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International Journal of Pharmaceutics 304 (2005) 178–184
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Development of sustained release gastroretentive drug delivery system for ofloxacin: In vitro and in vivo evaluation

Mahesh Chavanpatil *, Paras Jain, Sachin Chaudhari, Rajesh Shear, Pradeep Vavia

Mumbai University Institute of Chemical Technology, Pharmaceutical Sciences Division, Near Nathalal Parikh Marg, Matunga, Mumbai 400019, India

Received 5 June 2005; received in revised form 18 August 2005; accepted 22 August 2005

Abstract

Sustained release (SR)-gastroretentive dosage forms (GRDF) enable prolonged and continuous input of the drug to the upper parts of the gastrointestinal (GI) tract and improve the bioavailability of medications that are characterized by a narrow absorption window. A new strategy is proposed for the development of gastroretentive dosage forms for ofloxacin preferably once daily. The design of the delivery system was based on the sustained release formulation, with floating and swelling features in order to prolong the gastric retention time of the drug delivery systems. Different polymers, such as psyllium husk, HPMC K100M, crospovidone and its combinations were tried in order to get the desired sustained release profile over a period of 24 h. Various formulations were evaluated for buoyancy lag time, duration of buoyancy, dimensional stability, drug content and in vitro drug release profile. It was found that dimensional stability of the formulation increases with the increasing psyllium husk concentration. It was also found that in vitro drug release rate increased with increasing amount of crospovidone due to the increased water uptake, and hence increased driving force for drug release. The optimized formulation was subjected to stability studies at different temperature and humidity conditions as per ICH guidelines. In vivo studies were carried out for the optimized formulation in 24 healthy human volunteers and the pharmacokinetic parameters of developed formulations were compared with the marketed once daily (Zanocin) formulation. Based on the in vivo performance in a parallel study design in healthy subjects, the developed formulation shows promise to be bioequivalent to the marketed product (Zanocin). The percent relative bioavailability of developed formulation was found to be 97.55%.

Keywords: Ofloxacin; Sustained release; Psyllium husk; Gastroretentive

1. Introduction

Oral sustained release (SR)-dosage forms (DFs) have been developed for the past three decades due to their considerable therapeutic advantages (Hoffman, 1998). However, this approach has not been suitable for

* Corresponding author. Present address: Eugene Applebaum College of Pharmacy, 259, Mack Avenue, #3250, Detroit, MI 48201, USA. Tel.: +1 313 577 8892.
E-mail address: mcp_112000@rediffmail.com (M. Chavanpatil).

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doi:10.1016/j.ijpharm.2005.08.009

a variety of important drugs, characterized by a narrow absorption window in the upper part of the gastrointestinal tract, i.e. stomach and small intestine. This is due to the relatively short transit time of the DF in these anatomical segments. Thus, after only a short period of less than 6 h, the SR-DF has already left the upper gastrointestinal tract and the drug is released in non-absorbing distal segments of the gastrointestinal tract. This results in a short absorption phase that is often accompanied by lesser bioavailability.

It was suggested that compounding narrow absorption window drugs in a unique pharmaceutical DF with gastroretentive properties would enable an extended absorption phase of these drugs. After oral administration, such a DF would be retained in the stomach and release the drug there in a sustained manner, so that the drug could be supplied continuously to its absorption sites in the upper gastrointestinal tract. This mode of administration would best achieve the known pharmacokinetic and pharmacodynamic advantages of SR-DFs for these drugs (Hwang et al., 1998; Hoffman and Stepensky, 1999).

The need for gastroretentive dosage forms (GRDFs) has led to extensive efforts in both academia and industry towards the development of such drug delivery systems (Deshpande et al., 1996). These efforts resulted in GRDFs that were designed in large part based on the following approaches: (a) low density form of the DF that causes buoyancy above gastric fluid (Singh and Kim, 2000); (b) high density DF that is retained in the bottom of the stomach; (c) bioadhesion to the stomach mucosa (Moes, 1993); (d) slowed motility of the gastrointestinal tract by concomitant administration of drugs or pharmaceutical excipients (Rubinstein and Friend, 1994); (e) expansion by swelling or unfolding to a large size which limits emptying of the DF through the pyloric sphincter (Mamjek and Moyer, 1980).

The objective of present work was to develop gastroretentive formulation, which releases drug in the stomach and upper gastrointestinal (GI) tract, and form an enhanced opportunity of absorption in the stomach and upper GI tract rather than the lower portions of the GI tract. Example of substance whose bioavailability is strongly dependent on the local physiology in the GI tract and which preferably is absorbed in the higher sections of the intestine is ofloxacin. Ofloxacin is readily soluble in the acidic environment of the stomach. In the intestine, where neutral to slightly alkaline pH conditions prevail; however, precipitation of the active compound occurs, which adversely affects absorption in the lower sections of the intestine. There is a need for systems that reside in the stomach over a relatively long time and release the active compound there in a sustained manner (Sen and Kshirsagar, 2002). This necessitated the design and development of sustained release gastroretentive drug delivery system for ofloxacin using suitable polymers.

2. Materials and methods

2.1. Materials

Ofloxacin and psyllium husk were gifted by Macleoid Pharmaceuticals, India. HPMC K100M, PVP K30 and crospovidone were obtained as gift samples from M/s Rohm Pharma, Germany. Talc and magnesium stearate were gifted by M/s Bayer India Ltd., India. All other solvents and reagents were purchased from Ranbaxy chemicals, India, and were of analytical grade.

2.2. Methods

2.2.1. General description of the manufacturing process for sustained release formulation of ofloxacin

Typical sustained release formulations of ofloxacin are listed in Tables 1 and 2. Tablets were made by using psyllium husk (gelling agent), HPMC K100M (hydrophilic polymer), crospovidone (swelling agent), sodium bicarbonate (gas-generating agent) and beta-cyclodextrin (channeling agent). Tablets were made by using wet granulation process with PVP K30 (5%, w/v, isopropyl alcohol). Compression was done on a Cad-

Table 1
Formulation composition to study the effect of psyllium husk and HPMC K100M on in vitro release of ofloxacin

Composition (mg/tablet)	F1	F2	F3	F4	F5
Ofloxacin	400	400	400	400	400
Psyllium husk	75	100	125	100	100
HPMC K100M	40	40	40	30	50
Sodium bicarbonate	70	70	70	70	70
Crospovidone	200	200	200	200	200
PVP K30 (5% in IPA)	20	20	20	20	20

Table 2
Formulation composition to study the effect of crospovidone, sodium bicarbonate and betacyclodextrin on in vitro release of ofloxacin

Composition (mg/tablet)	F6	F7	F8	F9	F10	F11
Ofloxacin	400	400	400	400	400	400
Psyllium husk	100	100	100	100	100	100
HPMC K100M	40	40	40	40	40	40
Sodium bicarbonate	70	70	60	80	70	70
Crospovidone	0	100	200	200	200	200
PVP K30 (5% in IPA)	20	20	20	20	20	20
Betacyclodextrin	–	–	–	–	50	100

mach single station tablet press using caplet shaped punches.

2.2.2. In vitro release study

The release of ofloxacin from the tablets was studied using USP dissolution Apparatus I. The dissolution medium was phosphate buffer pH 1.2 for first 2 h, phosphate buffer pH 4.5 for next 2 h and pH 7.4 for remaining hours (Wang, 1978), the volume being 900 ml. The temperature was maintained at $37 \pm 0.5\,^{\circ}C$. The rotation speed was 100 rpm. Five milliliters of aliquot were withdrawn at predetermined time intervals of 1, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 24 h. The medium was replenished with 5 ml of fresh buffer each time. Sample was analyzed by using UV spectrophotometry at 291 nm.

2.2.3. Buoyancy lag time and the duration of buoyancy

The buoyancy lag time and the duration of buoyancy were determined in the USP dissolution Apparatus II in an acid environment. The time interval between the introduction of the tablet into the dissolution medium and its buoyancy to the top of dissolution medium was taken as buoyancy lag time and the duration of buoyancy was observed visually (Yang et al., 1999).

2.2.4. Dimensional stability

The dimensional stability of the formulations was studied using USP dissolution Apparatus II. The dissolution medium was phosphate buffer pH 1.2 for first 2 h, phosphate buffer pH 4.5 for next 2 h and pH 7.4 for remaining hours, and the volume being 900 ml. The temperature was maintained at $37 \pm 0.5\,^{\circ}C$. The rotation speed was 50 rpm. The dimensional stability of ofloxacin formulations was observed visually.

2.2.5. Bioequivalence studies

A total of 24 volunteers participated in the study. The volunteers were fasted overnight at least 10 h prior to dosing with water ad lib. Twelve volunteers were given the test formulation (F11) and 12 the marketed formulation (Zanocin) with water (parallel study design). At predetermined time interval, 5 ml of blood was withdrawn at 0, 2, 4, 6, 8, 10, 12, 18 and 24 h. Blood samples were collected from forearm vein using sterile disposable needle and syringe into sterile glass centrifuge tubes containing heparin. The samples were centrifuged immediately at 4000 rpm and the plasma was stored in light protected container at $-20\,^{\circ}C$ till the time of analysis.

The concentration of ofloxacin from plasma was measured by validated reversed phase HPLC. HPLC method was validated for linearity, accuracy, precision and extraction efficiency. To 0.5 ml of plasma, 2 ml of acetonitrile was added and vortexed for 5 min. This was centrifuged at 4000 rpm for 10 min. The supernatant was filtered through 0.45 μm filter and this solution was stored at $-20\,^{\circ}C$ till HPLC analysis. There was no drug loss due to the adsorption of drug on 0.45 μm filter during filtration process. The filtered solution was injected onto the HPLC column (Waters C_8 spherisorb, 5 μm, 250 mm); the signal was monitored at 293 nm. Mobile phase was prepared with methanol:acetonitrile:water containing 1.5 g phosphoric acid/l (5:5:80, v/v/v). Tetrabutyl ammonium hydrogen sulphate (0.2%) was used as an ion-pairing reagent in the mobile phase. The flow rate was maintained at 1.5 ml/min. The parameters employed to evaluate were C_{max}, t_{max}, K_{el} and $t_{1/2}$ values. C_{max} and t_{max} were read directly from the observed mean drug plasma concentration against time profile. K_{el} and $t_{1/2}$ were computed from observed mean plasma concentration against time profile. The extent of absorption from the test formulation relative to the marketed one was calculated as the relative bioavailability by using the formula given below:

$$\text{relative bioavailability} = \frac{\text{AUC}_{(0-\infty)} \text{ of test}}{\text{AUC}_{(0-\infty)} \text{ of marketed}} \times 100$$

3. Results and discussion

3.1. Effect of psyllium husk on in vitro drug release and integrity of formulations

Effect of different concentrations of psyllium husk on in vitro release of ofloxacin was studied. Initially, tablet containing 75 mg of psyllium husk (F1) could not retain its physical integrity for desired period (24 h) of time. As the concentration of psyllium husk increases (F2 and F3), it retains integrity up to desired period of time (24 h). It is important to maintain the physical integrity of the tablet in case of once daily formulations. If it does not maintain its physical integrity, tablet could be broken down into smaller fragments and escape from the upper part of the GI tract. Hence, an attempt was made in order to increase the physical integrity of the tablets using psyllium husk. Further, formulation (F1) provided higher burst drug release as compared to the marketed formulation as shown in Fig. 1. In case of F1 and marketed formulations, burst drug release after 2 h was 34.06 ± 0.52 and 31.08 ± 0.89%, respectively. Therefore, amount of psyllium husk was increased to 100 (F2) and 125 mg/tablet (F3). As the concentration of psyllium husk increases, initial burst drug release as well as drug release in the latter hours decreases as compared to the marketed formulation. In case of F2 and F3 formulations, burst drug release after 2 h was found to be 28.22 ± 0.96 and 24.22 ± 1.28%, respectively. In case of formulation F1 and F3, cumulative drug release at the end of 24 h was found to be 53.60 ± 1.86 and 47.20 ± 1.74%, respectively. This might be due to the gelling properties of psyllium husk. Psyllium husk forms thick gel at higher concentrations, which could have contributed to the decrease in drug release. Drug associated with the surface of tablet matrix could have also contributed to the initial burst release. As the surface associated drug was released, psyllium husk matrix could have contributed to the slower drug release over a period of 24 h. Formulation (F2) containing 100 mg of psyllium husk maintained its physical integrity for 24 h and showed similar pattern of drug release as compared to the marketed formulation, hence, selected for the further studies.

Ofloxacin is soluble in aqueous solution with pH between 2 and 5. It is sparingly to slightly soluble in aqueous solution with pH 7. Hence, developed formulations as well as marketed formulation could not release total amount of the drug into the dissolution medium by pH change method.

3.2. Effect of HPMC K100M on in vitro release of ofloxacin

Initially, HPMC K100M was tried in concentration of 30 mg/tablet (F4). The formulation provided higher burst drug release as compared to marketed formulation as shown in Fig. 2. In case of F4 and marketed formulations, burst drug release after 2 h was found to be 34.08 ± 0.92 and 31.08 ± 0.89%, respectively.

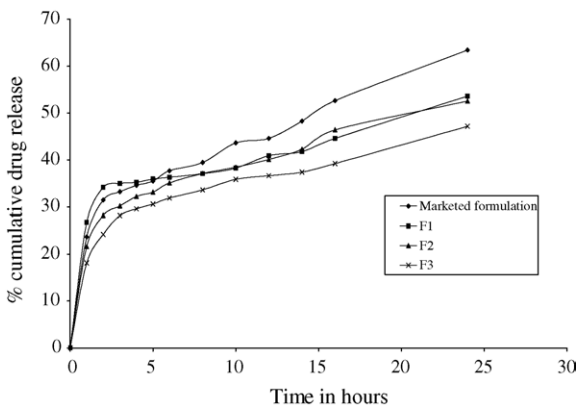

Fig. 1. Effect of psyllium husk concentration on in vitro drug release of ofloxacin ($n = 6$). Standard deviation was found to be less than 2% in all the in vitro drug release profiles.

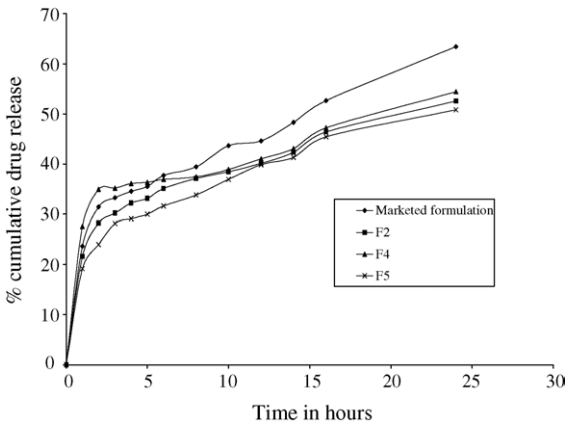

Fig. 2. Effect of HPMC K100M concentration on in vitro drug release of ofloxacin ($n = 6$). Standard deviation was found to be less than 2% in all the in vitro drug release profiles.

Therefore, amount of HPMC K100M was increased to 40 mg/tablet (F2), the formulation provided burst drug release comparable to the marketed formulation. Further increase in amount of HPMC K100M to 50 mg (F5) provided low burst drug release as compared to the marketed tablets. Formulation (F5) showed 24.01 ± 1.23% of burst drug release at the end of 2 h. High HPMC K100M content results in a greater amount of gel being formed. This gel increases diffusion path length of the drug. Its viscous nature also affects diffusion coefficient of the drug. As a result, reduction in drug release is obtained. Thus, 40 mg (F2) HPMC K100M was selected for further studies.

3.3. Effect of crospovidone on in vitro release of ofloxacin

In order to improve the release profile of the formulations, concentration of crospovidone was increased. Crospovidone acts as a swelling agent, which is capable of swelling greater than its original volume and preferably to at least twice its original volume when coming into contact with an aqueous fluid, such as gastrointestinal fluid. The swelling agent, which normally swells to several times its original volume in water, exhibits a controlled swelling in the presence of water-soluble hydrophilic polymers (Talwar and Staniforth, 2001, WO 01/64183). Three concentrations 0 mg (F6), 100 mg (F7) and 200 mg (F2) of crospovidone were tried. As the concentrations of crospovidone increases from 0 to 200 mg/tablet, drug release has increased as shown in Fig. 3. Formulation (F6), which does not contain crospovidine, showed 38.71 ± 1.64% of cumulative drug release at the end of 24 h. Formulation containing 100 mg (F7) showed 49.64 ± 1.32% cumulative drug release whereas formulation containing 200 mg (F2) showed 52.55 ± 1.02% cumulative release at the end of 24 h. As the concentration of crospovidone increases, water uptake capacity of the formulation increases (data not shown). This increases the porosity of the matrix, which results in increased drug release from the matrix system.

3.4. Effect of gas-generating agent on in vitro release of ofloxacin

As the concentration of sodium bicarbonate increases from 60 to 80 mg/tablet, drug release

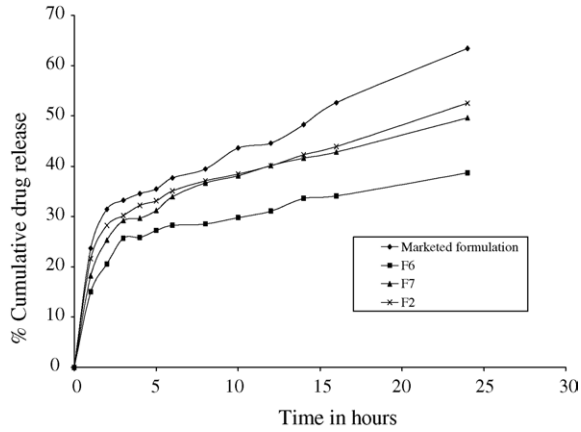

Fig. 3. Effect of crospovidone concentration on in vitro drug release of ofloxacin ($n = 6$). Standard deviation was found to be less than 2% in all the in vitro drug release profiles.

decreases as shown in Fig. 4. This might be due to the alkaline nature of sodium bicarbonate. Sodium bicarbonate creates an alkaline environment around the tablet. Ofloxacin is less soluble in alkaline environment that decreases the drug release from the formulation. Formulation containing 60 mg sodium bicarbonate (F8) showed 54.91 ± 1.30% cumulative drug release whereas formulation containing 80 mg of sodium bicarbonate (F9) showed 47.20 ± 1.35% cumulative drug release at the end of 24 h. Formulation containing 70 mg sodium bicarbonate (F2) showed

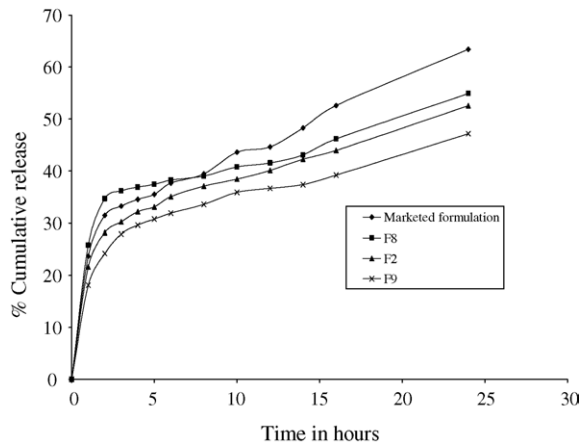

Fig. 4. Effect of sodium bicarbonate concentration on in vitro drug release of ofloxacin ($n = 6$). Standard deviation was found to be less than 2% in all the in vitro drug release profiles.

Table 3
Effect of sodium bicarbonate concentration on onset and duration of floating

Amount of sodium bicarbonate (mg)	Onset of floating (s)	Duration of floating (h)
60	40 ± 3	18
70	30 ± 4	24
80	25 ± 3	24

52.55 ± 1.02% cumulative drug release at the end of 24 h.

In such systems, sodium bicarbonate acts as a gas-generating agent. It generates gas when it comes into contact with an acidic environment of the stomach. This gas entraps into the matrix of water-soluble polymers and the formulation floats in an acidic environment of the stomach.

As the concentration of sodium bicarbonate increases from 60 (F8) to 80 mg/tablet (F9), floating lag time has reduced to 25 s. As the concentration of sodium bicarbonate increases from 60 to 80 mg/tablet, duration of floating has also been increased from 18 to 24 h as shown in Table 3. This might be due to the gas generated by 60 mg sodium bicarbonate might not be sufficient to keep formulation floating for prolonged period of time whereas in case of gas generated by 80 mg sodium bicarbonate was sufficient to keep formulation floating for 24 h.

3.5. Effect of betacyclodextrin on in vitro release of ofloxacin

In order to improve the drug release profile from the formulation, betacyclodextrin was added in to the formulation. As the concentration of betacyclodextrin increases from 0 to 100 mg/tablet, initial burst releases as well as drug release in the latter hours have been increased as shown in Fig. 5. In case of formulation containing 0 mg (F2) and 50 mg betacyclodextrin (F10), cumulative drug release after 24 h was 52.55 ± 1.02 and 57.90 ± 1.32%, respectively. The formulation containing 100 mg betacyclodextrin/tablet (F11) showed similar drug release as that of marketed formulation. Betacyclodextin dissolves rapidly from the tablet matrix into the medium and it creates porosity to the matrix, which results in increase in drug release from the tablet matrix. In vitro drug release studies were also carried out in pH 1.2 phosphate buffer, developed as well as marketed formulations released the total amount of the drug in pH 1.2 phosphate buffer for 24 h (data not shown).

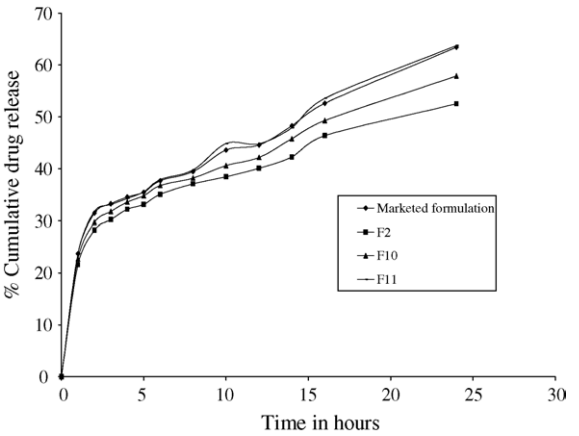

Fig. 5. Effect of betacyclodextrin concentration on in vitro drug release of ofloxacin ($n = 6$). Standard deviation was found to be less than 2% in all the in vitro drug release profiles.

3.6. Pharmacokinetic studies in healthy human volunteers

Pharmacokinetic studies were carried out in healthy human volunteers for test formulation (F11) and marketed once daily (Zanocin) formulation. C_{max} value for Zanocin was found to be 4.13 ± 0.487 μg/ml

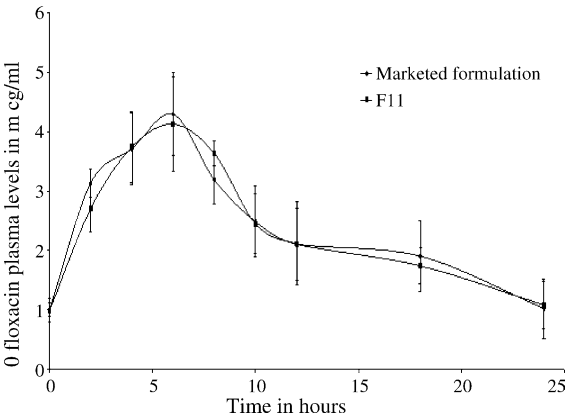

Fig. 6. Pharmacokinetic studies of marketed and developed formulation ($n = 12$).

whereas C_{max} value for F11 was found to be 3.87 ± 0.467 μg/ml as shown in Fig. 6. T_{max} values for both Zanocin and F11 formulation were found to be 6 h. AUC values for Zanocin and F11 were 64.48 ± 6.60 and 62.90 ± 6.65 μg/h ml, respectively. K_{el} values for Zanocin and F11 were 0.0737 ± 0.0056 and $0.00704 \pm 0.0057\ h^{-1}$. $T_{1/2}$ value for Zanocin was found to be 9.45 ± 0.718 whereas $T_{1/2}$ value for F11 was found to be 9.89 ± 0.899 h. The difference between the AUC values of F11 and marketed formulation were found to be insignificant ($P > 0.05$). The percent relative bioavailability of F11 formulation was found to be 97.55%.

4. Conclusion

We conclude that psyllium husk and HPMC K100M increases the dimensional stability of the formulations, which is necessary in case of once daily formulations. Sodium bicarbonate acts as a gas-generating agent, which is necessary in case of gastroretentive dosage forms. Crospovidone improved the drug release profile and swelling factor of psyllium husk based formulations. We also conclude that channeling agents, such as betacyclodextrin are useful to increase the initial burst release from psyllium husk based formulations. The optimized formulation was found to be stable at all the stability conditions. Based on the in vivo performance in a parallel study design in healthy subjects, the developed formulation shows promise to be bioequivalent to the marketed product (Zanocin).

Acknowledgements

We are thankful to University Grant Commission and NAFETEC, India, for providing financial assistance.

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