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Research Article

DESIGN AND DEVELOPMENT OF MULTIPARTICULATE DRUG DELIVERY OF METRONIDAZOLE FOR TARGETED DELIVERY TO COLON

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Abstract

The objective of the present study was to develop and evaluate a multiparticulate drug delivery system for colon targeted delivery of metronidazole. Metronidazole granules were prepared by wet granulation method using Eudragit RSPO followed by enteric coating with Eudragit S100. Solubility studies and drug excipients compatibility studies were carried out. The prepared granules were evaluated for drug content, percentage increase in weight and *in vitro* drug release. Drug content in all batches varied from 97.95% to 99.14%. All excipients were found to be compatible with the drug. Granules (D) containing 40% Eudragit RSPO showed optimum binding property. A significant difference of %CDR in phosphate buffer (pH 6.8) between the formulations was noticed with the least difference being seen in D4 (20% increase in weight after coating with Eudragit RSPO) as compared to D. ($P<0.01$). In addition D4 showed a sustained drug release with 98.99 % drug released at the end of 24 hours. Further formulation D4c (15% increase in weight after coating with Eudragit S100) did not or least release the drug (0.4116%) in stomach pH (0.1 N HCL) and upper parts of small intestine pH 6. Studies demonstrated that multiparticulate drug delivery system (D4c) can be used for the targeted delivery of metronidazole to the colon.

Keywords: Colon drug delivery, Eudragit RSPO, Eudragit S100, Metronidazole.

Introduction

Targeting of drugs to the colon is advantageous in the treatment of diseases like amoebiasis, ulcerative colitis, crohn's disease and colon cancer. In addition, it is of great potential for the oral delivery of peptides and proteins

like insulin, growth hormone etc., because of the favorable environment in colon in comparison to the upper gastrointestinal tract (Reddy et.al., 1999) along with the low diversity and intensity of the digestive enzymatic activities as well as the near neutral pH. Metronidazole is the drug of choice for treatment of intestinal amoebiasis caused by protozoan parasite *Entamoeba histolytica* (Tracy and Webster, 1996). The drug has to be delivered to the colon for its effective action against *E. histolytica* wherein the trophozoites reside in the lumen of the caecum and large intestine and adhere to the colonic mucus and epithelial layers (McCoy et al., 1994, Aminabhavi et al 2007). Conventional tablets of metronidazole provide minimal amount of drug for local action in the colon, still resulting in the relief of amoebiasis but with unwanted systemic side effects (Krishnaiah et al., 2002). Colonic delivery of metronidazole could prevent systemic side effects and subsequently a lower dose of the drug may be sufficient to treat protozoal infections (Sindhu et al., 2007). Recently, much emphasis is being laid on multiparticulate dosage forms over single unit preparations because of its advantages such as more uniform dispersion in the GI tract and uniform drug absorption, less inter- and intra- individual variability and more flexible formulation process. Because of their small particle size, multiparticulates can pass through the upper GI tract easily, they can reach the colon quickly, and are retained longer in the ascending colon (Davis et al., 1986). Therefore, a multiparticulate system has been selected for colon targeting of metronidazole. In addition, a sustained local action in the colon will help to reduce the dose and side effects. Hence, in this study a delayed and sustained release multiparticulate formulation in the form of granules using Eudragit polymers viz., RSPO (for the sustained effect) and S100 (for the delayed effect) was formulated and evaluated with respect to various *in vitro* evaluation tests.

Materials and Methods

Materials

Metronidazole was a kind gift sample from Lupin Research Park, Pune. Eudragit RSPO and Eudragit S100 were purchased from Rohm GmbH & Co, Mumbai. Triethyl citrate procured from Himedia Laboratories Pvt.Ltd. All other solvents and reagents used were of analytical grade.

Methods

Preformulation studies

Solubility studies of metronidazole: An excess quantity of metronidazole was taken in 10 mL of different buffer solutions in a shaking water bath (100 agitations/ min) for 24 h at room temperature. The solution was then passed through a whatmann (No. 1) filter and the amount of the drug dissolved was analyzed spectrophotometrically (UV-1601PC, Shimadzu, Tokyo, Japan) after suitable dilutions.

Drug-excipient compatibility studies

Differential scanning calorimetry (DSC) was performed using DSC-60 (Shimadzu, Tokyo, Japan) caloriemeter. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), thermal analyzer (TA 60) and operating software (TA 60). The samples (drug alone or mixture of drug and excipients) were heated in sealed aluminum pans under nitrogen flow (30 mL/min) at a scanning rate of 5 °C/min from 24±1 to 250 °C. Empty aluminum pan was used as a reference. The heat flow as a function of temperature was measured for the drug and drug-polymer mixture. The physical mixtures of drug with different excipients were also tested for compatibility studies by DSC analysis which were prepared by triturating drug and additives in a dried mortar for 5 min

Preparation of granules

Five batches of granules were prepared by wet granulation method to optimize the binder concentration. Eudragit RSPO was used as the sustained release excipient and ethanol as granulating agent. The compositions of the tablet formulations are given in Table 1.Dough mass were passed through sieve No: 10. After drying, the granules were passed through sieve No: 12 superimposed on sieve No: 16. The 12/16 fraction was taken to find out the optimum concentration of binder along with sustained release of drug.

Table-1: Composition of granule formulations.

Ingredients (mg)	A	B	C	D	E
Metronidazole	90	80	70	60	50
Eudragit RSPO	10	20	30	40	50

Coating of granules

The batch with optimized binder concentration was then coated with Eudragit RSPO to further sustain the drug release. The coating solution was prepared using Eudragit RSPO, Triethyl citrate, Talc and Ethanol. The composition of coating solution using Eudragit RSPO is given in Table 2. The coating solution was sprayed on to small batches of granules (2g) taken in a Petri dish, and being sprayed with the coating solution using a fine mist sprayer to obtain theoretical weight gains of 5%, 10%, 15% and 20% . The spraying was done with intermittent periods of drying followed by rotation of the granules. In between the coating solution was subjected to mixing using a cyclomixer to prevent the settling of talc particles. The granules optimized for sustained release property was then subjected to coating with Eudragit S100 which was to prevent the drug release in medium of less than pH of 7.0. The composition of coating solution using Eudragit S100 is given in Table 2. The same procedure as in the case of coating with Eudragit RSPO was followed.

Table-2: Composition of the coating solution using Eudragit RSPO and Eudragit S100.

Ingredients	Quantity	Quantity
Eudragit RSPO	4 g	-
Eudragit S100	-	4 g
Tri ethyl citrate	0.4 g (10% of Eudragit RSPO)	0.4 g (10% of Eudragit S100)
Talc	0.2 g (2% of Eudragit RSPO)	0.2 g (2% of Eudragit S100)
Ethanol	up to 100 ml	up to 100 ml

Evaluation of granules

Drug content of granules and weight of the granules

An amount of granules equivalent to 100 mg was taken and crushed. The drug content was determined using 100ml pH 6.8 buffer. It was filtered and analyzed spectrophotometrically at 320 nm. The percentage drug content was determined as per the following formula.

Percentage drug content = (Practical drug content x 100) ÷ Theoretical drug content

The practical percentage increase in weight of the granules coated with Eudragit RSPO and Eudragit S100 was calculated and tabulated

Theoretical percentage increase in weight= $\frac{A_w - O_w * 100}{O_w}$

Practical percentage increase in weight= $\frac{P_w - O_w * 100}{O_w}$

Where,

Aw= Anticipated weight after coating

Ow= Original weight before coating

Pw= Practical weight obtained after coating

Dissolution Studies

The *in vitro* dissolution study was carried out using USP Type I dissolution apparatus. The study was carried out in 900ml of phosphate buffer (pH 6.8) during the initial stages of study for 12- 24 hours. After coating with the enteric polymer Eudragit S100 the dissolution was carried out in phosphate buffer (pH 7.4) for 2 hours followed by phosphate buffer (pH 6.8) up to 12 hours in order to optimize the percentage coat of the enteric polymer which will prevent the release of the drug in the small intestine.

The final optimized formula was subjected to a test run in all the four dissolution media. The dissolution medium was kept in thermostatically controlled water bath, maintained at 37±0.5 °C. The paddle was lowered so that the lower end of the stirrer was 25 mm above from the base of the dissolution basket. A pre weighed amount of granules equivalent to 100 mg of the drug was introduced into the baskets. The basket was rotated at 100 rpm. At different time intervals, 5 ml sample was withdrawn and analyzed spectrophotometrically at 277 nm for 0.1N HCl and 320nm for the other phosphate buffers (pH 6.0, 7.4 and 6.8) for the drug release. At each time of withdrawal, 5 mL of fresh corresponding medium was replaced into the dissolution basket.

Results and Discussion

Solubility studies

The saturation solubility of metronidazole was found in all the four buffers used for the study. The result of solubility study of Metronidazole in various pH buffers is shown in Table 3. The solubility was highest in 0.1 N HCl and it decreased with increase in pH.

Table-3: Solubility of metronidazole in different solutions media.

Solutions	Solubility(mg/ml)
0.1N HCl	64.6 ± 0.014 mg/ml
Phosphate buffer pH 6.0	9.71 ± 0.020 mg/ml
Phosphate buffer pH 6.8	9.68 ± 0.016 mg/ml
Phosphate buffer pH 7.4	9.75 ± 0.025 mg/ml

All values are expressed as Mean ± SD, n=3.

Drug-excipient compatibility studies

The possible interaction between the drug and the excipients was studied by DSC. The results of DSC studies are given in Table 4 and in Fig. 1 and Fig. 2. There was no considerable change in the DSC endotherm values when metronidazole was mixed with excipients compared to that of pure metronidazole

Table-4: DSC Thermograms of Metronidazole and Metronidaozole + Excipient Mixture.

Composition	Melting Point(ºC)
Metronidazole	162.56
Metronidazole+Eudragit RSPO	161.85
Metronidazole+ Eudragit S100	161.62
Metronidazole+Eudragit RSPO+Eudragit S100	161.69

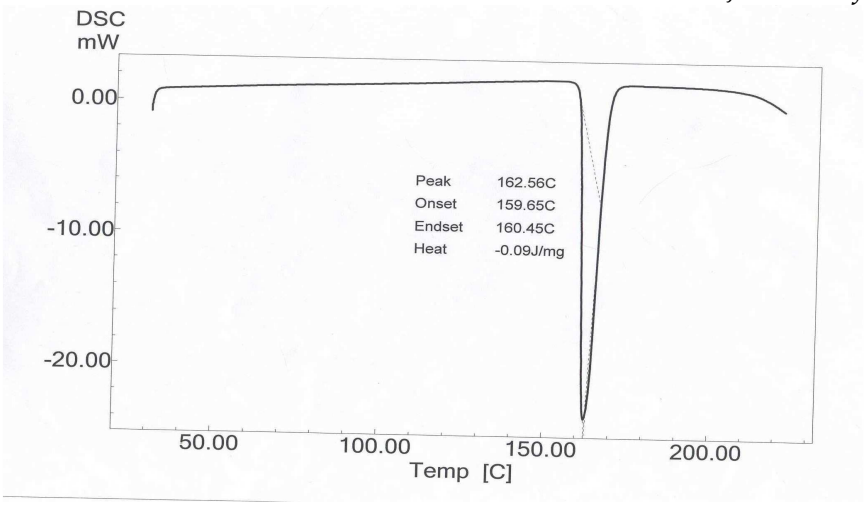

Fig. 1. DSC Thermogram of pure Metronidazole.

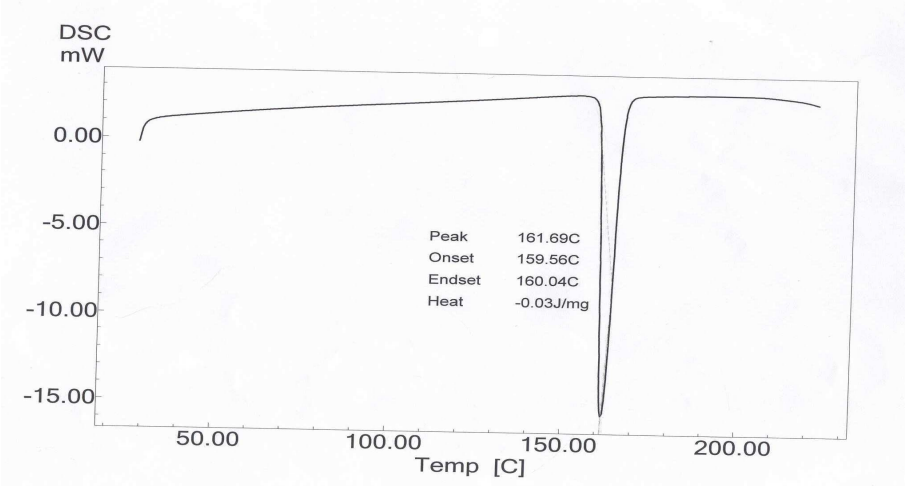

Fig. 2. DSC Thermogram of Metronidazole+Eudragit RSPO+Eudragit S100.

Evaluation of granules

Drug and Eudragit RSPO were mixed in different proportions and granules were prepared by wet granulation method. Granules containing **D** (60% metronidazole + 40% eudragit RSPO) showed good binding properties and was selected for further studies.

Drug content of granules and weight of the granules

Good uniformity in drug content was found among different batches and the percentage drug content varied from 97.95 to 99.14. The practical percentage increase in weight of the granules coated with Eudragit RSPO and

Eudragit S100 was calculated. The results of drug content and percentage increase in weight of granules are given in Table 5.

Table-5: Drug content and practical percentage increase in weight of the granules.

Formulation	Drug Content (mg)	Theoretical percentage increase in weight	Practical percentage increase in weight
D	98.25± 0.238	-	-
D1	99.14±0.206	5	4.86±0.185
D2	98.76±0.105	10	10.12± 0.234
D3	97.95±0.127	15	15.4± 0.197
D4	99.12±0.246	20	19.86± 0.164
D4a	97.98±0.267	5	5.16± 0.159
D4b	98.54±0.124	10	9.74±0.259
D4c	98.69±0.253	15	15.53±0.302
D4d	98.55±0.231	20	20.33±0.203

All values are expressed as mean± SD, n=3

D1,D2, D3 andD4 are coated with Eudragit RSPO and D4a, D4b,D4c,D4d are D4 coated with Eudragit S100 after coating with Eudragit RSPO

Dissolution Studies

The results of *in vitro* release studies of D to D4 in phosphate buffer (pH 6.8) for 24 hours are shown in Fig. 3. D3 showed a drug release of 85.17 % and 98.80 % and D4 showed a release of 81.24 % and 98.99 % at the end of 12 and 24 hours respectively. Since D showed drug release for only 7 hours so one way ANOVA was carried out for 7^{th} hour %CDR for the formulations D to D4 (D being uncoated drug RSPO granules and D1 to D4 being D coated with 5,10,15 and 20 % of weight increase with Eudragit RSPO coating solution respectively). A significant difference in %CDR between the formulations was noticed with the least difference being seen in D4 as compared to D. ($P<0.01$). In addition D4 showed a sustained drug release with 98.99 % drug released at the end of 24 hours. So formulation D4 was selected for further studies. *In vitro* drug release for the formulations coated with Eudragit S100 (D4a, D4b, D4c, D4d),carried out for the first two hours in phosphate buffer (pH 7.4) and then up to 12 hours

in phosphate buffer (6.8 pH) are shown in Fig. 4. One way ANOVA for %CDR at 2nd hour for formulation D4 to D4c showed a significant difference in percentage release with 21.612% CDR from D4 (no Eudragit S100 coating) & D4c showed the least release i.e. 5.8%. ($P<0.01$). This suggest that among all the formulations (D4a to D4d) D4c was will be able to minimize the drug release most efficiently while traversing through the lower small intestinal medium of pH 7.4 for 2 hours and was able to sustain the drug release for 24 hours. Further *in vitro* drug release study from D4c showed a release of 0.2337% in 0.1 N HCL medium and 0.1778% phosphate buffer (pH 6), giving a total of 0.4116% for the first three hours which suggests that formulation D4c will not or least release the drug in stomach and upper parts of small intestine.

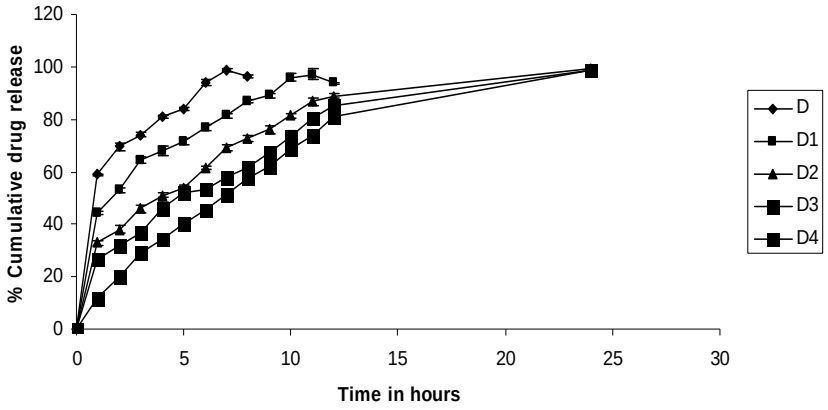

Fig. 3. *In vitro* release of metronidazole from D to D4.

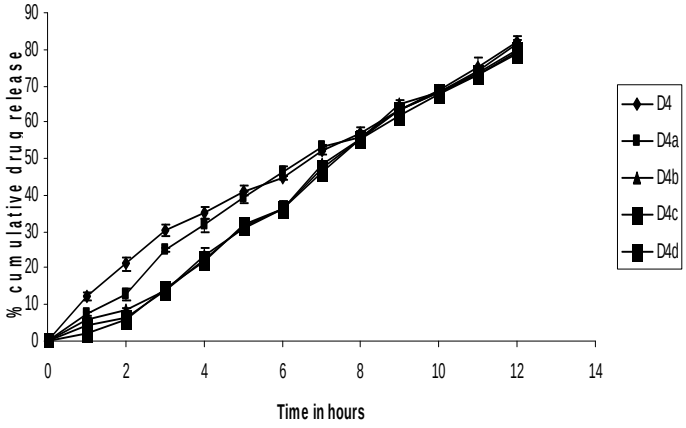

Fig. 4. *In vitro* release of metronidazole from formulation D4, D4a to D4d.

Conclusion

The present study demonstrated the successful preparation of sustained release multiparticulate formulation in the form of granules (D4c) for colon targeted delivery of metronidazole. However stability studies of the developed products are necessary to be carried out to establish the stability of developed product. Additionally, γ Scintigraphy is required to be done to confirm that the drug release starts only from lower small intestine

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