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Research Article

DEVELOPMENT AND *IN-VITRO* CHARACTERISATION OF CHITOSAN LOADED PACLITAXEL NANOPARTICLE

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ABSTRACT

Objectives: To meet the above aim the following objectives are undertaken: (1) Preparation of paclitaxel (PTX) loaded nanoparticles by different techniques, (2) *In-vitro* evaluations of the drug loaded nanoparticles and selection of optimized batch.

Methods: PTX loaded chitosan nanoparticles were prepared by Ionic-crosslinking technique. In this technique, chitosan was dissolved in 0.25%v/v acetic acid solution. To this above solution 0.84%v/v, glutaraldehyde solution was added dropwise under high-speed homogenizer at 17000 rpm for 1 hr.

Result: Particle size of prepared nanoparticle formulations was found to be 345.175±5.66-815.125±8.355 nm with low PDI between 0.456. The maximum entrapment of drug was found to be 88.57±2.533% with formulation F5. *In-vitro* release studies of the F5 formulation showed 57.8±1.735% release of drug after 24 hrs.

Conclusion: The prepared nanoparticles were evaluated for its particle size, zeta potential, drug entrapment efficiency, *in-vitro* drug release study, and surface morphology studies by scanning electron microscopy. The results of Fourier transform infrared studies of 1:1 physical mixture of drug and excipients confirmed the absence of incompatibility. Thus, the study concludes that PTX loaded nanoparticles were developed successfully by ionic crosslinking method, which is expected to enhance the oral bioavailability of PTX.

Keywords: Paclitaxel, Nanoparticles, Chitosan, Ionic-crosslinking, *In-vitro* release.

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INTRODUCTION

Nanoparticles are to be defined as a particulate dispersion or solid particles with a size range of 10-1000 nm. Here in nanoparticles, the drugs are entrapped, dissolved, encapsulated, or attached to the matrix of nanoparticles. Nanoparticles either nanosphere or nanocapsules depend on the method of preparation. Nanospheres are the matrix dosage form in which drug is uniformly and physically dispersed, while nanocapsules are the system were the drug is confined to its cavity surrounded by a unique polymer membrane [1]. Recently biodegradable polymeric nanoparticles, particularly those nanoparticles that are coated with hydrophilic polymer such as poly (ethylene glycol) known as long-circulating particles, which are used as potential drug delivery devices because of their ability to circulate for a prolonged period of time targeting a particular organ, ASA carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes. The major aims in designing nanoparticles as a delivery system are to achievea control particle size, surface properties and to release pharmacologically active agents to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen [2].

Paclitaxel (PTX) is a major anticancer chemotherapeutic agent, extracted from the bark of Pacific yew tree (*Taxus brevifolia).* PTX belongs to the class of Taxanes. PTX stops the cell division in late mitotic phase by preventing the microtubules destruction and inhibits the cell proliferation [3]. PTX is poorly water-soluble product with a molecular weight 853.91 g/mol. PTX has a potent antitumor effect against various human solid tumors, *viz*., ovarian cancer, breast cancer, and non-small cell lung cancer. In general, PTX is administered to the patients via an intravenous route. Due to its poor water solubility, some solubilizer or injection oil has to be used, which induces more toxicity such as hypersensitivity and peripheral neuropathy [3]. Clinical use of PTX is impaired due to inappropriate delivery vehicles. Hence, there is a need for the development of new formulation for delivery, efficient loading, and sustained release of poorly water-soluble drug PTX to increase its therapeutic efficacy and to decrease its side effects [4].

In this study, we prepared PTX loaded chitosan nanoparticles to improve its oral bioavailability. The usefulness of PTX loaded chitosan nanoparticles were evaluated for its particle size and polydispersity index (PI), zeta potential, *in-vitro* drug release, surface morphology by scanning electron microscopy (SEM) and drug-excipients compatibility studies by Fourier transform infrared (FT-IR).

MATERIALS AND METHODS

Material

PTX were obtained as a gift sample from MAC-CHEM Pvt. Ltd. (Mumbai, India), Chitosan and potassium dihydrogen phosphate from MERCK Pvt. Ltd. (Mumbai, India), glutaraldehyde from BALAJI drugs (India), sodium chloride from HIMEDIA Pvt. Ltd. (Mumbai, India), and disodium hydrogen phosphate from SPECTROCHEM Pvt. Ltd. (Mumbai, India). All other solvents and chemicals are of analytical grade.

Preparation of drug loaded chitosan nanoparticles

PTX loaded chitosan nanoparticles were prepared by Ionic-crosslinking method as per the composition shown in Table 1. PTX loaded chitosan nanoparticles were prepared by crosslinking with Glutaraldehyde solution. Here, 10 mg of PTX was dissolved in ethanol and 10-50 mg of Chitosan was dissolved in aqueous solution of 0.25% v/v acetic acid solution at various concentrations such as 2-10 mg/ml under magnetic stirring. 5 ml of 0.84% v/v glutaraldehyde aqueous solution was added dropwise to the above solution [5]. The solution was then homogenized at 17.000 rpm for 1 hr using high-speed homogenizer (IKA T25 digital Ultra Turrax, Germany). The resultant nanoparticles suspense was centrifuged at 12.000 × g for 30 minutes and the free nanoparticles were separated [6].

Drug-excipient compatibility study by FT-IR

This study was carried out to find out the compatibility between PTX and different excipients to be used in formulations [7]. Physical mixture in ratio 1:1 of drug-excipients was prepared and scanned from 4000cm^{-1} to 400 cm^{-1} in Bruker Alpha FT-IR spectrophotometer after placing the sample onto the sample holder. The spectra obtained were compared and interpreted for the functional group [8].

Characterization of prepared PTX nanoparticles

Particle size and PI

To analyze particle size, each formulation of drug loaded lyophilized nanoparticles was dispersed in deionized water, centrifuged for 5 minutes at 5000 rpm and filtered using 0.2 µm membrane filter. Particle size and PI were determined by using Malvern Zetasizer Nano S90 at a temperature of 25°C at a measuring angle of 90° to the incident beam [8].

Zeta potential study

The zeta potential of PTX nanoparticles was measured by a laser Doppler anemometer coupled with Zetasizer Nano ZS 90 (Malvern Instruments Ltd. UK). A potential of ±150 mV was set in the instrument. Disposable cuvette of 0.75 ml capacity was used for measurement [8].

Entrapment efficiency

Loading of PTX in chitosan nanoparticles was determined by extracting 5 mg nanoparticles with 1 mL acetone for 6 h. From this solution, 0.2 ml was diluted with phosphate buffer pH 6.8 and analyzed by ultraviolet (UV) spectrophotometer (Shimadzu UV-1800, Japan) at 230 nm against appropriate blank [9]. The entrapment efficiency was calculated using the following equation:

$$\%\ \text{Emtrapment efficiency} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of drug in the formulation}} \times 100$$

SEM of nanoparticles

The shape and surface characteristics of the nanoparticles were observed by SEM. The nanoparticle sample was thinly sprinkled onto a metal stub and vacuum coated with a thin layer of gold in an argon atmosphere. The SEM photomicrographs of the coated particles were obtained at 15 KV using a ZEISS, Germany, SEM [10].

***In-vitro* drug release**

Drug release from PTX loaded chitosan nanoparticles was determined in phosphate buffer pH 6.8. Nanoparticle suspension (1 mg/ml, 2 ml) was placed in a dialysis tube (cellulose membrane, Sigma Chemical Company, USA) and immersed in 10 ml of the release buffer in a 15 ml centrifuge tube and shaken in an incubator shaker set at 100 rpm. At predetermined time intervals, 1 ml of the buffer solution was removed from the tube and analyzed for drug content by UV spectrophotometer (Shimadzu UV-1800, Japan) at 230 nm against appropriate blank medium [11].

RESULTS AND DISCUSSION

Drug-excipients compatibility study

FT-IR spectroscopy study was carried out to test the compatibility of PTX with chitosan in the formulation shown in Fig. 1. FT-IR spectra of PTX showed the presence of characteristics band at 1243, 1704, 1733, 1172, 705 cm^{-1} due to C-O, C = O, C = O, C-F, and N-H functional groups. All this characteristics band also retained in 1:1 physical mixture of PTX-chitosan showed in Fig. 1. The results clearly revealed the incompatibility of drug with the excipients used in the formulation.

Physicochemical characteristics

The physicochemical characteristics of PTX loaded chitosan nanoparticles were briefly narrated in Table 2. The results showed that the particle size of the prepared nanoparticles varied from 349.17±5.665 to 815.12±8.355 nm with a low PI in the range of 0.234±0.020-0.456±0.022 as shown in Table 2. The size of nanoparticle was decreased with inmer concentration. The PI was found to be <0.5, which is a considered as proof of a homogeneous nanoparticle formulation.

The PTX loaded Chitosan nanoparticle formulations measured zeta potential value was found of 12.2±0.022 to 26.7±0.021 mV shown in Fig. 2. The net positive surface charge of all formulation may be due to the use of cationic polyelectrolyte (Chitosan) and the addition of glutaraldehyde. The average droplet of the selected formulation F5 by dynamic light scattering determination was found to be 26.7±0.021 mV. The average entrapment efficiency of PTX nanoparticle formulations was found to be 18.6±1.15, 36.9±0.34, 58.77±0.98, 79.17±3.04, 88.57±2.53 in the formulation F1, F2, F3, F4 and F5, respectively. The average entrapment efficiency of the formulations increased with increase in polymer concentration. The maximum entrapment efficiency 88.57±2.53 was observed at 1:5 drugs to polymer ratio in formulation F5; the change in drug entrapment may be due to poor aqueous solubility and high binding capacity of drug on the polymer surface.

SEM

SEM image of the selected formulation showed spherical and smooth surface with size measuring 300 nm. The image also confirmed the uniform distribution of nanoparticles which are presented in Figs. 3 and 4.

***In-vitro* drug release studies**

In-vitro release profile of PTX loaded chitosan nanoparticles in phosphate buffer pH 6.8 are shown in Fig. 5. Here sustained release of

Table 1: Composition of paclitaxel loaded chitosan nanoparticles

Formulations code	Chitosan (mg)	PTX (mg)	0.25% v/v acetic acid (ml)	0.84% v/v glutaraldehyde (ml)
F1	10	10	20	5
F2	20	10	40	5
F3	30	10	60	5
F4	40	10	80	5
F5	50	10	100	5

PTX: Paclitaxel

Table 2: Physicochemical characterization of PTX loaded chitosan nanoparticles

Formulations code	Chitosan: PTX ratios	Particle size (nm)	PI	Zeta potential (mV)	Entrapment efficiency (%)
F1	1:1	815.12±8.35	0.456±0.022	12.2±0.022	18.6±1.15
F2	1:2	668.34±4.75	0.410±0.034	15.5±0.012	36.9±0.34
F3	1:3	537.06±1.99	0.369±0.045	20.2±0.043	58.77±0.98
F4	1:4	446.17±3.66	0.289±0.024	22.4±0.024	79.17±3.04
F5	1:5	349.17±5.66	0.234±0.020	26.7±0.021	88.57±2.53

PTX: Paclitaxel, PI: Polydispersity index

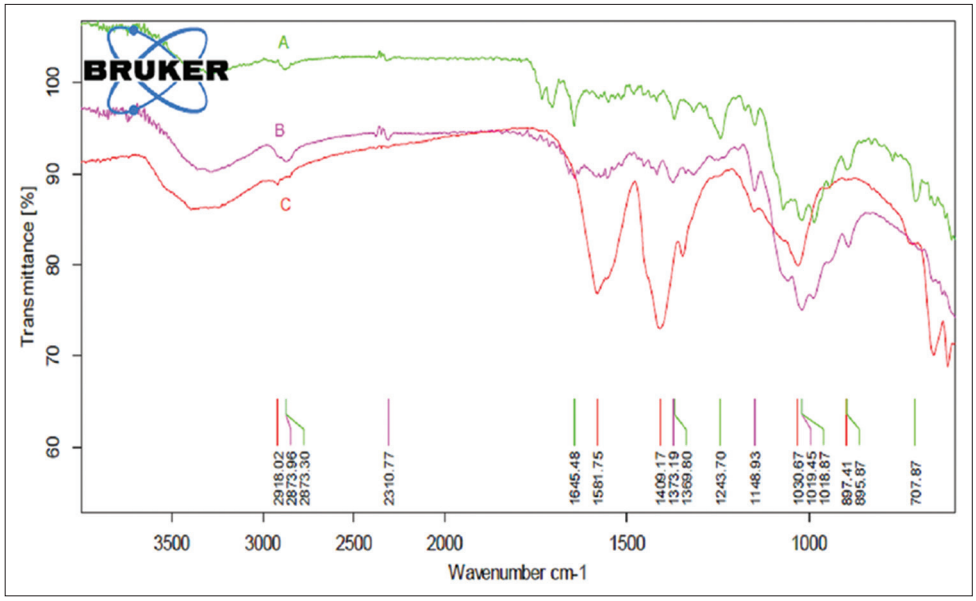

Fig. 1: Fourier transform infrared spectra of 1:1 physical mixture of Paclitaxel and Chitosan. (a) Paclitaxel -chitosan mixture, (b) Chitosan, and (c) Paclitaxel

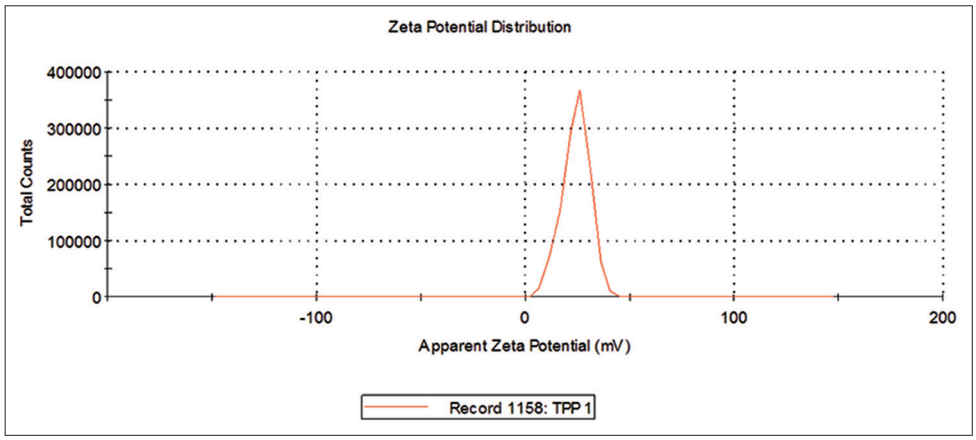

Fig. 2: Zeta potential of paclitaxel loaded chitosan nanoparticles

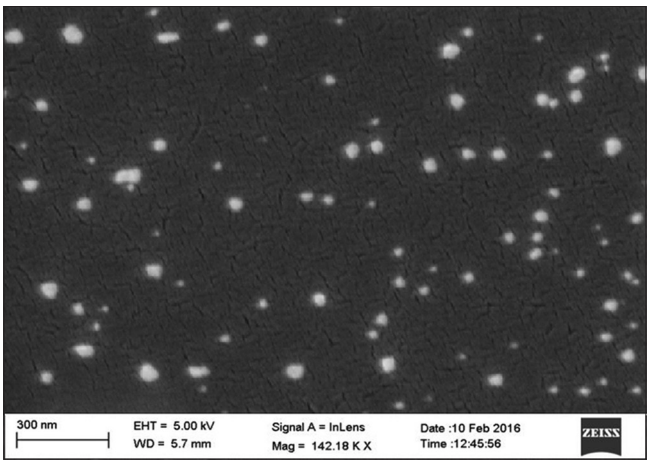

Fig. 3: Scanning electron microscopy of paclitaxel loaded chitosan nanoparticle

drug was observed from the formulation in phosphate buffer pH 6.8 for the duration of 24 hrs. Here, we found that with increased in concentration of polymer in formulation drug release was sustained for a long period, which may be due to the hydration capability of chitosan which on coming in contact with dissolution medium results to the formation of gelatinous mass that act as a retardant material for the drug to get diffused out. The cumulative % drug release from formulation F1, F2, F3, F4 and F5 are 86.4±1.05, 80.2±0.56, 78.4±0.68, 59.2±0.44 and 57.8±1.77, respectively. Among all the formulations, F5 formulation showed better sustained release profile of the drug for a period of 24 hrs in phosphate buffer pH6.8.

DISCUSSION

In this study, a nanoparticles with a positive zeta potential value between 12.2±0.022 and 26.7±0.021 mV and particle size range between 349.17±5.665 and 815.12±8.355 nm of PTX were prepared successfully using Ionic-crosslinking technique. Nanoparticle size depends primarily on the excess of polymer added into the system and *in vitro* release study revealed the sustained release of drug for 24 hrs. The FT-IR study results confirm the compatibility of PTX with the excipients used in formulations. Thus, the developed nanoparticles are expected to the improvement of the oral bioavailability of the drug.

CONCLUSION

The main aim of the research work was to develop and characterized the chitosan loaded PTX nanoparticle formulation by ionic crosslinking

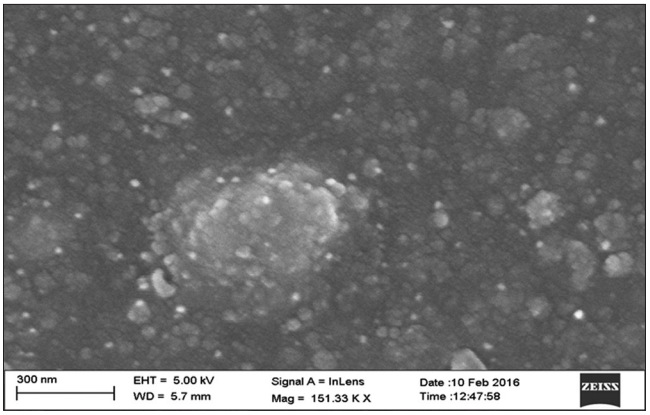

Fig. 4: Scanning electron microscopy of chitosan nanoparticles

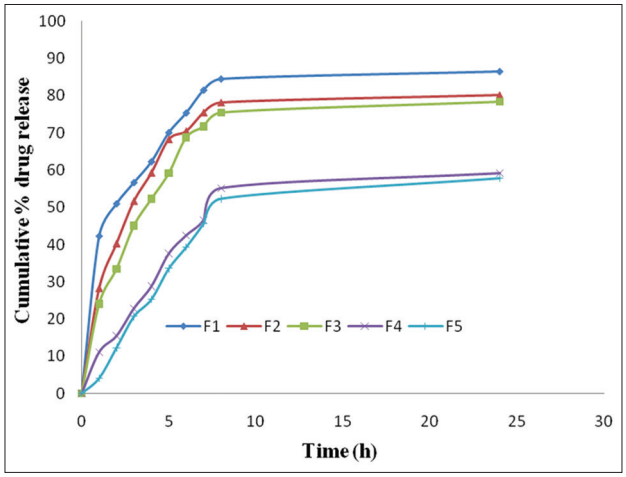

Fig. 5: *In-vitro* drug release profiles of nanoparticles in phosphate buffer pH 6.8

method. Glutaraldehyde was used as cross-linking agent. PTX is a hydrophobic anti-chemotherapeutic agent with poor water-solubility. PTX when given by parenteral route, its bioavailability gets decreased. Therefore to increase its bioavailability, the nanoparticle formulation is administered through oral route.

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