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Preparation And Evaluation Of Bedaquiline Loaded Microspheres By Ionic Gelation Technique.

J Adlin Jino Nesalin, Sachith MP, and Manu Kumar MS*.

Department Of Pharmaceutics, Bharathi College Of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India.

ABSTRACT

The Bedaquiline loaded Microspheres were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). Microspheres of different core: coat ratios were formulated and evaluated for process yield, loading efficiency, particle size, zeta potential, in vitro drug release, kinetic studies and stability studies. The chitosan Microspheres have a particle diameter ranging from approximately 344-243µm and zeta potential of 1.3 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 followed the first order and provided sustained release throughout 24 h. No appreciable difference was observed in the drug content of the product during the 3 months in which Microspheres were stored at 4˚C and room temperature. According to the data obtained, this chitosan-based delivery system opens new and exciting perspectives for drug carriers.

Keywords: Microspheres; Chitosan; Bedaquiline; Ionic gelation technique

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Corresponding author

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INTRODUCTION

Tuberculosis (TB), caused by the pathogen Mycobacterium tuberculosis, has been a significant cause of mortality in humans for millennia and is currently one of the top ten causes of death worldwide [1]. The incidence of drug-resistant TB is especially concerning and has been exacerbated by the absence of new treatment options developed for this disease over the last half-century. In 2012 the FDA approved Bedaquiline (BDQ, also referred to in the literature as Sirturo, For the treatment of multidrug-resistant TB, the first new drug to be approved for this disease in forty years. BDQ is a diarylquinoline drug that exerts a novel action mechanism, namely inhibition of mycobacterial ATP synthase. It displays excellent activity against both drug-sensitive and drug-resistant TB strains and was recently added to the World Health Organization's essential medicines [2-5].

Recently, 2 new drugs, Bedaquiline and Delamanid, were introduced for MDR-TB treatment. Bedaquiline belongs to a new class of anti-TB drugs known as diarylquinolines, which inhibit mycobacterial adenosine triphosphate synthases [6]. In a recent study, the crystal structure of the c-ring from Mycobacterium phlei (M. phlei) in a complex with BDQ was resolved, indicating that BDQ cannot bind to the ATP synthase of non-mycobacterial species [7].

Despite significant progress in reducing tuberculosis (TB) incidence and deaths in recent decades, multidrug-resistant tuberculosis (MDR-TB, defined as TB with resistance to at least isoniazid and rifampin) has become a significant obstacle to controlling this disease worldwide [8-9]. In 2018, there were an estimated 484,000 incidents of MDR-TB, with an estimated 214,000 deaths from MDR-TB globally that year.2 Therapy for MDRTB has been challenging due to the prolonged treatment duration required for treating this disease, limited therapeutic options and poor drug tolerability. Unfortunately, approximately 50% of MDR-TB patients experience unsuccessful treatment outcomes across all countries, according to recent World Health Organization (WHO) reports. Therefore, more chemotherapeutic interventions are needed [10].

Hence, the objective of the work was to formulate Chitosan Microspheres containing Bedaquiline by the Ionic gelation method and evaluate its physicochemical characteristics such as solubility, particle size, shape, and drug loading capacity, zeta potential, and in vitro release property.

MATERIALS AND METHODS

The Bedaquiline was received as a gift sample from Recipharm pharma services Pvt ltd., Karnataka, Chitosan main drug house(p)Ltd. New Delhi, India. Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide and hydrochloric acid 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 Microspheres were prepared 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 a syringe needle into 10 ml chitosan solution containing 50 mg of Bedaquiline. pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was carried for about 30 min. The obtained Microspheres suspensions were centrifuged at 12000 × g for 30 min using a C24 centrifuge. The formation of the particles as a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation) (Table 1) [11].

Characterization Of Prepared Microspheres

Differential scanning calorimetry (DSC)

A DSC study was carried out to detect possible polymorphic transitions during the crystallization process. DSC measurements were performed on a DSC DuPont 9900 differential scanning calorimeter with a thermal analyser [12].

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

The FT-IR spectra of pure Bedaquiline and chitosan Microspheres loaded with Bedaquiline were recorded using Shimadzu IR spectrophotometer, Model 840, Japan, to check drug-polymer interaction and stability of drug [13].

Practical yield

Ionic gelated Microspheres were collected and weighed to determine practical yield (PY) from the following equation.

$$PY(\%) = \frac{\text{Microparticales weight}}{\text{Theoretical mass(polymer+drug+TPP)}} \text{ X } 100$$

Drug entrapment efficiency

Microspheres equivalent to 5 mg Bedaquiline were crushed using a glass mortar and pestle. Then, they were suspended in 25 ml of phosphate buffer pH 7.4. 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 229 nm. The drug entrapment efficiency was calculated using the following formula [14].

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

Surface morphology study

Scanning electron microscopy (SEM) of the chitosan Microspheres was performed to examine the particle size and surface morphology. The Microspheres were mounted 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 Microspheres was determined by photon correlation spectroscopy (PCS, Coulter Counter model N4 MD, Coulter Counter Co. USA). The Microparticle dispersions were added to the sample dispersion unit containing a stirrer and stirred to reduce the aggregation between the Microspheres. The average volume-mean particle size was measured after experimenting triplicate.

Zeta potential

The Zeta-potential of drug-loaded Microspheres was measured by Zeta sizer (Microtrac). Microspheres samples were diluted with KCl (0.1 mM) and placed in an electrophoretic cell where an electrical field of 15.2 V/cm was applied to determine the zeta potential. Each sample was analysed in triplicate.

Determination of solubility

Drug solubility was determined by adding excess amounts of pure Bedaquiline, their physical mixture and microspheres in distilled water at 37 ± 0.5°C, respectively. The solution formed was equalize 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 the UV spectrophotometer method (UV 1601 A Shimadzu, Japan) at 229 nm, and the concentrations in µg/ml were determined. Each sample was determined in triplicate [15].

In vitro release studies

The In vitro release studies of Bedaquiline Microspheres were carried out in a USP paddle-type 2 dissolution test apparatus. 50mg Bedaquiline drug-loaded Microspheres were introduced into 900ml of the dissolution medium and stirred at 100 rpm at 37oC. At different time intervals, the solution was withdrawn, and absorbance was read at 229nm. After each withdrawal, an equal volume of the medium was replaced into the container to maintain sink condition.

Kinetic modeling

In order to understand the kinetics and mechanism of drug release, the result of in vitro drug release study of Microspheres was fitted with various kinetic equations 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 [16].

Stability studies

The stability study was carried out using batch F1. Formulation F1 was divided into 3 sets of samples and stored at 4˚C in a refrigerator, room temperature 40± 2˚C/75% RH in humidity control ovens. After 60 d drug content of all samples was determined by the method as in drug content. In vitro release study of formulation, F1 was also carried out after 60 d of storage [17].

RESULTS AND DISCUSSION

physicochemical characterization of Microspheres

Spherical Microspheres were formed spontaneously upon incorporating TPP solution into the chitosan solution under magnetic stirring. Chitosan Microspheres are obtained by ionic gelation, a simple process where particles are formed through electrostatic interactions between the positively charged chitosan chains and polyanions employed as crosslinkers. The FTIR spectrum shows no significant changes in the chemical integrity of the drug and indicates that the polymer and drug are compatible with each other.

Microspheres prepared by the ionic gelation technique were discrete, and through SEM analysis (Fig. 1), their mean size distribution was found to be 229 nm. Since the particle size is less than 1000µm, 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 reducing the dose delivered.

The drug entrapment efficiency of Microspheres containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 was found to be90%, 77%,72%,68.5%,65% (Table 1). Thus, there was a steady increase in the entrapment efficiency by increasing the polymer concentration in the formulation. The high entrapment efficiency is likely due to electrostatic interactions between the drug and the polymer. The Zeta potential of all formulated Microspheres was found to be1.3 mV, which indicates that they are stable.

Table 1: Formulation and physicochemical characterization of Bedaquiline Microsphere.

S. No	Batch code	Drug: carrier ratio	Entrapment efficiency (%)	Particle size (µm)
1	F1	1:1	90± 0.23	486± 5.04
2	F2	1:2	77± 0.56	409 ± 4.2
3	F3	1:3	72.1± 0.58	344± 8.9
4	F4	1:4	68.1± 0.42	289± 10.5
5	F5	1:5	65± 0.36	243± 10.7

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

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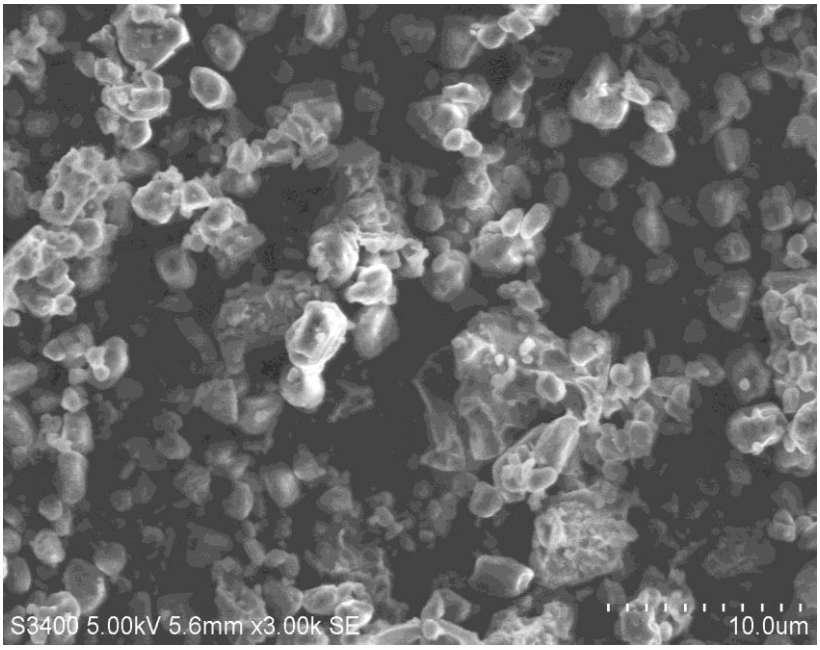

Figure 1: SEM of formulation F1.

In vitro release of Microspheres

Cumulative percentage drug released for F1, F2, F3, F4 and F5 after 24 h were found to be 84.8%,75.4%,73.16%,69.78%, and 67.33% respectively (Fig. 2). It was apparent that in vitro release of Bedaquiline showed a very rapid initial burst, followed by a prolonged drug release. An initial, fast release suggests that some drug was localized on the surface of the Microspheres.

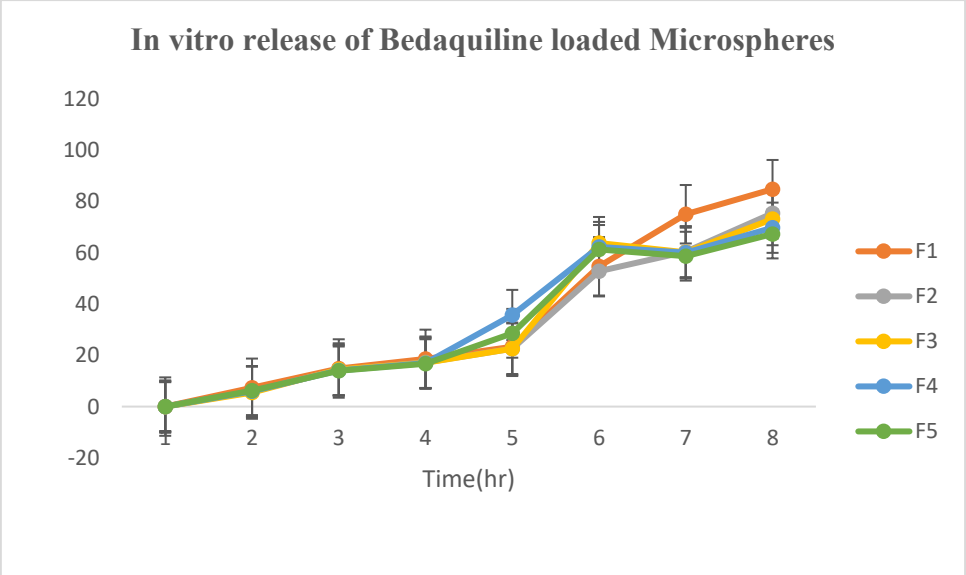

Figure 2: Cumulative release of Bedaquiline loaded Microspheres (mean ± SD, *n* = 3).

Kinetic studies

The corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, and Higuchi, respectively (Table 2) to describe the release kinetics of all five formulations. Higher R2 (coefficient of correlation) values indicate that the drug release from all formulations follows Zero-order release and the Higuchi model. Since it was confirmed as the Higuchi model, swelling and diffusion controlled the release mechanism. The Peppas model is widely used to confirm whether the

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release mechanism is Fickian diffusion, non-Fickian diffusion or zero-order. 'n' (release exponent of Korsemeyer- Peppas model) value could be used to characterize different release mechanisms. The 'n' values for all formulations were not less than 0.50, indicating that the release approximates the non-Fickian diffusion mechanism.

Stability studies

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

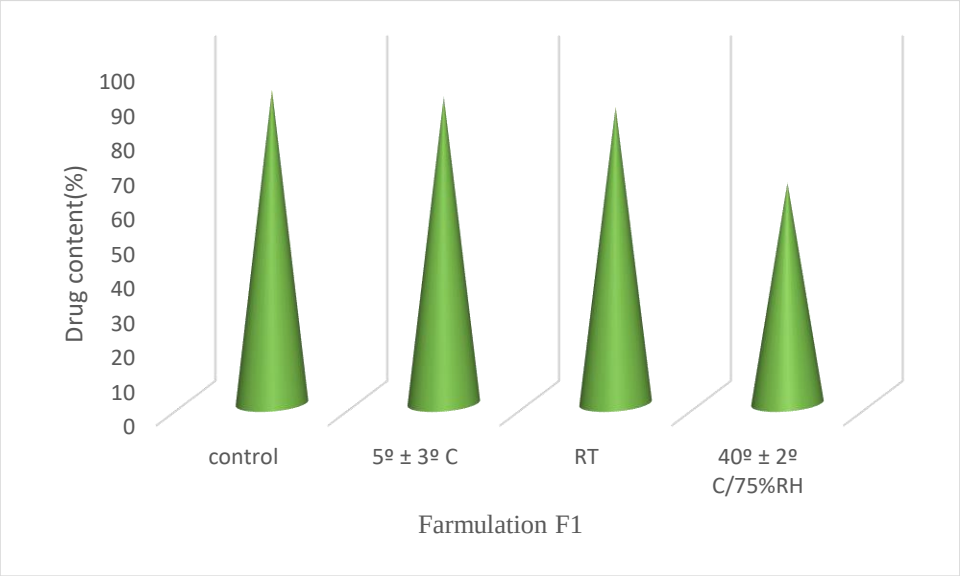

Figure 3: Stability study: comparison of drug content of formulation F1 at 4˚C, room temperature and 40 ± 2˚C/ 75% RH.

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

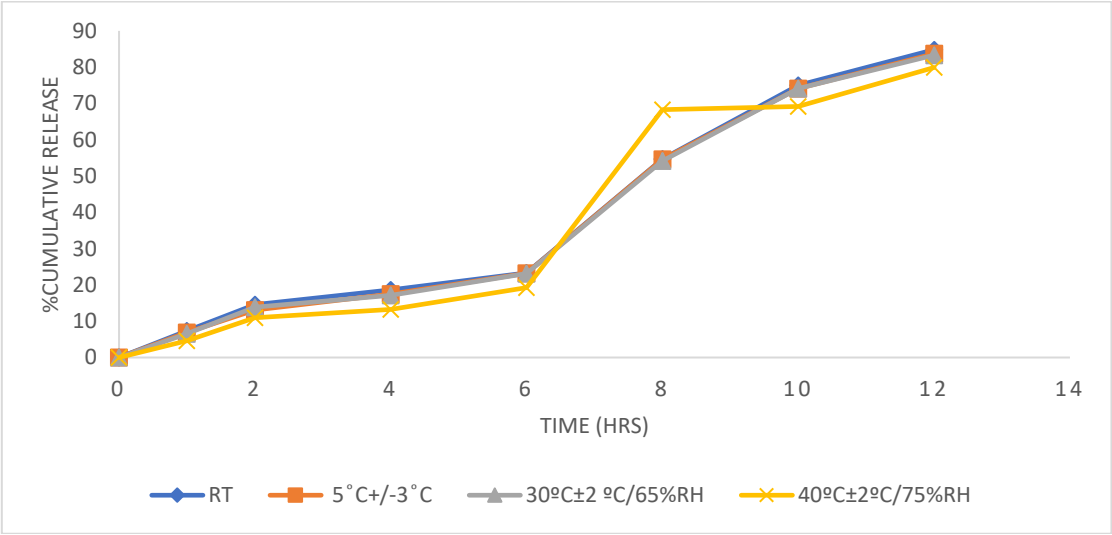

Figure 4: Stability study: comparison of in vitro drug release profile for Formulation F1 at 4˚C, room temperature and 40 ± 2˚C/75% RH after three months storage (*n* = 3).

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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	7.335	0.8654	92.6	1.966
2	0.3010	1.4142	14.85	1.1719	85.15	1.9301
4	0.6020	2	18.6206	1.2699	81.38	1.910
6	0.7781	2.4494	23.322	1.3677	76.67	1.884
8	0.9030	2.8284	54.733	1.7382	45.26	1.655
9	0.9542	3	75.047	1.8753	24.96	1.3972
12	1.0791	3.4641	84.82	1.9284	18.18	1.1812

Table 3: Correlation coefficients according to different kinetic equations of Ideal formulation (F1).

Formulation	F1
Cumulative drug release (%)	84.1
Zero order (r^2)	0.9356
First order (r^2)	0.877
Higuchi plot (r^2)	0.8157
Peppas plot (r^2)	0.826
'n' values	1.32

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 4°C and Room temperature. Still, there was a significant decrease in drug content when the formulation w s stored at 40 ± 2°C/75 RH because there might be chances for drug degradation that decreased the drug release at the higher temperature.

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 maximum drug content, and the closest in vitro release to previous data was found for F1 stored at 4°C and room temperature. The solubility and dissolution of the microspheres were improved significantly compared with its physical mixture and a pure sample of Bedaquiline. Stability results showed that prepared microspheres were stable for 3 months as per ICH guidelines. Hence, from the above result, Bedaquiline microspheres are a valuable technique to improve the solubility and dissolution of a poorly water-soluble drug-like Bedaquiline.

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