS nanoparticles suspensions were separated with a centrifuge at 1250 rpm for 30 min, and the drug encapsulation efficiency (EE) and loading capacity (LC) of CS nanoparticles were evaluated by measuring the UV spectrophotometer (Systronics, India) absorption of the supernatant. The corresponding calibration curves were made by testing the supernatant of blank CS nanoparticles. Each sample was measured in triplicate. RIF was measured in the λ_{max} value of 333 nm. The encapsulation efficiency and loading capacity of RIF of the CS and CS-PEG nanoparticles were calculated according to the following equations[46]

$$EE = \frac{W_t - W_f}{W_t} \quad \text{..... (1)}$$

$$LC = \frac{W_t - W_f}{W_n} \quad \text{..... (2)}$$

where W_t represents the total amount RIF; W_f is the amount of free RIF in the supernatant; and W_n is the weight of nanoparticles after freeze-drying. All measurements were performed in triplicate and the mean value was reported.

Evaluation of in vitro drug release

The evaluation of drug release was performed at 37 ºC using the dissolution tester (Disso test, Lab India, Mumbai, India) equipped with six paddles at a paddle speed of 100 rpm. About 900 ml of phosphate buffer solution (pH 3.4 and 7.4) was used as the dissolution media to stimulate the gastrointestinal tract (GIT) conditions. A 5 ml aliquot was used each time for analyzing the rifampicin content at a fixed time interval. The dissolution media were replenished with a fresh stock solution. The amount of rifampicin released was analyzed using a UV spectrophotometer (Systronics, India) at the λ_{max} value of 333 nm.

RESULTS AND DISCUSSION

Characterization of the prepared nanoparticles

Formation of combination drug loaded CS nanoparticles

The CS-RIF and CS-RIF-PEG nanoparticles were obtained in the average size range of ≅ 210 280 and ≅ 210 to 300 nm. With increasing of RIF encapsulation concentration, the size of the particle is gradually increased and also the coordination of PEG to RIF encapsulated CS again increased the size of the nanoparticles. The preparation of CS nano particles was based on the ionic interaction of a positively charged CS solution and negatively charged TPP solution[47]. The charge density of both CS and TPP solution has a great effect on the ionic interaction. The zeta potential is an important index for the stability of nanoparticles suspension. The zeta potential increases with the increasing of drug encapsulation, so the stability of the drug increased with the increasing of encapsulation. The zeta potential was found to be 30.4 and 34.1mV for 50% RIF encapsulated CS and CS-PEG respectively, indicated good stability. The data are shown in Table 1. It has been reported that the value of zeta potential less than -30 mV or higher than +30 mV can be used to assure the stability of nanoparticles suspensions. The values obtained for the prepared nanoparticles were above +30, so it was stable. The positive values obtained for zeta potential indicated that the nanoparticles surface was positively charged. This may be due to the availability of free NH_3^+ groups on the polymer.

Table 1: The particle size and zeta potential values of CS-RIF and CS-RIF-PEG

% of RIF Concentration	Particle size (nm) Mean ± SD*		ZP (mV) Mean SD*		ZP (mV) in 0.1 mol/l KCl	
	CS-RIF	CS-RIF-PEG	CS-RIF	CS-RIF-PEG	CS-RIF	CS-RIF-PEG
10	209±19.1	211±11.3	36±1.8	39.0±2.7	-2.67±0.14	-1.86±0.09
20	215±15.2	219±18.4	34±1.3	38.5±1.9	-2.91±0.32	-1.32±0.05
30	222±14.4	226±12.1	32±2.1	37.2±1.2	-3.04±0.9	-3.47±0.17
40	228±13.2	231±17.5	31.7±1.3	35.0±2.9	-3.15±0.18	-2.76±0.2
50	263±22.5	272±10.2	29.4±2.2	34.1±1.1	-3.42±0.07	-3.85±0.06

RIF: rifampcin, CS: chitosan, PEG: polyethylene glycol 600, TPP: sodium tripolyphosphate, SD: standard deviation for three determinations. *n = 3. the experiments were repeated twice.

Surface morphology

The surface morphology of nanoparticles was studied using SEM. Fig. 1 shows the SEM images of Rifampicin (a), Chitosan-drug (b), Chitosan-drug-PEG (c)nanoparticles. The SEM image of the RIF is crystalline shape and in the addition of CS, the CS-RIF SEM image is homogenous surface. Furthermore, the incorporation of PEG into the CS-RIF produced a smooth surface and compact structure and the CS-RIF and CS-RIF-PEG nanoparticles were found to be spherical in shape. The SEM pictures showed that the RIF encapsulated chitosan and Chitosan-PEG nanoparticles were found to be in the size range of ≅ 200 to 270 and ≅ 200 to 300 nm.

Fourier transmission infra red spectroscopy (FTIR)

Fig. 1(d) shows the FTIR spectra of rifampicin (RIF), chitosan-rifampicin (CS), and chitosan–rifampicin-poly ethylene glycol (CS–RIF-PEG) blend. The result of the FTIR indicated that rifampicin nanoparticles showed a sharp peak at 3,483 cm^{-1}corresponding to OH, N–CH3 band at around 2,878 cm^{-1}, characteristic absorption band at about 1,727 cm^{-1} for acetyl C=O, sharp peaks at 1,645 and 1568 cm^{-1} representing the furanone C=O and amide C=O groups, respectively. The FTIR result indicated that the purchased rifampicin was in the crystalline form. Agrawal et al.[48] reported using the IR spectrum as a qualitative tool for identifying the crystalline and amorphous forms of rifampicin. Chitosan is an amino glucose characterized by a small proportion of amide groups via an amide linkage with acetic acid. The FTIR of both CS-RIF and CS-RIF-PEG showed a characteristic carbonyl ketone band at 1,738 cm^{-1}, but OH band at 3,483 cm^{-1} is slightly shifted to 3426 and 3392 cm^{-1}. The carbonyl furanone and carbonyl amide of rifampicin were also evident in the drug-loaded CS-PEG nanopaticles without major shift, indicating that there was no chemical interaction between the drug and copolymer. However, the peak sizes were smaller that could be due to the smaller amount of rifampicin entrapped in the polymer matrix. A similar finding was also reported by Abdulla et al.[49] in the rifampicin-loaded mPEG5000–DSPE polymeric micelles.

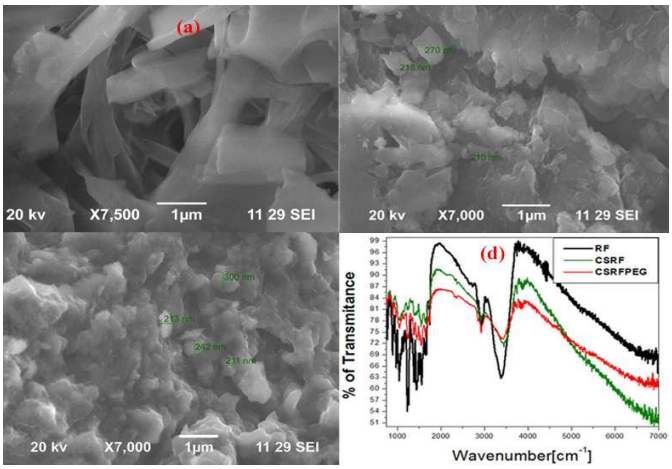

Fig. 1: SEM and FTIR images of rifampicin (a), CS-RIF (b), CS-RIF-PEG (c) and FTIR (d).

In-vitro drug release

In order to investigate the effect of pH on the swelling of composite chitosan and chitosan/PEG, we have measured the % cumulative release in both pH 3.4 and 7.4 media. Cumulative release data presented in Fig. 2 indicate that by increasing the pH from 3.4 to 7.4 of CS-RIF and CS-RIF-PEG, a considerable increase in the cumulative release is observed for both composites. From Fig. 2 it is seen that the 50% drug loaded CS-PEG polymer composites have shown longer drug release rates than the drug– CS composites. Thus, drug release depends upon the nature of the polymer matrix as well as the pH of the media. This suggests that the drugs in the blend can be used to be suitable for the basic environment of the large intestine, colon and rectal mucosa for which there are different emptying times. The CS-RIF-PEG binding polymer was taking much more time for release than the CS-RIF composite. The observed very slow release was significant because controlled release is required in the field of drug delivery. These results indicated that the CS-RIF-PEG nanoparticles are useful controlled delivery system for TB treatment. The electrostatic attraction between drug and CS-PEG polymer composite is higher than the drug and chitosan polymer composite. The SEM analysis Fig. 1 was also evidence for more electrostatic attraction of polymer and drug. This suggests that the drugs in the composites can be easily released into the basic than acidic environment. Because the electrostatic interaction of composites is more easily broken at pH 7.4 than at pH 3.4, leading to rifampicin being released more rapidly at pH 7.4 than 3.4.

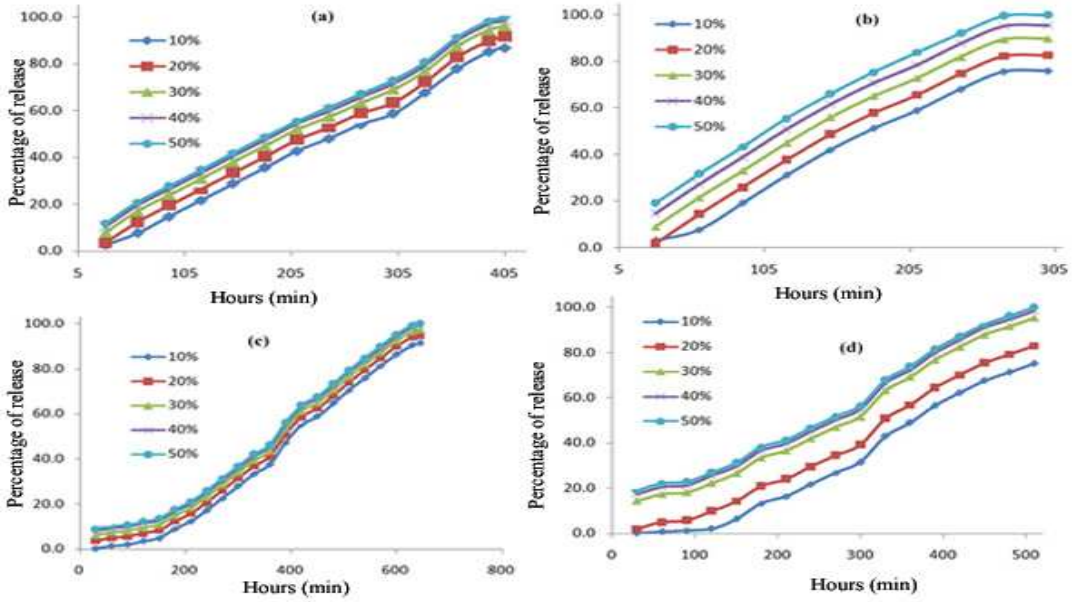

Fig. 2: In-Vitro analysis of rifampicin, (a) at pH 3.4 of CS-RIF, (b) at pH 7.4 of CS-RIF, (c) at pH 3.4 of CS-RIF-PEG and (d) at pH 7.4 of CS-RIF-PEG

Table 2: Encapsulation efficiency (EE) and loading capacity (LC) of CS nanoparticles

S. No.	% of RIF	CS-RIF nano particle		CS-RIF-PEG nano particle	
		% of EE	% of LC	% of EE	% of LC
1	10	43.0	8.9	58.0	9.2
2	20	53.0	18.1	62.0	18.5
3	30	58.7	27.5	67.3	28.0
4	40	62.0	37.0	69.0	37.5
5	50	62.6	46.3	72.8	47.3

Encapsulation efficiency and loading capacity of nanoparticles

The initial concentration of RIF plays an important role in the encapsulation efficiency and loading capacity of CS and CS-PEG nanoparticles (Table 2). When the concentration of RIF is increased, the EE of CS and CS-PEG polymer combination is increased. The CS-PEG nanoparticles encapsulation efficiency is slightly greater than the CS nanoparticles. However, the increase in RIF concentration leads to an increase of both EE and LC of CS-PEG nanoparticles. It was found that the EE and LC of CS-PEG 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-PEG nanoparticles is a more electrostatic attraction than CS nanoparticles.

CONCLUSION

Ion gelation technique is a suitable technique for preparing nanoparticles of chitosan and chitosan-PEG with a nano particle size range and also CS, CS-PEG nanoparticles loaded with RIF were successfully formulated by ionic gelation technique. The amount of the drugs had great effect on the drug loading and encapsulation efficiency, zeta potential and particle size of nanoparticles. The additional polymer combination and pH of the solution both affected drug release from the nanoparticles composites. Rifampicin loaded in the CS-PEG nanoparticles and the incorporated rifampicin slowly released from the nanoparticles at pH 3.4 than compared to CS nanoparticles. The result indicated that the RIF-conjugated with CS and CS-PEG polymers may be potential materials for drug loading and long-acting releasing anti-tuberculosis drugs. The presence of a chitosan and chitosan-PEG controlled the release of rifampicin from nanoparticles in which the drug was dispersed at a nanoparticles level. These interesting formulations deserve further characterization in terms of long term stability, kinetics of release, ex vivo/in vivo gene permeation, and possibly in vivo studies in the future.

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