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Research Article

Nano and Microparticulate Chitosan Based System for Formulation of Carvedilol Rapid Melt Tablet

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Abstract

Purpose: In the present study rapid melt tablets (RMT's) of carvedilol were prepared by using ionotropic-gelated chitosan nanoparticles using a spray-drying method. Carvedilol is beta-adrenergic antagonist and its oral bioavailability is about 25-35% because of first pass metabolism.

Methods: The spray-dried microparticles were formulated into RMT's using a wet granulation process. The Formulation and optimization of carvedilol loaded RMTs using nano and microparticulate chitosan based system (NMCS) was done by using 3^2 factorial designs.

Results: Drug entrapment efficiency of about 64.9 % (w/w) and loading capacity of 14.44% (w/w) were achieved for the microparticles, which were ranged from 1 µm to 4 µm in diameter. Results of disintegration tests showed that the formulated RMTs could be completely dissolved within 40 seconds. Dissolution studies suggested that Carvedilol is released more slowly from tablets made using the microencapsulation process compared with tablets containing Carvedilol that is free or in the form of nanoparticles.

Conclusion: Results shown that the development of new RMTs designed with crosslinked microparticle might be a rational way to overcome the unwanted taste of conventional RMTs and the side effects related to Carvedilol intrinsic characteristics. The development of Carvedilol NMCS using ludiflash as RMTs could be used as a promising approach for improving the solubility and oral bioavailability of water insoluble drug.

Introduction

Rapid melt tablets, in which drugs are administrated orally for direct ingestion without water, are particularly useful for paediatric, geriatric, psychiatric patients with dysphagia and travelling patients. These new forms of medicine are quite advantageous because they can be taken without any chewing or prior dispersion and dissolution processes.[1-3] Some of them (sublingual or buccal tablets) display a spontaneous deaggregation and fast absorption in the mouth upon contact with saliva.[4] Therefore, numerous studies on various compositions and manufacturing methods of rapid melt or orally disintegrating or dissolving tablets have been performed.[5,6] The rapidly disintegrating dosage forms usually have an unpleasant taste, local stimulation, and other side effects caused by short, intense exposure to high concentrations of the active agent.

Rapid melt tablet (RMTs) is a defined as solid dosage forms that contains medicinal substances, active ingredient and disintegrates rapidly within few seconds without water when placed on the tongue. The Biopharmaceutical classification system (BCS) class II drug is released, dissolved, or dispersed in the saliva, and then swallowed and absorbed across the GIT.[7] Oro-dispersible tablet (ODTs) also are known as orodisperse, mouth-dissolving, quick-dissolve, fast-melt, and freeze-dried wafers.

The advantage of spray-drying techniques for application to microencapsulation is that it is reproducible, rapid, and relatively easy to scale up. Microencapsulation is a new technique that can be used to control the drug release rate, increased stability, protect it from premature destruction, targeted release of encapsulated materials and avoid the unpleasant taste by entrapping the active drug into a microcarrier.[8] Controlled drug delivery (CDD) technology represents a widely studied area in the field of pharmaceutical sciences. The main aim when formulating CDD systems is to increase the effectiveness of drug therapy. The benefit could be in a form of increased therapeutic activity, reduced toxicity, avoidance of the first pass metabolism, elimination of a specific drug administration route (e.g. injections), or reduction in a dosing frequency.[9] Spherically shaped and surface-distorted microparticles (embedded with the drug) could lessen the unwanted effects of RMTs by controlling the drug release rate. Recently, a novel "nano and microparticulate" chitosan based system (NMCS)

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has been developed using a double emulsion like technique and evaluated for drug and gene delivery in specific regions of the gastrointestinal tract.[10,11] Spray-drying has been used to increase drug solubility and bioavailability of active substances, modified release.[12,13] Microparticles can be used for the controlled release of drugs, vaccines, antibiotics, and hormones.

One of the most commonly used excipient for microencapsulation purpose is chitosan (CS), it is a naturally occurring nontoxic, biocompatible, biodegradable, cationic polysaccharide. This hydrophilic polymer can easily cross-link with counter poly anions like TPP **(**Tripolyphosphate pentasodium) to control the release of drugs. CS is a mucoadhesive polymer with permeation enhancing properties which facilitate opening of the epithelial tight junction.[14,15] Chitosan based NMCS displays high drug entrapment efficiency and controlled drug release.[16,17]

The NMCS of water insoluble model drug was prepared using a spray-drying method. Carvedilol is a non-selective β-adrenergic blocking agent with α1-blocking activity decreases the heart rate, myocardial contractility and myocardial oxygen demand. It has vasodilating activity at alpha-1 receptors at higher doses calcium channel blocking activity may contribute. Carvedilol is used in the management of hypertension and angina pectoris, and as adjunct to standard therapy in symptomatic heart failure. The absolute bioavailibity is about 25% and elimination half-life is about 6 hrs. This is because of undergoing of drug to first pass metabolism in liver and gut wall.[18] Carvedilol is rapidly and extensively absorbed following oral administration. The most common side effects include dizziness, fatigue, hypotension, diarrhoea, asthenia, bradycardia, and weight gain. The immediate disintegration behaviour of conventional Carvedilol RMTs, result in initial "burst-released" drug could be absorbed very quickly and resulting in high serum concentration and immediate disintegrating non conventional controlled release tablet which may results in controlled serum concentration that is controlled steady state (CSS). Drug plasma concentrations remain inside the therapeutic range for a longer period of time compared with conventional RMT. So it was rationale to design and develop a drug delivery system for Carvedilol using NMCS-based RMTs.

Materials and Methods

Materials

Carvedilol was received as gift sample from Zydus Cadila Healthcare Ltd., Ankleshwar, India. Ludiflash was received as gift samples from BASF Mumbai, India. Tripolyphosphate pentasodium was purchased from Sigma–Aldrich Chemical Co., Ltd. Chitosan was over 75% deacetylated, polyvinyl pyrrolidone K30, ethanol, Magnesium stearate, Lactose, Xylitol, (Hi Media Laboratories Pvt. Ltd., Mumbai, India) Methanol, Microcrystalline cellulose (Merck specialties Pvt. Ltd., Mumbai, India.) were purchased for carrying out various experiments. All other chemical were commercially available and used as received.

Experimental Design

In the present study a 3^2 full factorial design was employed, containing 2 factors evaluated at 3 levels. The details of experimental conditions are shown in Table 1.

Table 1. Design parameters and experimental conditions for 3^2 full factorial design. Factors (independent variables), factor levels and responses (dependent variables) used in 3-level factorial experimental design.

Factors	Type of factors	Factor level used			Response	
		-1	0	1		
X1	Chitosan (gm)	0.200	0.400	0.600	Y1	Particle size (nm)
X2	Drug (gm)	0.250	0.500	0.750	Y2	Entrapment efficiency (%)

Preparation of Carvedilol loaded Chitosan nanoparticles by ionotropic gelation method

Ionotropic gelation for the Chitosan nanoparticles formation was performed as previously described.[19-22] Chitosan solution was prepared by dissolving the required amount of chitosan in dilute acetic acid (0.3%, v/v) at room temperature overnight and passed through a 0.22 μm filter to remove insoluble's. Various amounts of Carvedilol mentioned in Table 1 were dissolved into the 10 ml methanol. This solution was added drop wise with a syringe (type 25 G x1″) to the chitosan solution with constant stirring to produce a homogenous mixture. Then, Tripolyphosphate pentasodium (TPP) was dissolved in distilled water at 2 mg/ml (w/v). Thereafter, the ionic gelation of chitosan in the aqueous medium was achieved by the addition of TPP solution (3 ml/min) drop wise with a syringe. The above chitosan solution was subjected to constant stirring until colloidal chitosan nanoparticles formed then the colloidal solution was continuously stirred at (1000 rpm) for 60 minutes at room temperature to ensure complete cross-linking of chitosan. The ratio of Chitosan/Tripolyphosphate pentasodium (TPP) was kept at 6:1 (w/w) for the formulation of chitosan nanoparticles in both formulations and the product.

Preparation of Carvedilol loaded Chitosan microparticles by spray-drying

Chitosan microparticles loaded with various concentrations of carvedilol were prepared (as per Table 1) by an aqueous spray drying technique. The resulting aqueous mixture of chitosan nanoparticles colloidal solution (including free chitosan, nanoparticles and drug not entrapped) without centrifugation, was spray-dried (Lu-222 Advanced, Lab Ultima, Mumbai) at a feed rate of 6.0 ml/min. The spray-drying conditions were maintained like inlet temperature 100–105°C, outlet temperature 68–71°C, aspirator 45%, and pump feed 10%. Finally, the product was kept under vacuum for 48 hours and kept in desiccator at room temperature until use.

Compatibility Studies

Infrared Spectroscopy

Carvedilol further identified and conformed by using FTIR (Infinity, Shimadzu) and recorded spectrum in the range of 4000 cm^{-1} to 400 cm^{-1} using FTIR spectrophotometer. IR spectroscopy was also used to determine the molecular interaction between excipients and drug. Infrared spectra of crosslinked microparticles and tablet formulation. IR spectrum of drug was measured in the solid state as potassium bromide dispersion. The bands (cm^{-1}) have been assigned.

Differential scanning calorimetric (DSC)

The molecular state of the pure drug Carvedilol was evaluated by performing DSC analysis. The DSC curves of the samples were obtained by a differential scanning calorimeter (Mettler Toledo) and thermograms were obtained by heating at a constant heating rate of 10°C/min in the range of 20–350°C.

Characterisation of NMCS

Particle size analysis

Particle size analysis of colloidal solution of nanoparticles was carried out by Malvern Mastersizer using water as dispersion medium and quartz cuvetts as sample holder. The sample was scanned 100 times for determination of particle size.

Determination of % Yield

The percentage yield from the microparticles was determined with following formula.

$$\%\, Yield = \frac{\text{Weight of microparticles}}{\text{Total weight of solids}} \times 100$$

Determination of Drug encapsulation efficiency and loading capacity

For the colloidal solution (nanosuspension sample), the amount of drug entrapped was calculated as the difference between the total amount of carvedilol used and the amount presented in the centrifuged aqueous supernatant phase.[23] Then, the drug entrapment efficiency and loading capacity was determined directly from the spray-dried microparticles.[24] An accurately weighed amount of 20 mg spray-dried Carvedilol microparticle were dissolved into 100 ml of pH 6.8 with constant stirring for 6 hr at 1000 rpm by magnetic stirrer at 37 ± 1°C. After ultracentrifugation at 20,000 × *g* and 12°C for 30 min, the dissolved carvedilol in the supernatant was determined using a UV–Vis spectrophotometer method. Each sample was assayed in triplicate. The Carvedilol loading capacity (LC) of microparticles and the encapsulation efficiency (EE) of the process were calculated according to the following equation:

$$LC = \frac{WCarvedilol}{Wmp} \times 100$$

$$EE = \frac{WCarvedilol}{Wtotal\ drug} \times 100$$

Where,

W*Carvedilol* is the amount of Carvedilol in microparticles,

Wmp is the microparticle weight,

Wtotal drug is the total amount of Carvedilol added.

Scanning electron microscopy

The morphology and surface appearance of the nanoparticles and spray-dried carvedilol microparticles were studied by scanning electron microscopy (JEOL 5400, Tokyo, Japan). The powdered sample was placed on double-sided adhesive tape that had previously been secured on aluminium stubs and then observed using SEM operating at 5.0 kV after gold sputtering and coated under vacuum with gold in an argon atmosphere prior to observation.

In vitro Drug release from the microparticles[19,20]

In vitro drug release profiles of Carvedilol loaded microparticles were carried out as described here. About 30 mg of microparticles was placed into a dialysis membrane bag that was impermeable to molecular cut-off of 8 kDa. Then, the bag containing the Carvedilol microparticles was tied and put into 100 ml of phosphate-buffered saline (PBS, pH 6.8). The entire system was incubated at (37°C ± 1°C) with stirring at 50 rpm. At scheduled time intervals, 5 ml of the release medium was removed and replaced with the same volume of fresh PBS. The amount of Carvedilol in the release medium was determined by UV spectroscopy. All measurements were performed in triplicate.

Manufacture of optimized carvedilol chitosan microparticle based RMTs[21]

RMTs tablets were prepared by wet granulation compression method. First, the powdered carvedilol loaded NMCS and the various rapid melt tablet adjuvant (with appropriate amounts of lubricant, superdisintegrant (Ludiflash) 40%, corrigent (Xylitol) 1%, deaggregant (MicrocrystallineCellulose) 35% and diluent **(**Lactose) 5% for all formulation batches only difference is for F1 Conventional RMT., for F2 RMT with non-crosslinked microparticles and for F3 RMT with crosslinked microparticles. These materials were screened to produce a homogeneous mixture. The resulting homogeneous mixtures were passed through a 30-mesh sieve with openings of less than 600 μm in diameter and then granulated in a mortar using 10% (w/v) polyvinylpyrrolidone K30 ethanol solution (95%, v/v) as an adhesive. The obtained granulates were dried in a 60°C oven for 1 hour. The final granules were then passed through a 14-mesh sieve and stored in a desiccator at room temperature until use and lastly add magnesium sterate 1% before compression. The filtered dry granules (100 mg containing oral disintegrating microparticle tablets (RMTs) equivalent to 6.25 mg Carvedilol) was manually filled into the die and compressed using (Mini press 12SP, Karnavati Rinek tablet machine) single punch tablet press equipped with

concave faced 08 mm punches, at a suitable compression force to obtain tablet hardness of about 2±0.5 kg.

Evaluation Parameter Tablets

Pre-compression Parameters

The RMT's blends were evaluated for their bulk density, tapped density, carr's index, flow properties and hausner's ratio.

Post-compression Parameters

Hardness: The hardness of a tablet is indicative of its tensile strength (Kg/cm^2) and the tablet crushing load, which is the force, required breaking a tablet into pieces by compression. It was measured using a texture analyser.

In vitro disintegration test: In vitro disintegration tests are usually performed for quick dissolving tablets to determine the disintegration rate when they come in contact with the mucus and saliva. The in vitro disintegration test was carried out on one tablet in pH 6.8 at 37±1°C using the texture analyser. Attempts have been made to develop disintegration tests that better mimic the in vivo conditions of the oral cavity. Dor and Fix[24] developed a disintegration test using a texture analyzer, consisting of a 35mm tall flat-ended acrylic cylindrical probe with 2 kg load cell. The RMT was attached to a cylindrical probe and placed under a constant force to promote disintegration. The tablet was immersed into a defined volume of medium (200 µl) and the time for complete tablet disintegration versus distance traveled was determined. The limitation of this method is that one side of the tablet is attached to the probe and cannot interact with the immersion medium, whereas in the oral cavity the tablet will be moistened on all sides and this can enhance disintegration.[25]

Friability: Roche friabilator was used for the purpose. This device subjects a number of tablets to the combined effect of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm for 4 min dropping the tablets at a distance of 6 inches with each revolution. Pre-weighed 10 tablets were placed in the friabilator, which was then operated for 100 revolutions. Tablets were dusted and reweighed.

Weight Variation Test: Weigh individually 20 units selected at random or, for single dose preparations in individual containers, the contents of 20 units, and calculate the average weight. Not more than two of the individual weights deviate from the average weight and none deviates by more than twice that percentage.

Determination of Drug content of the Drug: Ten tablets were powdered and blend equivalent to 6.25 mg of drug was stirred with 100ml of (6.8 pH PBS) with constant stirring for 6 hr at 1000 rpm by magnetic stirrer at 37 ± 1°C. Then, 100 ml of pH 6.8 the solution was filtered and After ultracentrifugation at 20,000 × *g* and 12°C for 30 min, the dissolved carvedilol in the supernatant was determined using a UV/vis Spectrophotometer, Shimadzu-1800.

In Vitro Dissolution Test:[26] The dissolution tests of the prepared tablets were performed at 37±1°C using (Electro lab EDT-08Lx) the paddle method at 100 rpm with 500 ml phosphate buffer (pH 6.8) as a dissolution medium. At specified time intervals, an aliquot of 5 ml was withdrawn and replaced quickly with equal dissolution medium volume to maintain total volume constant. The withdrawn samples were filtered through 0.45 µm millipore filter. The filtrate was analyzed by UV/visible spectrophotometer at 241nm (UV/vis Spectrophotometer, Shimadzu-1800).

Results and Discussion

Drug-Excipients Interaction Study

The IR spectrum was measured in solid state as potassium bromide dispersion. IR spectra of carvedilol and its combination with excipients are shown in Figure 1. An IR spectrum of pure carvedilol showed the peaks 3345.89 cm^{-1} (N-H, str), 2995.87 cm^{-1} (C-H, str, Sp^2), 2923.56 cm^{-1} (C-H, str, Sp^3), and 1106 cm^{-1} (C-O, str). These peaks can be considered as characteristic peaks of carvedilol and were not affected and prominently observed in IR spectra of carvedilol along with excipients as shown in the (Figure 1) indicated no interaction between carvedilol and excipients.

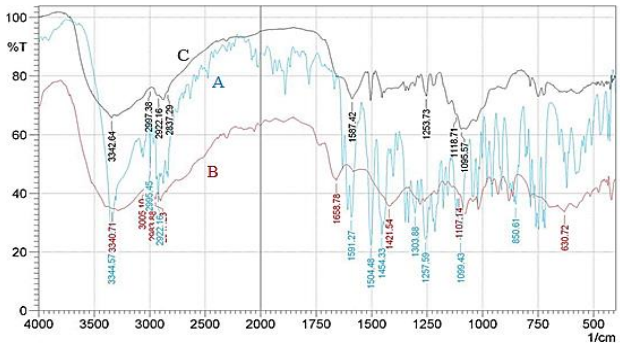

Figure 1. FTIR of A) Carvedilol pure B) Drug loaded microparticles with excipients (RMT) C) Drug loaded microparticles

Differential scanning calorimetric (DSC) study

The Differential Scanning Calorimetric study was carried out using Mettler Toledo Differential Scanning Calorimeter. Samples were placed in an aluminium crucible and the DSC thermograms were recorded at heating rate of 10°C/min in the range of 20–350°C. DSC studies revealed that endothermic peaks for pure Carvedilol were obtained at 118.38°C.

Evaluations of NMCS

Microparticles Prepared by ionotropic gelation method were evaluated particle size, loading capacity, entrapped efficiency and percentage yield. The results are shown in Table 2. Morphology of drug loaded microparticles observed by SEM is shown in Figure 2. The particle size of all prepared formulations was observed in the range of 164.2nm to 251.3nm. Entrapped efficiency of all the formulations was found to be in the range of 49 to 65%.

Percentage yield from all the batches was found to be in the range of 23.17 ± 0.81% to 42.05 ± 0.81%. Loading capacity of all the formulations was found to be in the range of 10.82 ± 1.78 to 14.44 ± 1.55%. Spherical, uniform, smooth-surfaced microparticles were successfully produced using the spray dying technique Figure 2.

In this case, a spray-drying technique was used to encapsulate the colloidal mixture of carvedilol chitosan nanoparticles and chitosan directly into powdered microparticles of carvedilol loaded microparticles (Figure 3). TPP is a nontoxic and multivalent anion that can form crosslinkages involving ionic interactions between the positively charged amino groups of chitosan and multivalent, negatively charged TPP molecules.[27,28] With the drop wise addition of TPP using syringe, the nanoencapsulation was processed with the electrostatic attraction. Meanwhile, with the incorporation of ion TPP to chitosan solution, the opalescence indicated the formation process of nanoparticles with a size range of 164.2nm to 251.3nm. The TPP reacted with a large amount of the chitosan, resulting in the formation of gelated nanoparticles. TPP-crosslinked chitosan nanoparticles and their conversion into spray-dried microparticles are expected to improve the particles' stability and applicability for controlled drug delivery. The spray-drying was used to dramatically improve drug encapsulation efficiency for the resulting microparticles. These various types of particles might provide an efficient carrier to load carvedilol into the matrix. The encapsulation efficiencies were 64.9% (Table 2) with satisfactory production yields. It can be seen in Table 2 that particle size were 188.2nm as the ratio of chitosan/drug reached 0.400:0.250 (w/w). Therefore, the drug entrapment percent can be adjusted by changing the ratio of carvedilol to chitosan, according to clinical needs.

Table 2. Characterisation of Particle size, Entrapped efficiency, Practical yield, Drug loading.

Batches	Particle size(nm)	Entrapped efficiency (%)	Percentage Yield*(%)	Drug Loading* (%)
F1	189.6	60.2	40.63 ± 0.81	13.33 ± 0.85
F2	188.2	64.9	42.05 ± 0.81	14.44 ± 1.55
F3	202.1	62.7	24.79 ± 0.34	13.93 ± 2.50
F4	251.3	49.6	39.16 ± 0.61	11.02 ± 1.74
F5	164.2	55.4	23.89 ± 1.24	12.31 ± 0.85
F6	223.4	50.7	35.51 ± 1.08	11.26 ± 1.22
F7	219.7	61.7	23.17 ± 0.81	13.71 ± 1.32
F8	231.5	48.7	36.60 ±1.06	10.82 ± 1.78
F9	219.1	64.2	37.95 ± 1.73	14.26 ± 2.51

* All values are mean ± SD, (n = 3)

Scanning Electron Microscopy study

The shape and surface morphology of drug loaded microparticles prepared by spray-drying were observed using SEM. As shown in Figure 2A, the spray-dried drug loaded microparticles were almost spherical, with a regular shape and a particle diameter range of 1–4μm. The observed small solid particles (Figure 2A) could have been formed from the spraying of a single minor droplet containing one or two chitosan colloidal nanoparticles. Most of the TPP-crosslinked Chitosan drug loaded microparticles were found to be spherical with smooth surfaces (Figure 2B).

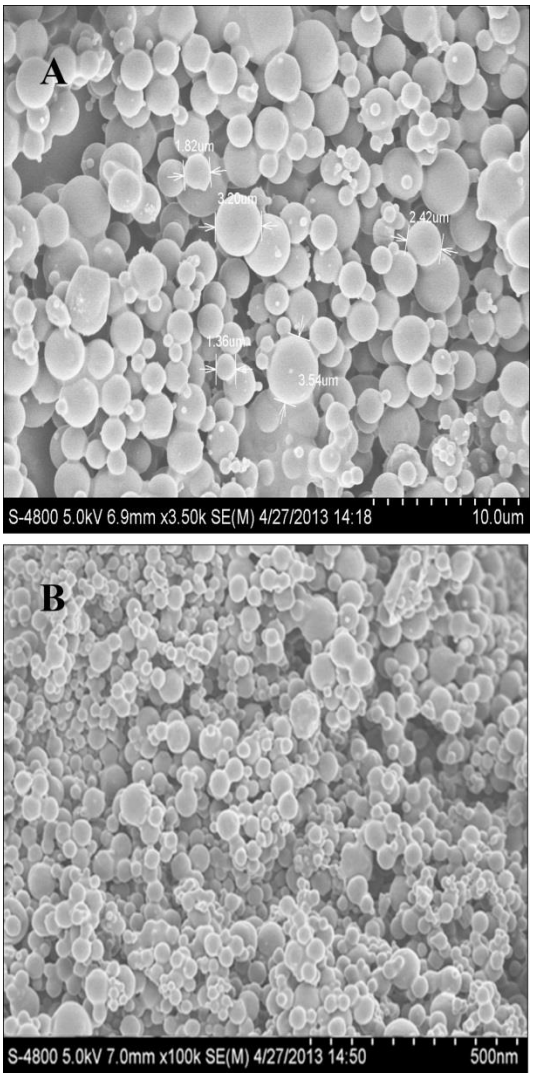

Figure 2. Scanning electron microscopy microphotograph of chitosan microparticle prepared by the spray drying method A) Drug loaded microparticle B) Chitosan nanoparticle.

From SEM photomicrographs, it can be speculated that carvedilol existed in very fine amorphous form with reduced particle size, increased surface area and closer contact between the hydrophilic polymer and the drug which may be influential in enhancing drug solubility and dissolution rate.[29]

To prepare chitosan nanoparticles as described the nanoparticles colloidal solution were transferred to eppendorf tubes and isolated by centrifugation (10,000 rpm, 1 h, 4°C). Supernatants were discarded and the nanoparticles were collected for further characterization. TPP was used to crosslink chitosan nanoparticles and microparticles. TPP reacts with compounds containing

primary amine groups, such as chitosan, to form covalently crosslinked networks and drug loaded nanoparticles and microparticles are shown in Figure 2. The incorporation of ion TPP to chitosan solution, the opalescence indicated the formation process of nanoparticles with a size range of 164.2nm to 251.3nm. Nanoparticles were spherical in shape (Figure 2B) and some aggregates are observed due the interactions between free amino and hydroxyl groups on the chitosan surface.

In vitro drug release behaviours of the spray-dried microparticles

Drug release of spray-dried carvedilol loaded microparticles could be prolonged in the presence of a proper amount of TPP used as a cross-linking agent. For the preparation of carvedilol loaded chitosan nanoparticles a chitosan/TPP ratio of 6:1 (w/w) was adopted for the ionotropic gelation process. The drug release rate was affected by cross-linking of CS microparticles with TPP.[28] and the TPP treatment of chitosan microparticles was expected to improve their stability and applicability in controlled drug delivery.

The in vitro Carvedilol release profiles were obtained by relating the amount of Carvedilol released from microparticles to the amount of Carvedilol encapsulated (Figure 3A). Displays the drug release profiles of spray-dried Carvedilol loaded microparticles and the effect of the crosslinked (TPP) on release rate. As shown in Figure 3A, the release rate of noncrosslinked microparticles was significantly faster (*P*, 0.05) than that of crosslinked microparticles during the first 90 min. The cumulative release percentage from the noncrosslinked microparticles exceeded 79% within 30 minutes. In contrast, only about 58% of the drug was released from microparticles crosslinked with TPP in 30 minutes (Figure 3A). Statistical analysis of Carvedilol release values before 90 min shows that there is a significant effect (*P*, 0.05) of the TPP. The addition of TPP is probably effective to occupy chitosan aminogroups. The crosslinking of chitosan microparticles with TPP could retard the release rate because of the decreased water solubility of chitosan in this formulation, which could be important for maintaining further sustained drug release.

Therefore, the degradation and enzymatic digestion of Chitosan nanoparticles would be required for accomplishing the release process.[30] The delayed drug release from nanoparticle entrapped microparticles and local stimulation of the formulated tablets in the mouth. It was apparent that drug release showed a rapid initial burst effect in vitro. As shown in Figure 3A, about 84% of drug was released from the spray-dried carvedilol loaded microparticles within 90 min. This initial burst release occurred because the drug was dispersed close to the surface of the particles. This portion of absorbed drug can be easily desorbed from the outer layer and will then diffuse out into solution. After 90 min, similar drug release behavior for the two formulated microparticles (*P*, 0.05) could be found in (Figure 3A). Generally, noncrosslinked chitosan microparticles cannot be kept suspended in water because of their swellability and dissolvability.

A controlled release dosage form can provide increased clinical value compared to conventional formulations, due to an improved therapeutic effect and increased patient compliance resulting from a reduced dosing frequency and a more constant or prolonged therapeutic effect and possible to enhanced bioavailability.

Pre-compression Parameters

Pre-compressional parameters of Conventional RMTs**,** Non-crosslinked microparticles RMTs, Crosslinked microparticles RMTs like angle of repose, bulk density, tapped bulk density, compressibility index, hausner's ratio of tablets shown in Table 3.

From the above result all batches showed good flow properties. Compressibility of all batches was less than 21% indicates fair to passable compression properties. Hausner's ratio < 1.25 for all batches indicates that good flow properties.

Post compression Parameters

Post compressional Parameters of Conventional RMTs (FA)**,** Non-crosslinked microparticles RMTs (FB), Crosslinked microparticles RMTs (FC) are following in (Table 3). The hardness of the tablet was found between 1.0 – 2.0 kg/cm^2 which have good mechanical strength. The tablet thickness was found to be 3.1-3.25 mm, which provided good uniformity, friability of tablet was found below 1% indicating good mechanical resistance. The drug content found in the range of 60-90%, disintegration time of all batches was found in the range of 28-40 sec.

Dissolution characteristics

The Figure 3B shows the in vitro drug release profiles of conventional RMTs, NMCS-based RMTs, and noncrosslinked RMTs. For the conventional RMTs, 94.6% of the drug was released within only 3 minutes. In contrast, 70% or less of the carvedilol was released from the two prepared RMTs in 30 minutes, and the time for 90% of drug release to occur was more than 90 minutes (Figure 3B). The statistical analysis shows that the drug released very quickly from conventional RMTs ($P < 0.01$) compared with that from the other two RMT formulations. Formulation of a microencapsulated drug into RMTs, as in the present case, might result in this controlled, sustained release. (Figure 3B) shows that the drug release rate of RMTs formulated with crosslinked microparticles (NMCS-based RMTs, 63% in 30 minutes and 85% in 90 minutes) was slower (*P* , 0.05) than that of RMTs made with noncrosslinked microparticles (noncrosslinked RMTs, 70% in 30 minutes and 91% in 90 minutes). This behaviour might be due to the retardant effect of TPP, in accordance with the behaviour of the microparticles described previously (Figure 3B). The controlled drug release could alleviate problems such as premature destruction of the tablet, intense exposure to high concentrations of the active

agent. Finally, it is important to note that the carvedilol-NMCS, including novel RMTs, could potentially improve compliance with drug administration regimens and promote a remarkably steady rate of drug absorption.

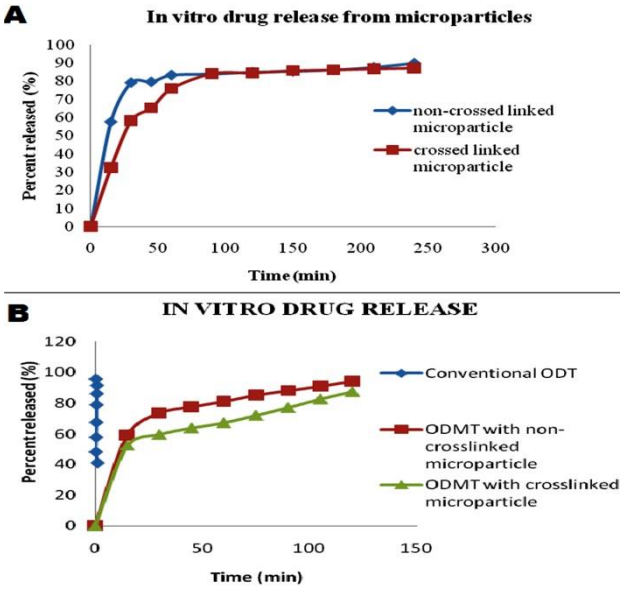

Figure 3. In vitro drug release profiles for A) Noncrosslinked and crosslinked chitosan microparticles. B) Orally disintegrating tablets (RMTs) formulated with different drug dispersion states; ODMT= orally disintegrating microparticle tablet. (RMT)

Experiments of 3-level factorial design

Experimental design

A 3-level factorial design was used to study the effect of two variables on characteristics of NMCS such as Particle size, Entrapment efficiency. Dependent and in dependent variables along with their levels are listed in Table 1. Experimental design of different batches of NMCS is summarized.

Mathematical modelling

Mathematical relationship was generated between the factors (independent variables) and responses (dependent variables) using the statistical package Design-Expert. First step in mathematical modeling was fitting the experimental data to appropriate model. A suitable model was selected by software on the basis of different parameter obtained from regression analysis such as p-value, adjusted R^2, predicted R^2 and Predicted Residual Sum of Square (PRESS) value (Table 4). ANOVA was applied for estimating the significance of model, at 5% significance level. If more than one model was significant ($p < 0.05$) for the response, the adjusted R^2 and PRESS value of the model were compared to select the best mathematical model for that response. Focus on maximizing the value of adjusted R^2 and predicted R^2. Low PRESS value indicated adequate fitting of model. General quadratic equation for two independent variables is as follow:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2$$

Abbreviation, β0 is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs. β1to β5 are all coefficients calculated from the observed experimental values of Y. X1 and X2 are the coded levels of factors. The terms X1, X2 and Xi2 (i ϵ {1, 2}) represent the interaction and quadratic terms, respectively. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Synergistic effect and antagonistic effect of factor were indicated by positive sign and negative sign in front of that factor term, respectively.

Table 3. Precompression Parameters and Post compression Parameters

Pre compression Parameters	Formulation Batch		
	F1	F2	F3
Angle of Repose(o)	28.01	29.74	24.48
Bulk Density (g/ml)	0.4461	0.4328	0.4558
Tapped Density (g/ml)	0.5370	0.5370	0.5535
Compressibility Index (%)	16.93	19.40	17.65
Hausner Ratio	1.20	1.24	1.21
Post compression Parameters			
Weight Variation (mg)*	90±2	100±1.52	100±1
Hardness (kg/cm²)*	1.2±0.25	1±0.5	1.5±0.32
Thickness (mm)*	3.1±0.15	3.1±0.20	3±0.2
Friability (%)	0.3703	0.7299	0.5154
In-vitro disintegrating time (Sec)*	28.66±3.05	33±2	40±2
Drug content (%)*	90.16±0.20	65.4±1.25	63.06±1.10

* All values are mean ± SD, (n = 3)

Table 4. Fit summary of model for the measured responses together with model summary statistics of responses to select suitable model to fit data.

Source	Y1 (Particle size)		Y2 (Entrapment Efficiency)	
	F value	P value	F value	P value
Linear vs. mean	0.0829	0.0001	0.2253	0.0005
Anadratic vs 2FI	0.2833	0.0055	0.6701	0.0040

Model summary statistics						
Response	Linear			Quadratic		
	Adj. R^2	PRESS R^2	PRESS	Adj. R^2	PRESS R^2	PRESS
Particle size	0.4187	0.0884	5790.03	0.6829	0.2579	7013.13
Drug content	0.1087	0.1800	403.67	0.1652	3.4377	1518.03

Particle size analysis

From the p-values presented in Table 4, linear model and quadratic model was found to be significant for particle size. Quadratic model was selected on the basis of maximum value of adj. R^2 and low PRESS value indicating adequate fitting of model (Table 4). Quadratic model was significant with model f-value of 10.55 (p-value <0.0001). The quadratic equation generated by software is as follows:

$$Y1 = 209.90 - 10.62X_1 + 20.28X_2 + 38.46X_1X_2 + 140X_1^2 + 210X_2^2$$

Equation reveals that both factors (X1 and X2) affect Particle size characteristics significantly. Equations also indicated that the effect of the change in Drug seems to be more pronounced in comparison with that of the change in conc. of chitosan. Since the coefficient of factor X2 has a larger value than that of factor X1. The combined effect of factors X1 and X2 can further be elucidated with the help of response surface plots (Figure 4A), which demonstrated that Y1 varies in a linear fashion with the effect of both parameter. However, the steeper ascent in the response surface with Drug (X2) – instead of Chitosan conc. (X1) – is clearly discernible from response surface plots, indicating that the effect of Drug is comparatively more pronounced than that of Chitosan conc. From this discussion, one can conclude that the Particle size may be changed by appropriate selection of the levels of X1 and X2. (Figure 4B) shows a linear relationship between the observed response values and the predicted values indicating the correctness of the model.

Entrapment efficiency

From the p-values presented in Table 5, quadratic model was found to be significant for entrapment efficiency. Quadratic model was selected on the basis of maximum value of adj. R^2 and low PRESS value indicating adequate fitting of model (Table 4). Quadratic model was significant with model f-value of 16.53 (p-value < 0.0003). The quadratic equation generated by software is as follows:

$$Y2 = 57.57 - 3.72X_1 - 2.92X_2 + 16.32X_1X_2 + 64X_1^2 + 32.09X_2^2$$

Equation reveals that both factors (X1 and X2) affect Entrapment efficiency characteristics of particle size significantly. Equations also indicated that the effect of the change in Chitosan concentration seems to be more pronounced in comparison with that of the change in Drug. Since the coefficient of factor X1 has a larger value than that of factor X2. The combined effect of factors X1 and X2 can further be elucidated with the help of response surface plots (Figure 4C). However, the steeper ascent in the response surface with Chitosan (X1) – instead of Drug (X2) – is clearly discernible from response surface plots, indicating that the effect of Chitosan is comparatively more pronounced than that of Drug. From this discussion, one can conclude that the Entrapment efficiency may be changed by appropriate selection of the levels of X1 and X2. (Figure 4D) shows a linear relationship between the observed response values and the predicted values indicating the correctness of the model.

Table 5. Analysis of variance (ANOVA) table for measured responses

Model	Y1		Y2	
	f-value	p-value	f-value	p-value
Model	4.38	0.0728	1.93	0.0221
X_1	2.20	0.1981	2.34	0.0003
X_2	8.03	0.036	1.47	0.0001
X_1X_2	0.148	0.0001	3.56	0.0320
X_1^2	5.21	0.0217	0.030	0.002
X_2^2	3.01	0.0012	8.92	0.0211

Stability studies of optimized formulation

The stability studies of NMCS RMT were carried for 3 months and it was found the formulations were sufficiently stable for said period at 40°C and 75 % R.H.

Conclusion

Ludiflash, a novel polymeric carrier was investigated for the solubility enhancement and improving bioavailability of water insoluble Carvedilol drug using spray drying method. The carvedilol entrapped NMCS were successfully prepared using an ionotropic gelation process combined with a spray-drying method. The results for particle size at several intervals within the

range of 100nm to 300 nm. The spherically shaped NMCS was formulated with satisfactory drug loading capacity and encapsulation efficiency. The developed RMT formulation has advantageous characteristics that distinguish it from commonly used RMTs. The RMTs can be formulated not only for oral dispersibility but also one of most advantageous is it shows the delayed release that takes place far from the buccal region. So, it is a novel method to prepare rapid melt tablet which will be able to mask the drug taste in the oral cavity. NMCS represent an example of rapidly dispersible/slowly releasing tablets that are an alternative to traditional RMTs. The presence of disaggregating of Ludiflash makes it possible to produce a hard tablet that can still disaggregate within seconds and be considered "quickly dispersible".

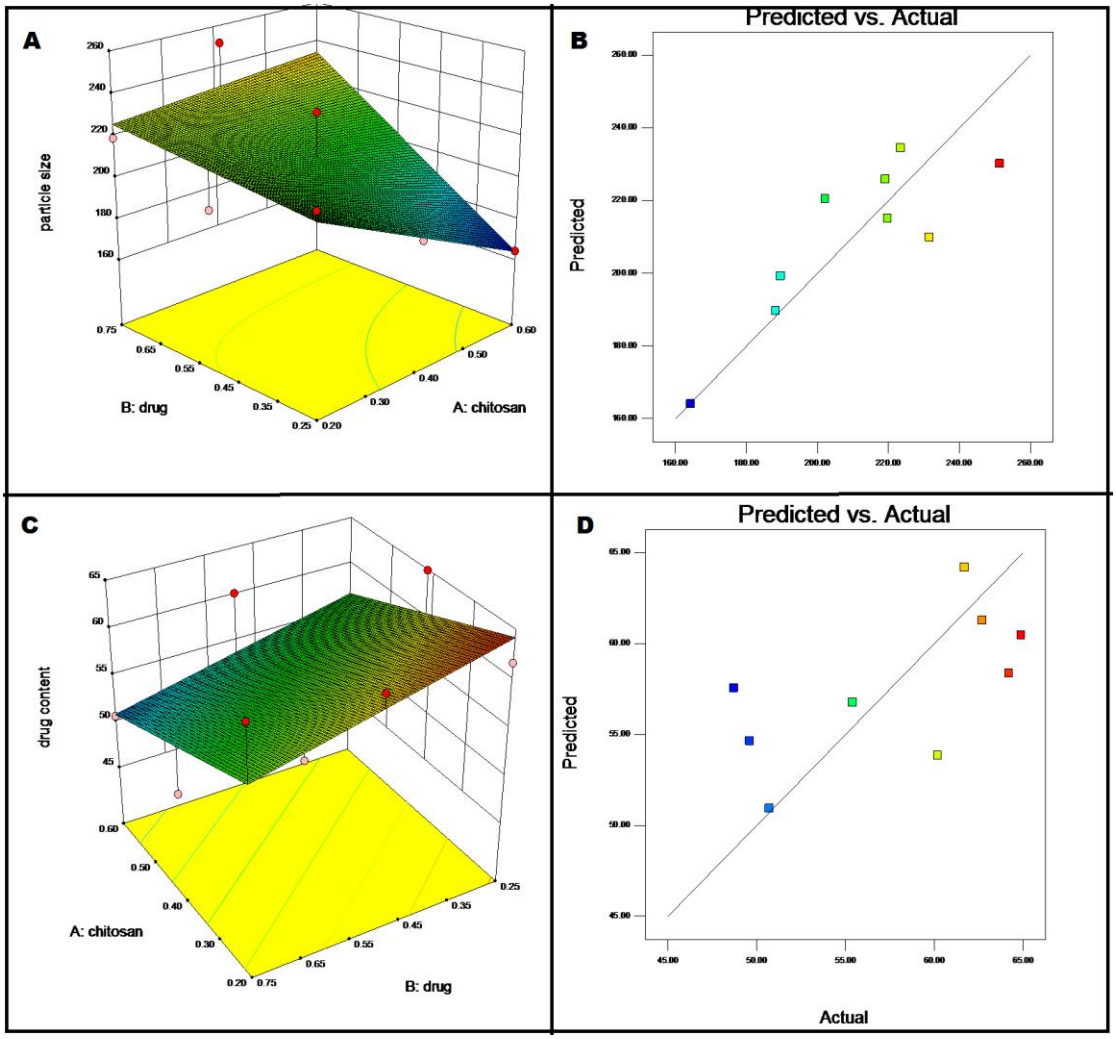

Figure 4. (A) Response surface plot showing the effect of Chitosan conc. and Drug on Particle size (Y1); (B) Linear plot between observed and predicted value of Y1. (C) Response surface plot showing the effect of drug and chitosan concentration of entrapment efficiency (Y2) (D) linear plot between observed and predicted value of Y2.

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Ethical Issues

Not applicable.

Conflict of Interest

Authors declare no conflict of interest.

Abbreviations

FA: Conventional RMTs**; FB:** Non-crosslinked microparticle RMTs and **FC:** Crosslinked microparticle RMTs

Referances

1. Chang RK, Guo X, Burnside B, Couch R. Fast-dissolving tablets. *Pharm Technol* 2000;24(1):52-8.
2. Habib W, Khankari R, Hontz J. Fast-dissolve drug delivery system. *Crit Rev Ther Drug Carrier Syst* 2000;17(1):61-72.
3. Fini A, Bergamante V, Ceschel GC, Ronchi C, De Moraes CA. Fast dispersible/slow releasing ibuprofen

tablets. *Eur J Pharm Biopharm* 2008;69(1):335-41. doi: 10.1016/j.ejpb.2007.11.011
4. Hisakadzu S, Yunxia B. Preparation, evaluation and optimization of rapidly disintegrating tablets. *Powder Technol* 2002;122(2-3):188-98. doi: 10.1016/s0032-5910(01)00415-6
5. Mizumoto T, Masuda Y, Yamamoto T, Yonemochi E, Terada K. Formulation design of a novel fast-disintegrating tablet. *Int J Pharm* 2005;306(1-2):83-90. doi: 10.1016/j.ijpharm.2005.09.009
6. Fukami J, Yonemochi E, Yoshihashi Y, Terada K. Evaluation of rapidly disintegrating tablets containing glycine and carboxymethylcellulose. *Int J Pharm* 2006;310(1-2):101-9. doi: 10.1016/j.ijpharm.2005.11.041
7. European Directorate for Quality of Medicines. Pharmaeuropa. 1998 [6 February 2007]; Available from: http://www.pheur.org.
8. Watts PJ, Davies MC, Melia CD. Microencapsulation using emulsification/solvent evaporation: an overview of techniques and applications. *Crit Rev Ther Drug Carrier Syst* 1990;7(3):235-59.
9. Kolakovic R, Laaksonen T, Peltonen L, Laukkanen A, Hirvonen J. Spray-dried nanofibrillar cellulose microparticles for sustained drug release. *Int J Pharm* 2012;430(1-2):47-55. doi: 10.1016/j.ijpharm.2012.03.031
10. Bhavsar MD, Tiwari SB, Amiji MM. Formulation optimization for the nanoparticles-in-microsphere hybrid oral delivery system using factorial design. *J Control Release* 2006;110(2):422-30. doi: 10.1016/j.jconrel.2005.11.001
11. Bhavsar MD, Amiji MM. Gastrointestinal distribution and in vivo gene transfection studies with nanoparticles-in-microsphere oral system (NiMOS). *J Control Release* 2007;119(3):339-48. doi: 10.1016/j.jconrel.2007.03.006
12. Cal K, Sollohub K. Spray drying technique. I: Hardware and process parameters. *J Pharm Sci* 2010;99(2):575-86. doi: 10.1002/jps.21886
13. Sollohub K, Cal K. Spray drying technique: II. Current applications in pharmaceutical technology. *J Pharm Sci* 2010;99(2):587-97. doi: 10.1002/jps.21963
14. Onishi H, Machida Y. Biodegradation and distribution of water-soluble chitosan in mice. *Biomaterials* 1999;20(2):175-82. doi: 10.1016/s0142-9612(98)00159-8
15. Rao SB, Sharma CP. Use of chitosan as a biomaterial: Studies on its safety and hemostatic potential. *J Biomed Mater Res* 1997;34(1):21-8. doi: 10.1002/(sici)1097-4636(199701)34:1<21::aid-jbm4>3.0.co;2-p
16. Li FQ, Fei YB, Chen X, Qin XJ, Liu JY, Zhu QG, et al. Anchoring of ulex europaeus agglutinin to chitosan nanoparticles-in-microparticles and their in vitro binding activity to bovine submaxillary gland mucin. *Chem Pharm Bull (Tokyo)* 2009;57(10):1045-9. doi: 10.1248/cpb.57.1045
17. Li FQ, Ji RR, Chen X, You BM, Pan YH, Su JC. Cetirizine dihydrochloride loaded microparticles design using ionotropic cross-linked chitosan nanoparticles by spray-drying method. *Arch Pharm Res* 2010;33(12):1967-73. doi: 10.1007/s12272-010-1212-3
18. Saraswatihi R, Nagasamy VD, Sangeetha S, Krishnan PN. Development and *in-vitro* characterization of terbutaline sulphate buccal films. *Int J Chem Sci* 2007;5(5):2402-10.
19. Li FQ, Ji RR, Chen X, You BM, Pan YH, Su JC. Cetirizine dihydrochloride loaded microparticles design using ionotropic cross-linked chitosan nanoparticles by spray-drying method. *Arch Pharm Res* 2010;33(12):1967-73. doi: 10.1007/s12272-010-1212-3
20. Fei YB, Li FQ, Hu J. Preparation of chitosan nanoparticles loaded with HB vaccine by ionotropic gelation-homogenization process. *Pharm Care Res* 2008;8(2):119-22.
21. Li FQ, Yan C, Bi J, Lv WL, Ji RR, Chen X, et al. A novel spray-dried nanoparticles-in-microparticles system for formulating scopolamine hydrobromide into orally disintegrating tablets. *Int J Nanomedicin* 2011;6:897-904. doi: 10.2147/IJN.S17900
22. Li FQ, Su H, Wang J, Liu JY, Zhu QG, Fei YB, et al. Preparation and characterization of sodium ferulate entrapped bovine serum albumin nanoparticles for liver targeting. *Int J Pharm* 2008;349(1-2):274-82. doi: 10.1016/j.ijpharm.2007.08.001
23. Li FQ, Hu JH, Lu B, Yao H, Zhang WG. Ciprofloxacin-loaded bovine serum albumin microspheres: preparation and drug-release in vitro. *J Microencapsul* 2001;18(6):825-9. doi: 10.1080/02652040110055298
24. Dor PJ, Fix JA. In vitro determination of disintegration time of quick-dissolve tablets using a new method. *Pharm Dev Technol* 2000;5(4):575-7. doi: 10.1081/pdt-100102041
25. Abdelbary G, Eouani C, Prinderre P, Joachim J, Reynier J, Piccerelle P. Determination of the in vitro disintegration profile of rapidly disintegrating tablets and correlation with oral disintegration. *Int J Pharm* 2005;292(1-2):29-41. doi: 10.1016/j.ijpharm.2004.08.019
26. Fini A, Bergamante V, Ceschel GC, Ronchi C, De Moraes CA. Fast dispersible/slow releasing ibuprofen tablets. *Eur J Pharm Biopharm* 2008;69(1):335-41. doi: 10.1016/j.ejpb.2007.11.011
27. Shu XZ, Zhu KJ. A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery. *Int J Pharm* 2000;201(1):51-8. doi: 10.1016/s0378-5173(00)00403-8
28. Anal AK, Stevens WF, Remunan-Lopez C. Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin. *Int J Pharm*

2006;312(1-2):166-73. doi: 10.1016/j.ijpharm.2006.01.043
29. Nepal PR, Han HK, Choi HK. Enhancement of solubility and dissolution of coenzyme Q10 using solid dispersion formulation. *Int J Pharm* 2010;383(1-2):147-53. doi: 10.1016/j.ijpharm.2009.09.031
30. Janes KA, Fresneau MP, Marazuela A, Fabra A, Alonso MJ. Chitosan nanoparticles as delivery systems for doxorubicin. *J Control Release* 2001;73(2-3):255-67. doi: 10.1016/s0168-3659(01)00294-2