Research Article

The Potential of TPP Chitosan Nanoparticles as Carrier for Poorly soluble Rosiglitazone Maleate

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ABSTRACT

The objective of this study is to develop biodegradable sub-micron chitosan nanoparticles loaded with rosiglitazone maleate for intravenous drug targeting. The rosiglitazone maleate loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tri polyphosphate anions (TPP). The effects of chitosan concentration and sodium tri polyphosphate on the physicochemical properties of the nanoparticles were studied. The particle size and zeta potential of nanoparticles were determined, respectively by transmission electron microscopy (TEM) and a zeta potential analyzer. It was found that the nanoparticles showed a size distribution in the range of 86 nm - 110 nm with spherical morphology and smooth surface structure. The encapsulation efficiency decreased with the increase of chitosan concentration. These studies showed that chitosan can form complex with tri polyphosphate anions to form stable cationic nanoparticles for subsequent rosiglitazone maleate. In vitro release studies showed a burst effect at the beginning and then a sustained release characteristic for 32 h. The study on the drug to polymer ratio showed a linear relationship between the concentration of drug and percentage drug loading.

Keywords: TPP, chitosan, rosiglitazone maleate, TEM.

INTRODUCTION

In the recent past, substantial scientific and technological advancements have been made in the research and development of rate controlled oral drug delivery systems to counter the short comings of physiological adversities of conventional drugs and its administration.[1] The rate controlled oral drug delivery system has given impetus to significant advancements in the pharmaceutical engineering of novel dosage forms such as nanoparticles, which are solid colloidal polymeric carriers than 1 μm in size.[2,3] These nanoparticles offers great advantages right from helping to increase the stability of drugs, proteins and up to controlled drug release properties. Several attempts have been made, towards developing biodegradable polymeric nanoparticles as potential drug delivery devices. In addition to the inherent property of reduced cytotoxicity, biodegradable polymeric nanoparticles have been found to be extremely effective in controlled and targeted drug release, and time-controlled drug delivery system.[4]

Diabetes mellitus is a vast growing public health problem throughout the world because of its association with increased cardio vascular mortality. So, the present exercise is focused towards anti-diabetic treatments.

Rosiglitazone maleate, a fast and short acting thiazolidinedione analog, is chosen as the model drug candidate for polymeric nanoparticles formulation. It has short half-life 3-4 h and has poor absorption characteristics in the upper intestinal tract. Although the drug is highly insulin sensitive, it suffers major drawbacks such as hepatic toxicity, anemia, G.I.T disturbances, oedema, liver toxicity and muscle cramps.[5.] Nano drug delivery system enclosed anti-diabetic drug which improve the therapeutic efficacy of the drug with pre-determined controlled, prolonged duration. Thus, the adverse effects, due to conventional dose can be surmounted. Recently many studies are focused on safety issues of manufactured nano materials to minimize or eliminate their nano toxicity before they are being widely used.[6-9] Chitosan (CS) is the second abundant polysaccharide and a cationic polyelectrolyte present in nature. Chitosan is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine and can be derived by the partial deacetylation of chitin. It is a biodegradable, biocompatible and hydrophilic polymer of low toxicity.[10] It is a material found in abundance in shells of crustacea such as lobsters, prawns and crabs. It is insoluble under alkaline and neutral conditions, but soluble with inorganic and organic acids such as hydrochloric acid, lactic acid, acetic acid and glutamic acid under acidic conditions. Therefore, the major goal of the present work is to create a new kind of biodegradable nanoparticles for the incorporation of rosiglitazone maleate and to evaluate their potential as delivery system. The factors that influence the preparation of nanoparticles were analyzed and their release property was examined. Hence in the present study we have made an attempt to

optimize and to check the suitability and potentiality of natural carrier such as chitosan for diabetic drugs.

MATERIALS AND METHODS

Rosiglitazone maleate was obtained as a gift sample by Torrent Pharmaceuticals Ltd, Ahmedabad and chitosan was procured as a gift sample from Central Institute of Fisheries Technology (degree of deacetylation 85%). Sodium tri polyphosphate was purchased by Qualikens fine Pvt Ltd., New Delhi and Tween 80 LR was purchased by S.D. Fine Chemicals Ltd, Mumbai. Dialysis membrane was purchased from Himedia laboratories (P) Ltd., Mumbai. All other reagents used were of analytical grade.

Development of polymeric nanoparticles

Chitosan nanoparticles were prepared by ionic gelation method.[11] Chitosan was dissolved in acetic aqueous solution at various concentrations (1.0, 1.2, 1.44, 1.6, 2.0, 2.5, 3.0 mg/ml). The concentration of acetic acid in aqueous solution was, in all case 1.5 times that of chitosan. Under magnetic stirring, at room temperature, 4 ml sodium tri polyphosphate aqueous solution with various concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) was added into 10 ml chitosan solution, respectively. The drug rosiglitazone maleate was dissolved in the chitosan solution. Tween-80 (%) solution was added to get a final suspension of chitosan nanoparticles. The suspension was stirred for 1h using a magnetic stirrer. Three kinds of phenomena were observed: solution, aggregates and opalescent suspension. The zone of opalescent suspension was further examined as nanoparticles.

Physicochemical characterization of nanoparticles

Fourier-transform Infrared Spectroscopy

Fourier-transform infrared (FTIR) spectra of the chitosan and cross-linked chitosan were obtained from 8201 PC, Schimadzu (Tokyo, Japan). The pellets were prepared on a KBr press. The spectra were scanned over the wave number range of 4600 to 400 cm^{-1}. The pellet was placed in the light path and the spectrum was obtained.

Particle morphology

Particle morphology was analyzed by a transmission electron microscope (TEM, Margagni-268-D, FEI,

Netherlands) using an acceleration voltage of 200 kV. Specimens were prepared by dropping the sample solution on to an upper grid, and then a drop of 2% uranyl acetate was added to give negative stain. The grid was then allowed to stand for 1 min and excess staining solution was removed by draining. The specimens were air dried and examined using TEM.[12,13]

Particle size analysis

Particle size of the nanoparticles was determined using a Zeta sizer 3000 HS (Malvern Instruments, Malvern, UK). Samples were diluted with phosphate buffer saline pH 7.4 and the measurements were performed at a scattering angle of 90º and at a temperature of 25 °C. The diameter is calculated from the autocorrelation function of the intensity of light scattered from particles, assuming a spherical form for the particles. The polydispersity index (PdI) is a measure of dispersion homogeneity and ranges from 0 to 1. Values close to 0 indicate a homogeneous dispersion while those greater than 0.3 indicate high heterogeneity or broad size distribution.[14]

Zeta potential

The particle charge was quantified measuring the zeta potential using a Zeta sizer 3000HS (Malvern Instruments, Malvern, UK). Samples were diluted with phosphate buffer saline pH 7.4. For the measurements samples were placed in the electrophoretic cell, where a potential of 150 mV was established. The zeta potential value was calculated by the software using Smoluchowski's equation. The results are presented as the mean of 3 determinations± S.D.[14]

Entrapment efficiency

Each suspension was centrifuged at 15,000 g for 40 min at 2°to 4°C to separate the free drug in the supernatant from the drug incorporated in the nanoparticles. Concentration of Rosiglitazone maleate was determined by visible spectrometry at 243.5 nm. The amount of the drug incorporated in nanoparticles was calculated from the difference in drug concentrations between the supernatant and the original given concentrations.[15] The entrapment efficiency was calculated according to the following equation:

$$\text{The entrapment efficiency (\%)} = \frac{\text{Weight of the total drug} - \text{weight of free drug}}{\text{Weight of total drug}} \times 100$$

Effect of surfactant on the drug loading capacity

The effect of surfactant on drug loading capacity was determined by adding various concentrations of surfactant. The batch of nanoparticles with minimum drug loading was selected for this study. After the addition of surfactant in the Chitosan solution, the other steps were followed as it is described in the preparation of the drug loaded nanoparticles. Drug loading capacity of the batch without addition surfactant was checked and it was then compared with the percentage drug loading after addition of surfactant.[16]

In vitro release studies of drug loaded batches by dialysis membrane

In-vitro release of all batches was carried out by employing a diffusion cell (float a lyzer). A quantity of nanoparticles suspension which is equivalent to 4 mg of drug was taken in a Himedia dialysis membrane (cut off 14 KDa) fixed to one end of the apparatus to result a permeation cell. The nanoparticles were taken in the cell and the cell was immersed in a beaker containing 50 ml of phosphate buffer (pH 7.4) as receptor compartment. The cell was immersed to a depth of 1 cm below the surface of the receptor

solvent. The medium in the receptor compartment was agitated continuously using a magnetic stirrer and a temperature of 35±2ºC was maintained within the diffusion chambers. 1 ml of the sample from receptor compartment was taken at various prefixed intervals of time period of over 24h and each time fresh buffer was reintroduced in the receiver chamber. The withdrawn sample was estimated spectrophotometrically at 243.5 nm.[15, 17]

Evaluation of in vitro release kinetics

In order to investigate the mechanism of release, the data were analyzed with the following mathematical models: zero order kinetic (Eq. 1), first order kinetic (Eq. 2) and Higuchi kinetic (Eq. 3).

$$Qt = Ko\ t \qquad\qquad (1)$$

$$\ln Qt = \ln Q0 - K1t \qquad\qquad (2)$$

$$Qt = Kht1/2 \qquad\qquad (3)$$

The following plots were made: Qt vs. t

(Zero order kinetic model),

ln (Q0 −Qt) vs. t (first order kinetic model) and Qt vs. t1/2 (Higuchi model),where Qt is the percentage of drug released at time t, Q0 is the initial amount of drug present in the formulation and K0, K1 and Kh are the constants of the equations. Further, to confirm the

mechanism of drug release, the first 60% of drug release was fitted in Korsmeyer–Peppas model (Eq. 4)

$$Mt/\ M_{\infty} = Kp\ tn \qquad\qquad (4)$$

Where

Mt/M = are the fraction of the drug release at time t, Kp is the rate constant and "n" is the release exponent. The value of "n" is used to characterized if ferent release mechanisms and is calculated from the slope of the plot of log of fraction of drug released (Mt/M) vs. log of time.[18]

Stability

The nanoparticles formulations were stored for stability profile for 1 month in different environment conditions such as 2˚- 8˚C, room temperature and 45˚C. The stability of drug loaded nanoparticles was evaluated every week in terms of its drug leakage into the storage medium buffer saline pH 7.4.[14,19]

RESULTS AND DISCUSSION

Development of polymeric nanoparticles

The polymeric nanoparticles were prepared by ionic gelation method with four different ratios of polymer. This method is comparatively easy to prepare than the other techniques. This mild technique involves the mixing of two aqueous solutions at ambient temperature without sonication or using organic solvents.[20] Various formulation were made with different initial concentrations of chitosan (1-6 mg /ml) and sodium tripolyphosphate solutions (1-5

mg/ml) to establish preparation conditions at which were formed and more over 3 mg/ml chitosan concentration and 4.6 mg/ml sodium tripolyphosphate was choosen for the study, as reported in literature.[11]

Chitosan was dissolved in acetic aqueous solution, added TPP under magnetic stirring at room temperature. The drug and Tween-80 solution was added to get a final suspension of chitosan nanoparticles. The polymeric nanoparticles were prepared in four different ratios of polymer viz. 1:1, 1:2, 1:3 and 1:4. However, based on the nanoparticle recovery and drug entrapment efficiency, among the four different ratios, 1:3 ratio is selected as the best ratio than the other three. The other three ratios produced low drug entrapment which causes high drug wastage during the preparation procedure itself and showed low nanoparticle recovery, and poor yield. These have been repeatedly tried for three times, for reproducibility and for consistency.

Physicochemical characterization of Nanoparticles

Prepared nanoparticles were characterized for their FTIR, particle size, surface properties, morphology, entrapment efficiency, stability and *in vivo* release study.
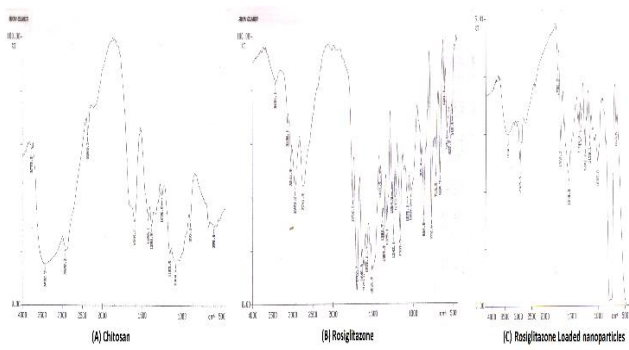
FTIR spectra of chitosan, rosiglitazone maleate and rosiglitazone–loaded nanoparticles

A characteristic band at 3449 cm^{-1} is attributed to –NH2 and –OH groups stretching vibration and the band for amide I at 1655 cm^{-1} is seen in the infrared spectrum of chitosan (Fig. 1A). In chitosan-TPP nanoparticles, the 1655 cm^{-1} peak of –NH2 bending vibration shifts to 1554 cm^{-1} and a new sharp peak 1645cm^{-1} appears. The disappearance of the band could be attributed to the linkage between the phosphoric and ammonium ions. The cross-linked chitosan also showed a peak for P = O at 1155 cm^{-1}. Many researcher also observed similar results in their study of formation of chitosan nanoparticles and chitosan film treated with phosphate.[21,22,23] So we suppose that the tripolyphosphoric groups of TPP were linked with ammonium groups of chitosan in nanoparticles. Compared with the spectrum of rosiglitazone maleate (Fig. 1B), in the spectrum of rosiglitazone maleate -loaded nanoparticles (Fig. 1C), the absorption peak of 1718 cm^{-1} (carboxyl group absorption peak) disappears and a new shoulder peak 1453 cm^{-1} (salt of carboxyl) appears. The results indicate that the presence of the electrostatic interactions between carboxyl groups of rosiglitazone maleate and amino groups of chitosan.
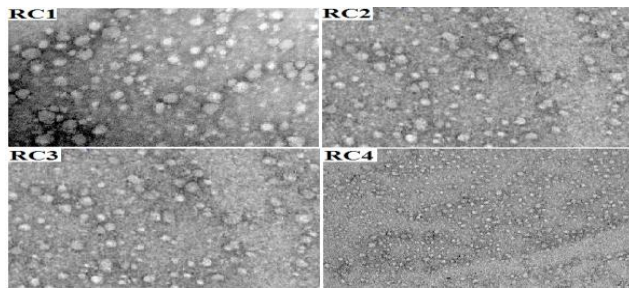
Morphology of nanoparticles

Particle morphology was analyzed using TEM using an acceleration voltage of 120 kV. Results showed that the surface of nanoparticles were uniform and of definite shape. The particles morphology was showed in Fig 2.

Figure 1: FTIR spectra of: (A) chitosan, (B) Rosiglitazone and (C) Rosiglitazone loaded nanoparticles.

Figure 2: TEM of Rosiglitazone loaded chitosan nanoparticles (RC1 RC2 RC3 and RC4)

Zeta size

Particle size is often used to characterize nanoparticles, because it facilitates the understanding of the dispersion and aggregation.[24] Nanoparticles produced were of submicron size and had low poly dispersity which indicates relatively narrow particle size distribution for Rosiglitazone loaded chitosan preparations. The mean diameter and poly dispersity index (PDI) of polymeric nanoparticles was found to be 1120 nm and 0.858 PDI. The raw data was correlated to Z average means size by cumulative analysis. Z-average (d.nm) of chitosan nanoparticles (RC3) was found 1120, shown in Fig 3.
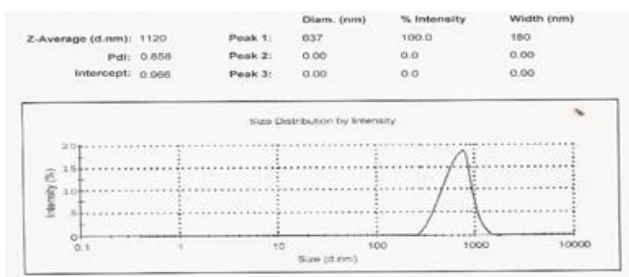
Figure 3: Zeta size of Rosiglitazone loaded chitosan nanoparticles of 1:3 ratio

Zeta potential

Zeta potential is used to characterize the surface properties of nanoparticles and colloidal drug delivery system. Zeta-potential of chitosan nanoparticles (RC3) was found 28.0 mV, shown in Fig 4.
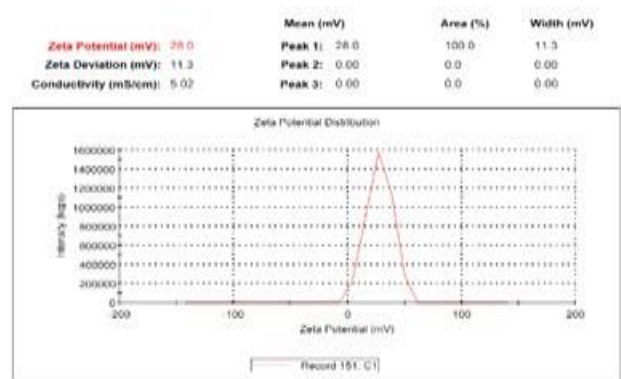
Figure 4: Zeta potential of Rosiglitazone loaded chitosan nanoparticles (RC3)

Entrapment efficiency in nanoparticles

The entrapment efficiency of chitosan nanoparticles loaded with drug was found for different ratio of polymer showed in Table 1. The study was focused on the encapsulation efficiency of drug by cross linking method using sodium triphosphate as cross linking agents. On the basis of *in vitro* release studies it was found that a better-sustained and prolonged release was obtained in case of STPP and encapsulation efficiency of the RC3 was found to be 90.61%. It was assumed that the intensive ionic interaction between the polymer and the drug may lead to an increase in entrapment of the drug in nanoparticles.

Table 1: Entrapment efficiency of chitosan nanoparticles

SN	Formulation Code	Drug Polymer ratio	% Entrapments efficiency
1	RC1	1:1	91.41±0.37
2	RC2	1:2	89.06±1.26
3	RC3	1:3	90.61±0.27
4	RC4	1:4	88.78±0.26

Effect of Chitosan/TPP concentration on the encapsulation of rosiglitazone maleate

When TPP concentration was 1 mg/ml, too high chitosan concentration (4 mg/ml) made encapsulation extremely difficult and too low chitosan concentration (0.5 mg/ml) made some aggregates with large diameter form. The formation of nanoparticles is only possible within some moderate concentrations of chitosan and TPP. As for gelation between TPP solution of 1 mg/ml and chitosan solution of 1–3 mg/ml, we usually observed that some opalescent suspension was formed, which was further examined as nanoparticles. Table1 shows that increase in chitosan concentration led to decrease of encapsulation efficiency of rosiglitazone maleates.

It has been previously reported that the highly viscous nature of the gelation medium hinders the encapsulation of drug in the study of chitosan microspheres.[25] So it was supposed that relatively lower viscosity of chitosan with lower concentration (such as 1–3 mg/ml) and 4.6 mg/ml sodium tripolyphosphate promotes the encapsulation of rosiglitazone maleate and gelation between chitosan and TPP.

Effect of surfactant on drug loading capacity

Because of larger surface area and attractive force between the particles, the chance of possible aggregation is high in small sized particles. To overcome such aggregations, which were not solicited, an addition of a surfactant in the preparation was necessary. Tween-80 appeared to be the most suitable surfactant in reducing aggregation between nanoparticles.

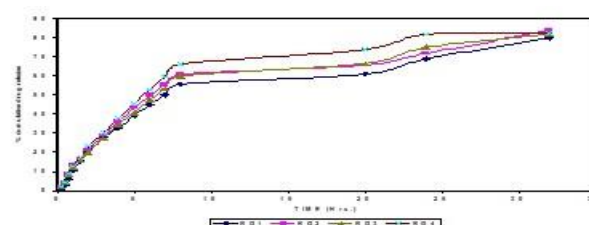### *In vitro* release of rosiglitazone maleate from the nanoparticles

Figure 5 displayed the release profile of rosiglitazone maleate from chitosan nanoparticles. It was apparent that rosiglitazone maleate release *in vitro* showed a very rapid initial burst, and then followed by a very slow drug release. Zhou reported about microspheres and revealed that the release involves two different mechanisms of drug molecules diffusion and polymer matrix degradation.[26]

The burst release of drug is associated with those drug molecules dispersing close to the microsphere surface, which easily diffuse in the initial incubation time. The hypothesis is also suitable for rosiglitazone maleate release from nanoparticles. Rosiglitazone maleate nanoparticles diffuse easily through the surface or the pore of nanoparticles in a short time because of its small size. Therefore, the rapid dissolution process suggests that the release medium penetrates into the particles due to the hydrophilic nature of chitosan and dissolves the entrapped rosiglitazone maleate. In addition, the nanoparticles with huge specific surface area can adsorb rosiglitazone maleate.

In vitro release profiles obtained for each formulation showed three phases compositions:

(1) A first initial burst release of 30%, due to the drug desorbed from the particles surface,

(2) A plateau for the following 8 h, resulting from the only diffusion of the drug dispersed in the polymer matrix,

(3) A constant sustained release of the drug, resulting from the diffusion through the polymer wall as well as its erosion, so the first burst release is also possibly due to the part of rosiglitazone maleate desorbed from nanoparticle surface.

However, the drug which was covalent bound to the nanoparticles continues to release in a sustained and prolonged manner and the total amount of drug release in terms of percentage entrapment was almost 67% from chitosan nanoparticles at the end of 32 h. In order to determine the release model which best describes the pattern of drug release, the *in vitro* release data were substituted in zero order, first order and diffusion-controlled release. The release pattern followed the Korsmeyer Peppas equation.

Figure 5: *In vitro* release profile of rosiglitazone loaded chitosan nanoparticles of 1:3 ratio

Stability

Nanoparticles made with chitosan were stored at 4°C, room temperature and 45°C for one month. It could be deduced that no aggregation occurs during the storage and the formulations exhibit a good stability (Fig.6). After 3 months study, aggregation of nanoparticles was observed and nanoparticles were not found under nanometer size range (Tab. 2).
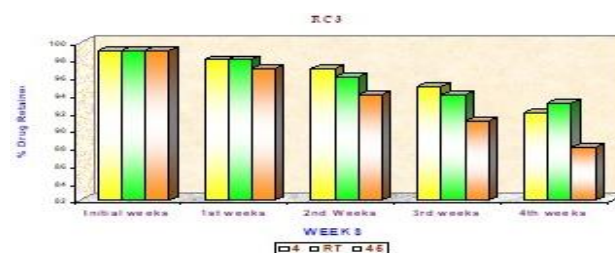
Figure 6: Stability study of rosiglitazone loaded chitosan nanoparticles of 1:3 ratio

Table 2: Particle size distribution initial and after 3 month

Initial particle size		Particle size after 3 months	
TEM (nm)	Zeta size (nm)	TEM (nm)	Zeta size (nm)
86±18.2	1120	110±19.6	----

CONCLUSION

This work shows a systematic study on the chemical physical characterization of nanoparticles formed by chitosan chains electrostatically linked by ionic interactions. This kind of particles is of great interest for pharmaceutical applications. Rosiglitazone loaded chitosan nanoparticles were successfully formulated by ionic gelation method. Different investigations on preparation, characterization and *in vitro* release of nanoparticles were carried out and performance of the formulations was evaluated. The proposed rosiglitazone loaded chitosan nanoparticles illustrates an effective way, to prolong drug release. The developed nanoparticles are safer and are the need of the hour for pharmaceutical industry as an alternative drug delivery system for the treatment of highly prevalent and chronic disease like type II diabetes mellitus. To optimize this drug delivery system, and for deeper understanding of different mechanisms, further studies are still required. Accordingly, the next step of this work has been planned to optimize various parameters which influence efficacy and bioavailability *in vivo*.

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