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Preparation And Evaluation Of Ezetimibe Loaded Nanoparticles By Ionic Gelation Method.

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ABSTRACT

The ezetimibe loaded nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). Nanoparticles of different core: coat ratio were formulated and evaluated for process yield, loading efficiency, particle size, zeta potential, in vitro drug release, kinetic studies and stability studies. The chitosan nanoparticles have a particle diameter ranging approximately 289–344 nm and a zeta potential 25.9 mV. There was a steady decrease in the entrapment efficiency on increasing the polymer concentration in the formulations. The in vitro release behaviour from all the drug loaded batches were found to follow first order and provided sustained release over a period of 24 h. No appreciable difference was observed in the drug content of product during 60 d in which nanoparticles were stored at 5˚C and room temperature. According to the data obtained, this chitosan- based delivery system opens new and interesting perspectives as drug carriers.

Keywords: Nanoparticles; Chitosan; Ezetimibe; Ionic gelation technique

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INTRODUCTION

Hyperlipidemia has been categorized as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases [1]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the main cause of death [2]. Hyperlipidemia is distinguished by elevated serum total cholesterol, low density lipoprotein, very low-density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia related lipid disorders are considered to cause atherosclerotic cardiovascular disease [3]. The causes of hyperlipidemia include inherited defects in lipid metabolism and hypercholesterolemia due to diet, drugs or diseases belongs to statin family of cholesterol lowering agents and is widely used to treat coronary heart disease, dyslipidaemia and hypercholesterolemia [4]. Ezetimibe is the first lipid-lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol without affecting the absorption of fat-soluble nutrients [5]. FDA has recently recognized ezetimibe as a novel medicine for the treatment of various disease conditions most particularly cardiovascular disease [6]. Ezetimibe is classified as BCS class-II drug, owing to its sparingly water soluble, yet extraordinary permeation capability. it has extremely low dissolution in gastrointestinal (GI) fluids, and exceedingly irregular bioavailability owing to its hydrophobic nature [7].

Hence, the objective of work was to formulate chitosan nanoparticles containing ezetimibe by ionic gelation method, evaluate its physicochemical characteristics such as solubility particle, size shape, zeta potential, drug loading capacity and in vitro release characteristics.

MATERIALS AND METHODS

The Ezetimibe was received as a gift sample from Recipharm pharmaservices pvt ltd., karnataka, India. Potassium dihydrogen phosphate, disodium hydrogen phosphate sodium hydroxide and sodium acetate were purchased from Thermo fisher scientific India Pvt. Ltd., Bangalore, India. The distilled water was produced in our research laboratory with a distillation unit.

Method Of Preparation

Chitosan nanoparticles were formulated by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25, v/v) at different concentrations such as 1.0, 2.0, 3.0, 4.0, 5.0 mg/ml. Under magnetic stirring at room temperature, 5 ml of 0.84% (w/v) TPP aqueous solution was added dropwise using syringe needle into 10 ml chitosan solution containing 10mg of Ezetimibe. pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was carried about 30 min. The prepared nanoparticles suspensions were centrifuged at 12000 × g for 30 min using C24 centrifuge. The formation of the particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation) (Table 1) [8].

Characterization Of Prepared Microspheres

Differential scanning calorimetry (DSC)

A DSC study was performed to detect possible polymorphic transition during the crystallization process. DSC measurements were performed on a DSC DuPont 9900, differential scanning calorimeter with a thermal analyser.

Fourier transform infra-red spectroscopy (FT-IR) Analysis

The FT-IR spectra of pure Ezetimibe and chitosan nanoparticles loaded with Ezetimibe were recorded using Shimadzu IR spectrophotometer, Model 840, Japan, to check drug polymer interaction and stability of drug [9].

Practical yield

Nanoparticles were collected and weighed to determine practical yield (PY) from the following equation

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$$PY(\%)= \frac{\text{Nanoparticles weight}}{\text{Theoretical mass (polymer+drug+TTP)x100}}$$

Drug entrapment efficiency

Nanoparticles equivalent to 5mg Ezetimibe were crushed using a glass mortar and pestle. Then, they were suspended in 25 ml of acetate buffer pH 4.5. After 24 hrs., the solution was filtered and 1 ml of the filtrate was diluted 10 times and analysed for the drug content by UV-visible spectrophotometer at 232nm. The drug entrapment efficiency was calculated using the following formula [10]:

$$\text{Entrapment efficiency}= \frac{\text{Actual drug content}}{\text{Theoretical drug content}} X\ 100$$

Surface morphology study

Scanning electron microscopy (SEM) of the chitosan nanoparticles was carried out to examine the particle size and surface morphology. The nanoparticles were arranged on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron microscope under magnification of 7500–20000 ×.

Particle size distribution

The particle size distribution of the nanoparticles was determined by photon correlation spectroscopy (PCS, Coulter Counter model N4 MD, Coulter Counter Co. USA). The nanoparticle dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the nanoparticles. The average volume-mean particle size was calculated after performing the experiment in triplicate.

Zeta potential

The Zeta-potential of drug loaded nanoparticles were measured by Zeta sizer (Malvern Zetasizer 3000HS, UK). To determine the zeta potential, nanoparticles samples were diluted with KCl (0.1 mM) and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analysed in triplicate [11].

Determination of solubility

Drug solubility was determined by adding excess amounts of pure Ezetimibe and nanoparticles in distilled water at 37 ± 0.5°C, respectively. The solution formed were equilibrated under continuous agitation for 24 h and passed through a 0.8 µm membrane filter to obtain a clear solution. The absorbance of the samples was measured using UV spectrophotometer method (UV 1601 A Shimadzu, Japan) at 232nm and the concentrations in µg/ml were determined. Each sample was determined in triplicate [12].

In vitro release studies

In vitro release studies were carried out by using dialysis tubes with an artificial membrane. The prepared ezetimibe nanoparticles were re-dispersed in 5 ml of acetate buffer pH 4.5 and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 150 ml of acetate buffer pH 4.5. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ±1˚C. 5ml sample of receptor compartment was taken at various intervals of time over a period of 24 h and each time 5 ml fresh buffer was replaced. The amount of drug released was determined spectrometrically at 232nm [13].

Kinetic modelling

In order to understand the kinetic and mechanism of drug release, the result of in vitro drug release study of nanoparticles was fitted with various kinetic equation like zero order (cumulative% release vs. time), first order (log% drug remaining vs. time), Higuchi's model (cumulative% drug release vs. square root of time), Peppas plot (log of cumulative% drug release vs. log time). R2 (coefficient of correlation) and

k (release rate constant) values were calculated for the linear curve obtained by regression analysis of the above plots.

Stability studies

The stability study was carried out using the batch F1. Formulation F1 was divided into 3 sets of samples and stored at 5˚C in refrigerator, room temperature, 45 ± 2˚C/75% RH in humidity control ovens. After 60 d drug content of all samples were determined by the method as in drug content. In vitro release study of formulation F1 was also carried out after 60 d of storage [14].

RESULTS AND DISCUSSION

physicochemical characterization of nanoparticles

Nanoparticles were formed spontaneously upon the incorporation of TPP solution to the chitosan solution under magnetic stirring. Chitosan nanoparticles are obtained by ionic gelation which is a simple process, where particles are formed by means of electrostatic interactions between the positively charged chitosan chains and polyanions employed as cross linkers. The FTIR spectrum shows that there were no significant changes in the chemical integrity of drug and also indicates that the polymer and drug are compatible with each other. Nanoparticles prepared by ionic gelation technique were found to be discrete and through SEM analysis (Fig. 1), their mean size distribution was found to be 232nm. Since the particle size is less than 1000nm, this drug delivery system can be used for parenteral formulations, drugs administered by such routes will achieve direct systemic delivery, thereby avoiding first pass hepatic metabolism and reaching a reduction in the dose delivered. The drug entrapment efficiency of nanoparticles containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 was found to be78.9%, 75.6%,74.3%,73.2%,71.1% (Table 1). Thus, there was a steady increasing in the entrapment efficiency on decreasing the polymer concentration in the formulation. The high entrapment efficiency is likely due to electrostatic interactions between the drug and the polymer. Zeta potential of the ideal formulation was found to be 1.3 mV, which indicates that they are stable.

Table 1: Formulation and physicochemical characterization of Ezetimibe nanoparticles.

S. No	Batch code	Drug: carrier ratio	Entrapment efficiency (%)	Particle size (µm)
1	F1	1:1	77.2± 0.23	409± 5.04
2	F2	1:2	74.1± 0.56	344 ± 4.2
3	F3	1:3	72.9± 0.58	289± 8.9
4	F4	1:4	71.5± 0.42	243± 10.5
5	F5	1:5	69.4± 0.36	204± 10.7

Mean ± SD (n=3). F1, F2, F3, F4 and F5 represent formulations 1 to 5, respectively, etc.

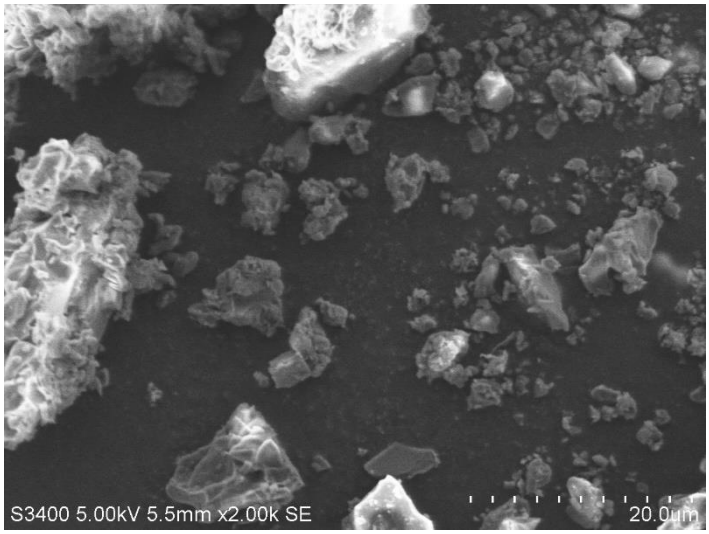

Figure 1: SEM of Formulation F1

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In vitro release of nanoparticles

Cumulative percentage drug released for F1, F2, F3, F4 and F5 after 24 h were found to be 78.9%,75.6%,74.3%,73.2%, and 71.1% respectively (Fig. 2). It was apparent that in vitro release of ezetimibe showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggests that some drug was localized on the surface of the nanoparticles.

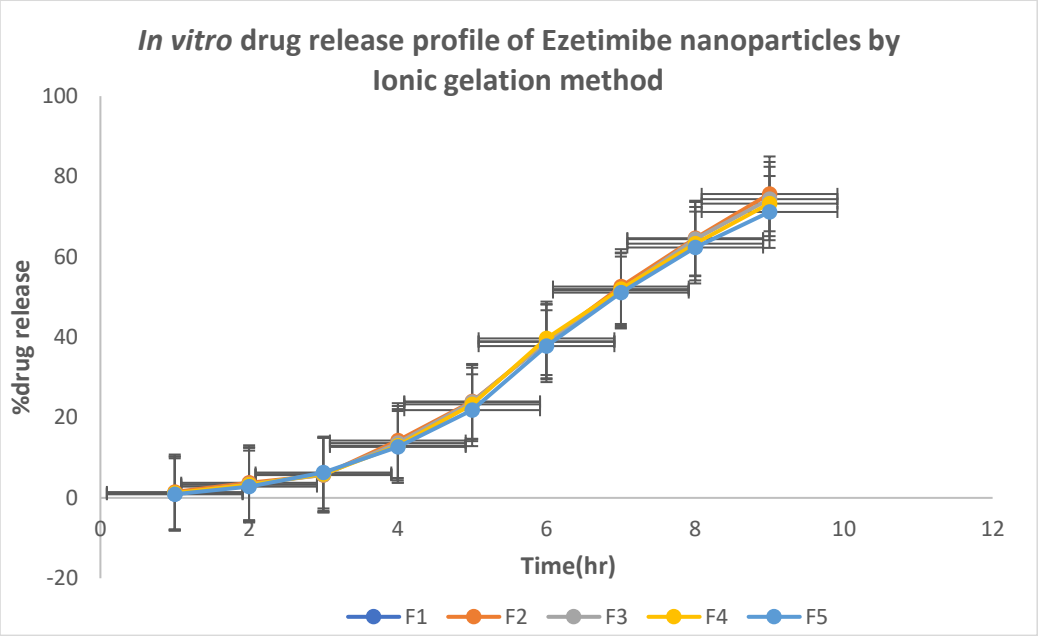

Figure 2: Cumulative release of ezetimibe loaded nanoparticles (mean ±SD, n=3)

Kinetic studies

In order to describe the release kinetics of all five formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, and Higuchi, respectively (Table 2)and(Table3). As indicated by higher R^2 (coefficient of correlation) values, the drug release from all formulations follows Zero order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, Non-Fickian diffusion or zero order. 'n' (release exponent of Korsmeyer- Peppas model) value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be not less than 0.50. This shows that the release approximates Non-Fickian diffusion mechanism.

Stability studies

The results of drug content of ideal formulation F1 after 3 month of stability testing at different storage conditions were shown in Fig. 3. In vitro release profiles for the same formulation stored at different storage conditions were also showed in Fig. 3.

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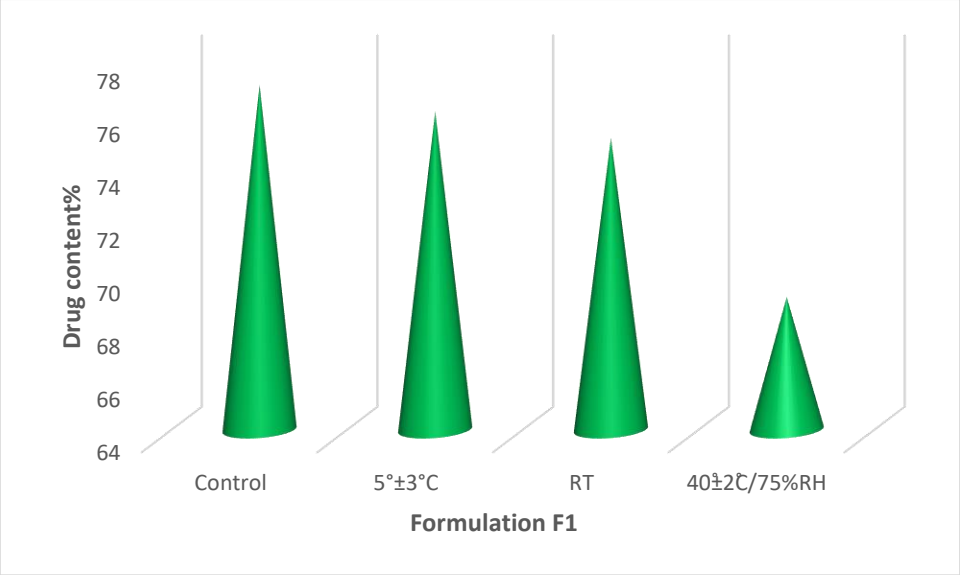

Figure 3: Stability study: comparison of drug content of formulation F1 at 4˚C, room temperature (29˚C) and 45 ± 2˚C/ 75% RH.

The results of drug content of ideal formulation F1 after 3 month of stability testing at different storage conditions were shown in Fig. 3. In vitro release profiles for the same formulation stored at different storage conditions were also showed in Fig. 4.

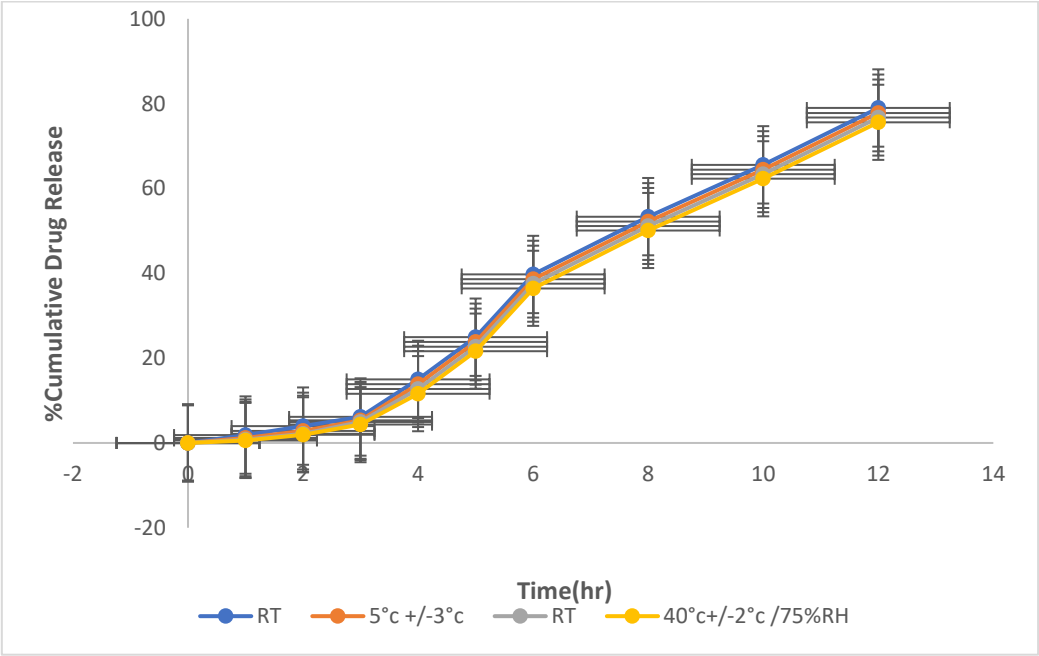

Figure 4: Stability study: comparison of in vitro drug release profile for Formulation F1 at 4˚C, room temperature (32˚C) and 45 ± 2˚C/75% RH after three months storage.

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Table 2: Correlation coefficients according to different kinetic equations.

Time (Hr)	Log T	SQRT	%CDR	log%CDR	%Drug remaining	log % drug remaining
0	0	0	0	0	100	2
1	0	1	1.8666	0.2710	98.1334	1.9910
2	0.3010	1.4142	3.9725	0.5990	96.0275	1.9823
3	0.4771	1.7320	6.1263	0.7871	93.8737	1.9688
4	0.6020	2	14.9808	1.1755	85.0192	1.9295
5	0.6989	2.2360	24.9361	1.3968	75.0639	1.8754
6	0.7781	2.4494	39.7255	1.5990	60.2745	1.7801
8	0.9030	2.8284	53.3663	1.7272	46.6337	1.6686
10	1.000	3.1622	65.5711	1.8167	34.4289	1.5369
12	1.0791	3.4641	78.9725	1.8974	21.0275	1.3227

Table 3: correlation coefficients according to different kinetic equations of the ideal formulation F1

Formulation	F1
Cumulative drug release (%)	78.9
Zero order (r^2)	0.981
First order (r^2)	0.9349
Higuchi plot (r^2)	0.7747
Peppas plot (r^2)	0.9764
'n' values	1.7305

On comparing this data with the previous data of F1, it was observed that there was a slight decrease in drug content when the formulation was stored at 5˚C and Room temperature, but there was significant decrease in drug content when the formulation w s stored at 40 ± 2˚C/75 RH because at higher temperature, there might be chances for drug degradation that decreased the drug release.

CONCLUSION

Based on drug content, drug entrapment efficiency, particle size morphology, zeta potential and in vitro release, formulation F1 was selected as an optimum formulation. Stability studies were carried out for the selected formulation F1. The stability studies showed that maximum drug content and closest in vitro release to previous data was found for F1 stored at 5˚C and room temperature. The solubility and dissolution of the nanoparticles was improved significantly compared with its physical mixture and pure sample of Ezetimibe. Stability results showed that prepared nanoparticles stable for 3 months as per ICH guidelines. Hence, from the above result it can be concluded that Ezetimibe nanoparticles is a useful technique to improve the solubility and dissolution of poorly water-soluble drug like Ezetimibe.

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