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PREPARATION AND EVALUATION OF BEDAQUILINE LOADED MICROSPHERES BY TWO DIFFERENT TECHNIQUES

J. Adlin Jino Nesalin and Manu Kumar M. S.*[1]

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

***Corresponding Author: Manu Kumar M. S.**
Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar-571422, Maddur Taluk, Mandya District, Karnataka, India.

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ABSTRACT
The present study is an attempt to prepare and evaluate Microspheres of Bedaquiline by using ethyl cellulose and Chitosan as polymers for treating Tuberculosis. The Bedaquiline loaded Microspheres were prepared by ionic gelation technique and solvent evaporation method. The drug and physical mixture were characterized by FTIR, their results indicates that there was no interaction between the drug and polymer. Changing the polymer concentration ratio will significantly affect the in-vitro drug release. The formulated Microspheres were evaluated for solubility, percentage yield, scanning electron microscopy, particle size, and in-vitro release. Among ten formulations FS5 of solvent evaporation method and F1of ionic gelation technique were selected as ideal formulation. The short-term stability studies on the optimized formulations indicate that there were slight physical or chemical changes in the formulations during the 3-month study period time. According to the data obtained from Bedaquiline loaded microspheres. Comparative study of two different methods shows that Ionic gelation method is better alternative to solvent evaporation method.

KEYWORDS: Microspheres, Tuberculosis, Chitosan, Ethyl cellulose, Bedaquiline, Methods.

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] Bedaquiline shows poor bioavailability when administered orally, major reason for poor bioavailability is poor drug solubility characteristics, followed by its first pass metabolism.

Hence, the objective of the work was to formulate Chitosan and Ethyl cellulose Microspheres containing Bedaquiline by Solvent evaporation method and 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, Ethyl cellulose, 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

Ionic gelation technique

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]

Solvent evaporation method

This is the method widely used in the microencapsulation process. Concisely the polymer ethyl cellulose was dissolved in methanol to get a clear solution. The drug Bedaquiline was added and dissolved in the polymer solution. The resultant mixture was then stirred at 900 rpm for 1 hour to evaporate the volatile substance. The formed microspheres were collected and air dried for 3 hours and stored in desiccator for further use.[12]

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.[13]

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.[14]

Practical yield

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

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

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.[15]

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

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.[16]

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 37°C. 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). R**2** (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 batch F1 and FS5. Formulation F1and FS5 was divided into 3 sets of samples and stored at 5±2˚C in a refrigerator, room temperature, 40± 2˚C/75% RH in humidity control ovens. After 3months drug content of all samples was determined by the method as in drug content. In vitro release study of formulation, F1 and FS5 was also carried out after 3months 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. Ethyl cellulose Microspheres are obtained by solvent evaporation method which is a simple process, 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.

Microspheres prepared by Solvent evaporation technique and ionic gelation technique were found to be discrete and through SEM analysis (Fig. 1), (fig. 2) their mean size distribution was found to be 344 µm and 409 µm. 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 reaching a reduction in the dose delivered.

The drug entrapment efficiency of Microspheres containing drug: polymer in various ratios of F1 1:1, 1:2, 1:3, 1:4 and 1:5 was found to be 90%, 77%, 72%, 68.5%, 65% (Table 1) and. Thus, there was a steady increase in the entrapment efficiency by decrease 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 be 1.3 mV, which indicates that they are stable. And FS5 1:1, 1:2, 1:3, 1:4 and 1:5 was found to be 77%, 54.55%, 53%, 76%, 82% (Table 2). Thus, there was a steady increase in the entrapment efficiency on increasing 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 all formulated Microspheres was found to be 1.6mV, 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	77± 0.23	243± 5.04
2	F2	1:2	74± 0.56	289 ± 4.2
3	F3	1:3	53.1± 0.58	344± 8.9
4	F4	1:4	76± 0.42	409± 10.5
5	F5	1:5	82± 0.36	486± 10.7

Mean ± SD (*n* =3). FS1, FS2, FS3, FS4 and F5 represent formulations 1 to 5, respectively, etc.

Table 2: 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.

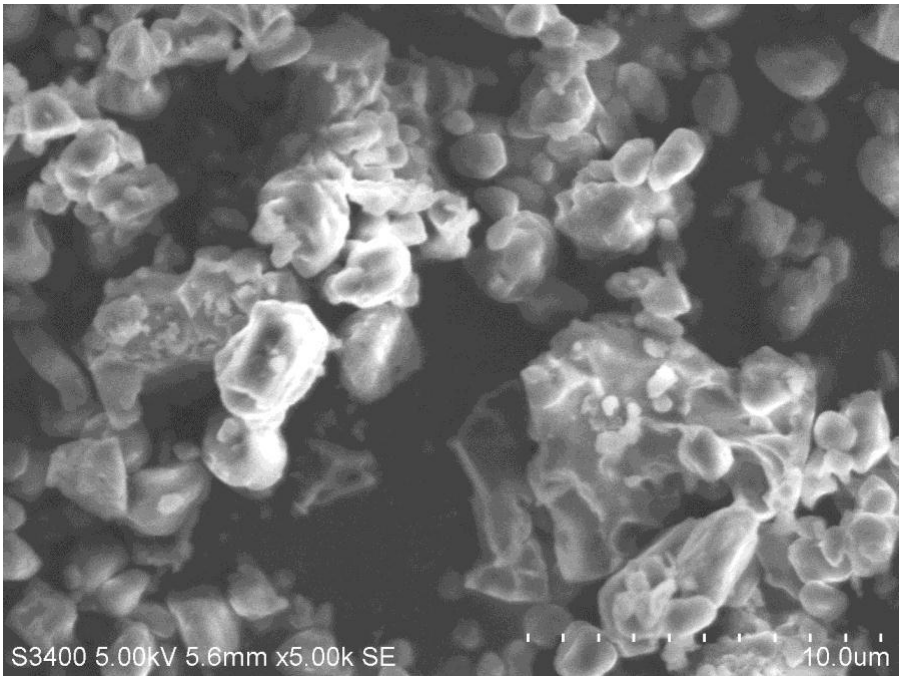

Fig. 1. SEM of formulation FS5.

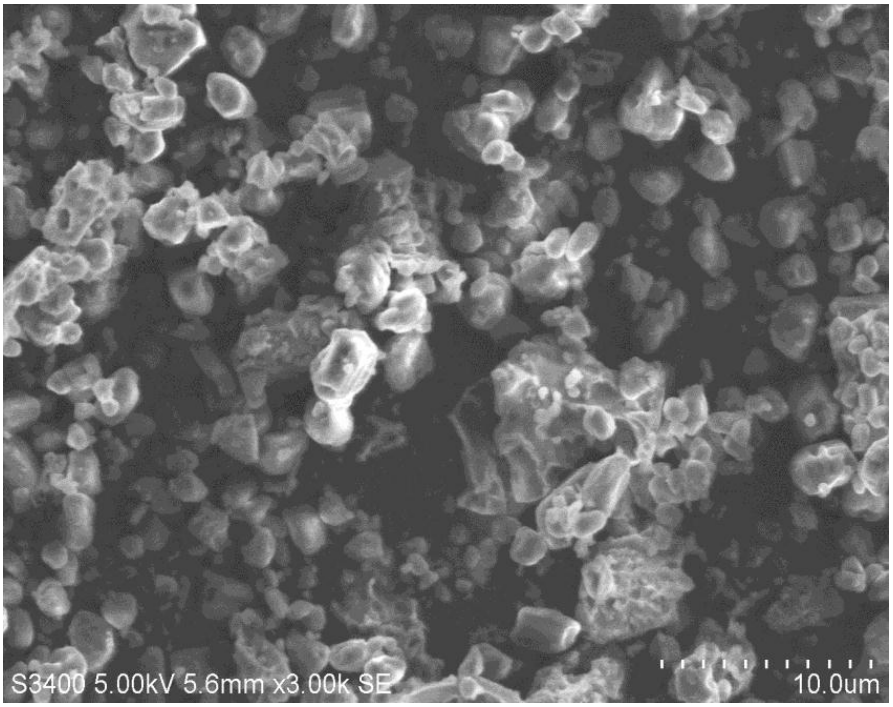

Fig. 2. SEM of formulation F1.

In vitro release of Microspheres

Cumulative percentage drug released for FS1, FS2, FS3, FS4 and FS5 after 24 h were found to be 32.16%, 39.68%, 40.43%, 42.88%, and 54.16%, respectively (Fig.3). It was apparent that in vitro release of Bedaquiline showed a slow initial burst, and then followed by Increase drug release. An initial, slow release suggests that some drug was localized on the surface of the Microspheres. And 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.4). 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.

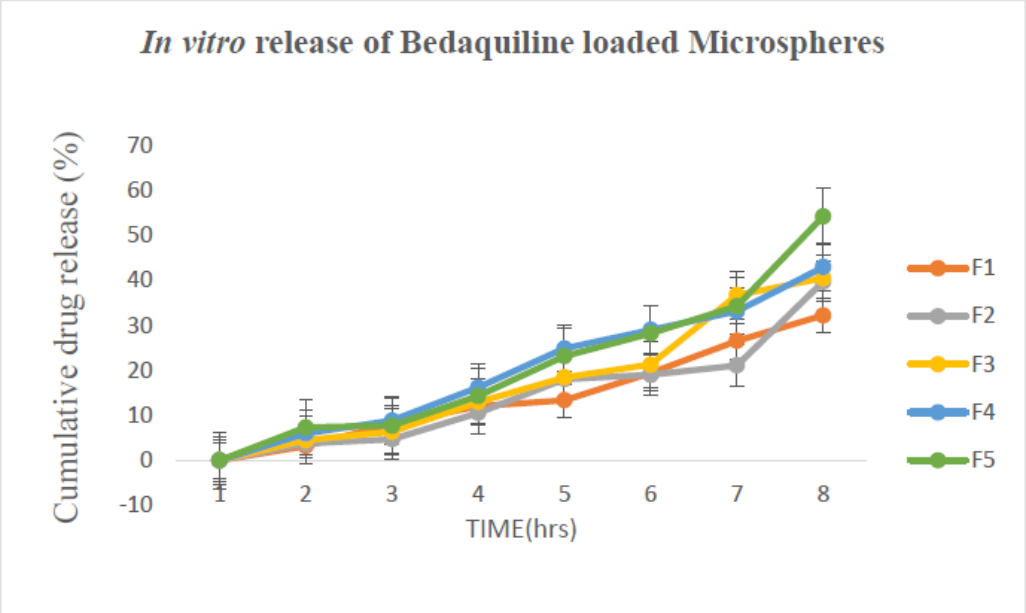

Fig. 3. Cumulative release of Bedaquiline loaded Microspheres (FS1-FS5) (*n*=3)

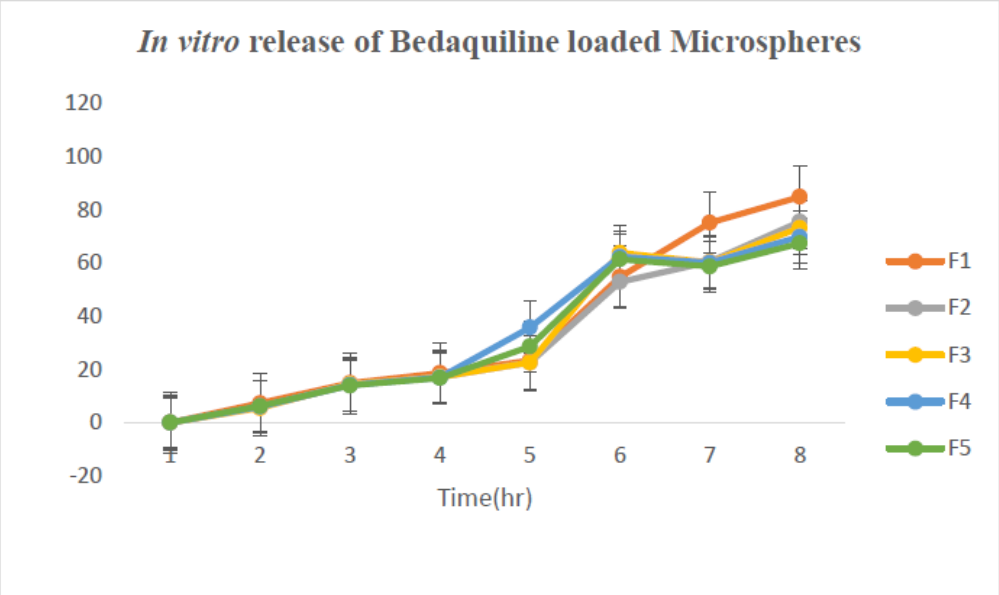

Fig. 4. Cumulative release of Bedaquiline loaded Microspheres(F1-F5) (mean ± SD), (*n* = 3).

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 3) (Table 4). As indicated by higher R2 (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 Korsemeyer- Peppas model) value could be used to characterize different release mechanisms. The 'n' values was were found to be more than 0.89. Which indicates indicates that the super case 2 II transport mechanism where drug release release occurs by swelling and relaxation of polymer chains.

Stability studies

The results of drug content of ideal formulation FS5 and F5 after 3 month of stability testing at different storage conditions were shown in (Fig. 5) and (Fig. 6) In vitro release profiles for the same formulation stored at different condition.

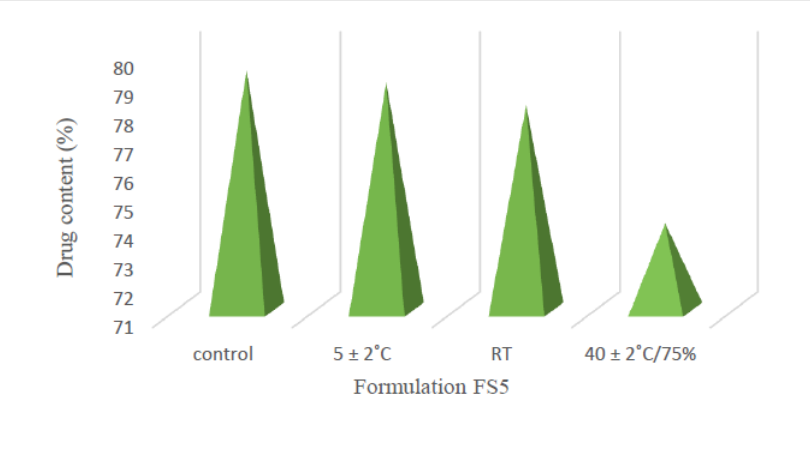

Fig. 5. Stability study: comparison of drug content of formulation FS5 at 5±2˚C, room temperature, and 40 ± 2˚C/ 75% RH.

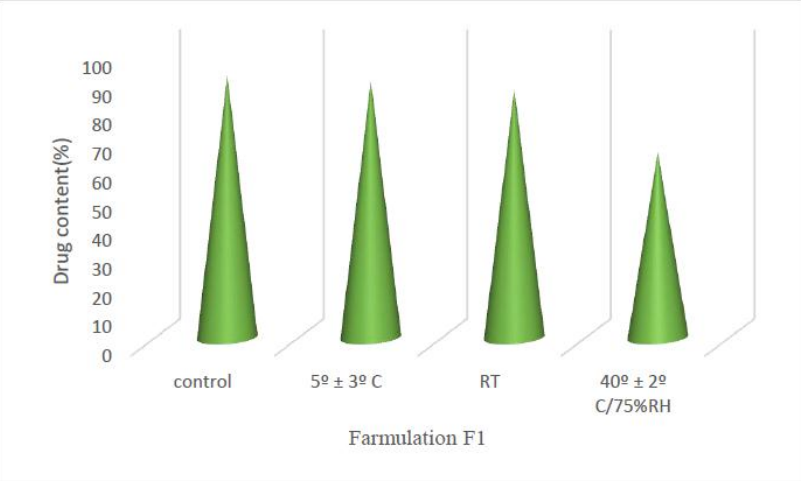

Fig. 6. Stability study: comparison of drug content of formulation F1 at 5±2°C, room temperature and 40 ± 2˚C/ 75% RH.

The results of drug content of ideal formulation FS5 And F1 after 3 month of stability testing at different storage conditions were shown in Fig. 7. In vitro release profiles for the same formulation stored at different storage conditions were also showed in Fig. 8

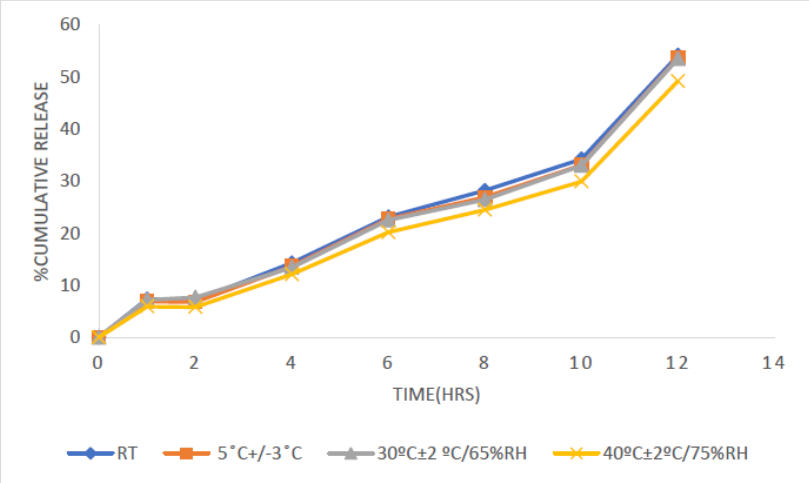

Fig. 7. Stability study: comparison of *in vitro* drug release profile for Formulation FS5 at 5±2˚C, room temperature (32˚C) and 40 ± 2˚C/75% RH after three months storage.

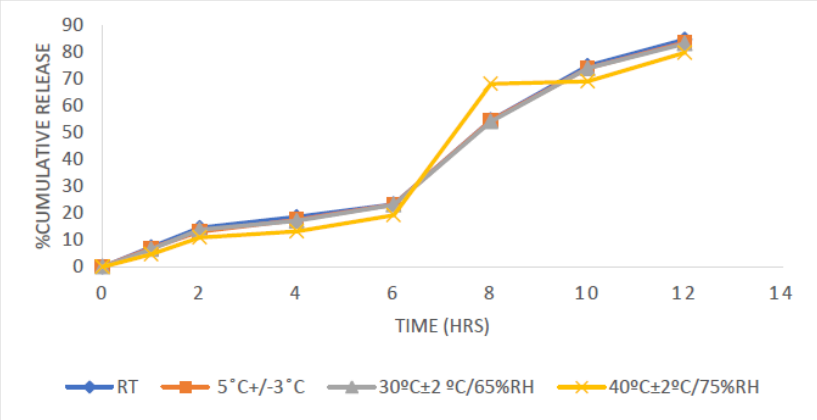

Fig. 8. Stability study: comparison of *in vitro* drug release profile for Formulation F1 at 5±2˚C, room temperature and 40 ± 2˚C/75% RH after three months storage (*n* = 3).

Table 3: Correlation coefficients according to different kinetic equations (FS5).

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	92.66	1.966
2	0.3010	1.4142	7.11	0.887	92.28	1.964
4	0.6020	2	14.29	1.1550	85.706	1.933
6	0.7781	2.4494	23.13	1.3641	76.866	1.8857
8	0.9030	2.8284	28.2	1.450	71.77	1.856
9	0.9542	3	34.231	1.534	65.76	1.817
12	1.0791	3.4641	54.16	1.7337	45.84	1.6612

Table 4: Correlation coefficients according to different kinetic equations(F1)

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

Formulation	FS5	F1
Cumulative drug release (%)	54.16	84.1
Zero order (r2)	0.9407	0.9356
First order (r2)	0.8992	0.877
Higuchi plot (r2)	0.8512	0.8157
Peppas plot (r2)	0.803	0.826
'n' values	1.125	1.32

On comparing this data with the previous data of FS5, it was observed that there was a slight decrease in drug content when the formulation was stored at 5±2°C and Room temperature, but there was significant decrease in drug content when the formulation was stored at 40 ± 2˚C/75 RH because at higher temperature, there might be chances for drug degradation that decreased the drug release. And F1, it was observed that there was a slight decrease in drug content when the formulation was stored at 5±2˚C and Room temperature. Still, there was a significant decrease in drug content when the formulation was 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

The present study demonstrated the successful preparation of Microspheres of the Bedaquiline with ethyl cellulose and Chitosan polymer was prepared by the solvent evaporation method and Ionic gelation

method. Based on Solubility, drug entrapment efficiency, drug content, zeta potential, particle size morphology and in-vitro release formulation FS5 and F1 were selected as optimized formulations. And by comparing both optimized formulation the F1 formulation shows good results in all evaluation tests than FS5 formulation. Hence the ionic gelation technique was considered to be the best method to prepare Bedaquiline microspheres since it improves the solubility and dissolution of a poorly water-soluble drug-like Bedaquiline.

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