Research Article
ISSN: 0974-6943

Dinesh Chandra ***et al. / Journal of Pharmacy Research 2009, 2(7),1230-1233***
Available online through
www.jpronline.info

Formulation and Evaluation of Satranidazole Microspheres For Colon Targeted Drug Delivery

Dinesh Chandra*[1],Indranil Kumar Yadav[2], Durga Jaiswal[2], Niladry Ghosh[2], Hari Pratap Singh[3], Aushutosh Mishra[3], Arundhati Bhattacharya[1], Meenakshi Bajpai[1] and D.A. Jain[4]

[1.]*Division of Pharmaceutical Research, Raj Kumar Goel Institute of Technology, Ghaziabad-201003, India*
[2.]*Rajiv Gandhi College of Pharmacy, Nautanwa, Maharajganj-273164, India.*
[3.]*A.N.D. College of Pharmacy. Babhnan, Gonda- 271313, India.*
[4.] *Institute of Pharmacy, Bhagwant University- Ajmer, India*

Received on: 27-02-2009; Accepted on: 29-04-2009

ABSTRACT

The purpose of this research was to develop and evaluate Eudragit based microspheres exploiting pH sensitivity property and specific biodegradability for colon targeted delivery of Satranidazole. Eudragit based microspheres were prepared by oil-in-oil solvent evaporation method using different drug- polymer ratios (1:1 to 1:5), stirring speeds (1200-1400 rpm) and emulsifier concentrations (0.5%-1.0% wt/vol). Differential scanning calorimetry, study of the physical mixtures of drug and polymer revealed no drug-polymer interaction. All formulations were evaluated for particle size and shape, swellability and percentage drug entrapment. The yield of preparation and the encapsulation efficiencies were high for all Eudragit microspheres. The in vitro drug release study of optimized formulation was also performed in simulated gastrointestinal fluids (SGF). The release profile of satranidazole from Eudragit microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. It is concluded from the present investigation that Eudragit microspheres are promising controlled release carriers for colon-targeted delivery of satranidazole.

Keywords: pH sensitive polymer, Eudragit L 100, Colon specific drug delivery, Microspheres, Satranidazole.

INTRODUCTION

Delivery of drugs to the colon via, the oral route is valuable in treating diseases of the colon (ulcerative colitis, amoebiasis, chron's disease, carcinomas & infections) whereby high local concentration can be achieved while minimizing side effects that occur because of release higher up in the gastrointestinal tract or because of unnecessary systematic absorption [1].

There are several approaches, which is utilized in achieving colon targeting include use of pH-sensitive polymer, time-dependent formulation, bacterial degrading coating material, biodegradable polymer matrix and hydrogels and prodrug.[2]

Multiparticulate approaches tried for colonic delivery include includes formulations in the form of pellets, granules, microparticles and nanoparticles. The use of multiparticulate drug delivery systems in preference to single unit dosage forms for colon targeting purposes dates back to 1985 when Hardy and co-workers showed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these systems are capable of passing through the GI tract easily, leading to less inter- and intra subject variability[3].

The coating of pH-sensitive polymers to the tablets, capsules or pellets provide delayed release and protect the active drug from gastric fluid. The polymers used for colon targeting, however, should be able to withstand the lower pH values of the stomach and of the proximal part of the small intestine and also be able to disintegrate at the neutral of slightly alkaline pH of the terminal ileum and preferably at the ileocecal junction[4].

There are several publications based on drug-containing microspheres using the Eudragit series of polymers as the encapsulating materials [3]. The Eudragits are a family of polymers based on acrylic and methacrylic acids suitable for use in orally administered drug delivery systems. These polymers are available in various grades possessing a range of physicochemical properties.

The objective of the study is to develop controlled and colon targeted drug delivery system of satranidazole for the treatment of chronic amoebiasis by using Eudragit L 100 as a pH- sensitive polymer. By directly targeting the drug to colon, the maximum concentration of drug reaches and increases the residence time of drug in colon. Because of many of the protozoan especially Entamoeba histolytica remains confined in the large intestine, which necessitates high intracolonic drug concentration.

MATERIALS AND METHOD

MATERIALS

Satranidazole was obtained as a gift sample from Alkem Laboratories Mumbai, India. Eudragit L-100 was procured as a gift sample from Degusssa Darmstadt, Germany. All other solvents and reagents used were of analytical grade.

***Corresponding author.**
Tel.: +91-9455250869,+91- 9369171274
E-mail: neel_pharma@rediffmail.com, dcmpharm@rediffmail.com

METHOD

Prepration of Eudragit microsphere [5,6]

The Eudragit microspheres were prepared emulsion solvent evaporation method. Satranidazole and Eudragit L100 were dissolved in an ethanol: acetone (4:1) mixture, then emulsified in to liquid paraffin oil solution containing (0.5%-1% span-80 w/v) to form the oil in oil emulsion. The system was maintained under agitation speed of 1200 – 1400 rpm at room temperature for 4 hours to allow for the evaporation of solvent. Finally, the microspheres were filtered, washed with n-hexane, and air-dried overnight. Formulation variables are shown in Table 1.

All formulations were evaluated for particle size and shape, swellability and percentage drug entrapment. The particle size was examined by digital photomicroscope.

Scanning Electron Microscopy

The shape and surface morphology of Eudragit microspheres and were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope[7].

Drug-excipient interaction study

In order to find out the possible interactions between satranidazole and the polymer, differential scanning calorimetry (DSC) analysis were carried out on their physical mixtures[8].

Swellability[7]

A known weight (100 mg) of various Eudragit microspheres were placed in enzyme-free simulated gastric fluid (pH 1.2) and allowed to swell for the required period of time at 37ºC ± 0.5ºC in the dissolution apparatus (USP XXIII, model DT-06, Erweka, Germany). The microspheres were periodically removed and blotted with filter paper; then their change in weight (after correcting for drug loss) was measured until attainment of equilibrium. The swelling ratio (SR) was then calculated using the following formula:

$$SR = \frac{Wg - Wo}{Wo}$$

Where, SR indicates swelling ratio; Wo, initial weight of microspheres; and Wg, final weight of microspheres.

Percentage drug entrapment

10 milligram of microspheres were weighed and dissolved in 10 ml of water. This solution was shaken with the help of wrist action shaking machine for 5 hrs and then kept for 24 hrs. Then it was filtered. The filtrate was assayed by UV spectrophotometer at 318 nm and percentage drug entrapment was determined.

in vitro dissolution test:

After the evaluation and optimization of all formulation, best five formulations were selected on the basis of their percentage drug entrapment for the dissolution test. In vitro release study of microspheres was performed in pH progression medium at 37ºC ± 0.5ºC. The drug dissolution test of microspheres was performed by the paddle method (model DT-06, Erweka, Darmstadt, Germany) specified in USP XXIII. Microspheres (100 mg) were weighed accurately and gently spread over the surface of 900 mL of dissolution medium. The content was rotated at 100 rpm. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 hours using 0.1 N HCl. After 2 hours, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and maintained up to8 hours. The samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a microfilter. The rate of drug release was analyzed using UV spectrophotometer (Shimadzu 1700, Japan). The receptor volume was maintained constant by replacing equivalent amount of SGF.

Kinetic treatment of dissolution data: [9,10]

In order to describe the kinetics of the release process of drug in the different formulations, zero- order ($Q_t = Q_0 + K_0 t$), first- order ($\ln Q_t = \ln Q_0 + K_1 t$), Higuchi ($Q_t = K_H t^{1/2}$) and Korsmeyer- Peppas ($Q_t/Q_8 = Kt^n$) models were fitted to the dissolution data of optimized formulations EF6 using linear regression analysis. A value of n = 0.5 indicates case I (Fickian) diffusion or square root of time kinetics, $0.5<n<1$ anomalous (non- Fickian) diffusion, n=1 Case –II transport and n>1 Super Case II transport.

Accelerated stability studies:

Accelerated stability study was carried out to observe the effect of temperature and relative humidity on optimized formulation (EF6), by keeping at 40°C, in airtight high-density polyethylene bottles for three months, at RH 75±5%. Physical evaluation and *in vitro* drug release was carried out each month for three months.

RESULTS AND DISCUSSION:

Eudragit microspheres of satranidazole were successfully prepared by Solvent evaporation technique. The result shown in Table no 1 indicates that Uniform and almost spherical microspheres were obtained at higher stirring speed (1400 rpm) as shown in scanning electron photomicrographs of batch no. EF6 (Fig.1) while microspheres produced at less stirring speed (1200 rpm) are irregular and aggregated in shape, this is because at higher speed resistance towards the subdivision of dispersed phase into smaller particle is reduced[7]. In the DSC spectra of Satranidazole and Eudragit –L mixture, a sharp endothermic peak of fusion of Satranidazole is observed at 186.92^{O}C which is corresponding to its melting point and show no interaction (Fig. 2).

The mean diameter of Eudragit microspheres varied from 26.30 ± 1.6µm to 32.54 ± 1.6µm with varying polymer concentration. A higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres as reported by Pongpaibul et al.[11] The result also indicate that batches prepared at low surfactant concentration (0.5%w/v), the emulsifier may not be sufficient to cover the droplet resulting in coalescence, however at high concentration (1.0%w/v) free flowing spherical microspheres are obtained. Increased surfactant concentration led to the formation of particles with a lower mean geometric diameter. Increasing Span 80 concentration from 0.05% to 1.0% wt/vol led to stabilization of the emulsion droplets avoiding their coalescence, resulting in smaller microspheres.[12]

The percentage drug entrapment varied from 24.66% ± 2.5% to 73.12 %± 1.4%with varying emulsifier concentration and polymeric solution concentration from 0.5% to 1.0% and 10% to 15% respectively

Fig.1: Scanning electron microscopic photograph of Eudragit L 100 based satranidazole microspheres.

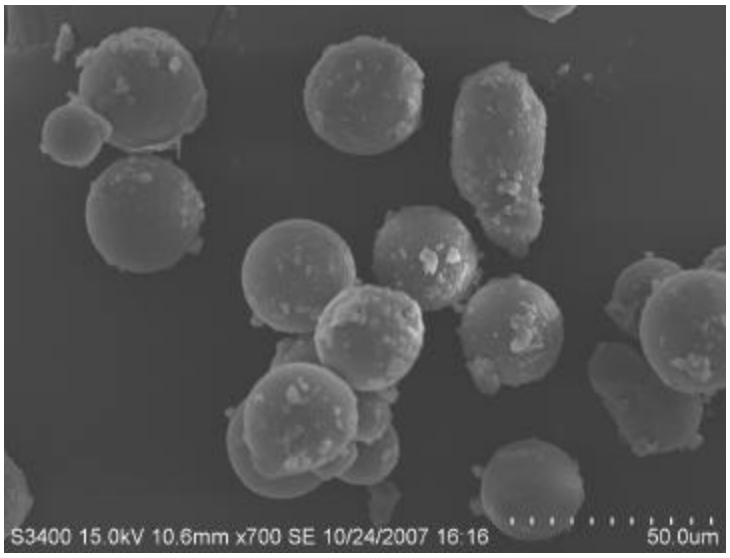

Fig.2: Differential scanning calorimetry (DSC) spectra of satranidazole and Eudragit L 100.

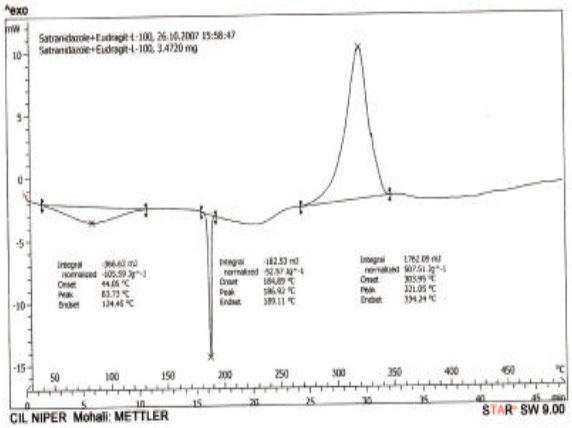

Fig. 3: Comparative study of *in vitro* dissolution profile of optimized formulations (◆) EF4, (■) EF6, (▲) EF7, (×) EF12 and (✱) EF13.

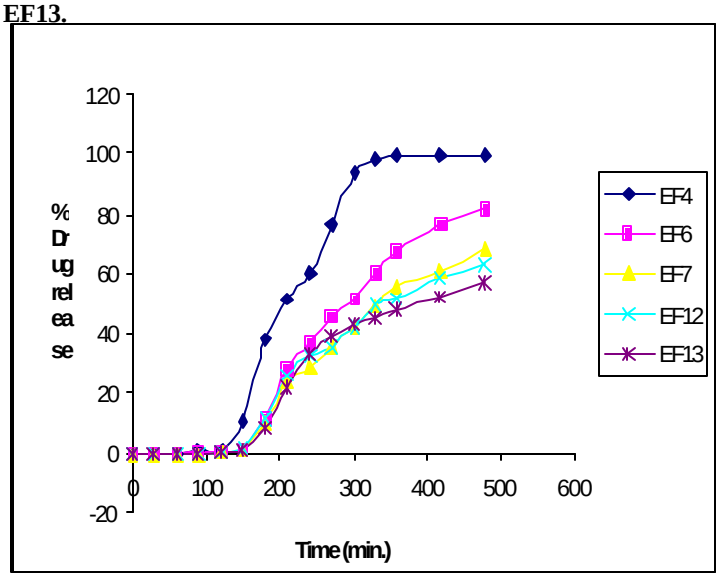

Table 2: Physical properties of Satranidazole microspheres

Batch no.	Mean Diameter (mm)	Shape of microspheres	Percentage Drug Entrapment	Degree of Swelling
EF1	26.30 ± 1.6	Irregular and aggregated	24.66 ± 2.5	0.04 ± 0.01
EF2	27.78 ± 1.4	Irregular and aggregated	31.57 ± 2.4	0.08 ± 0.01
EF3	27.07 ± 1.2	Spherical	41.76 ± 1.4	0.13 ± 0.02
EF4	27.87 ± 2.0	Spherical	72.68 ± 2.8	0.15 ± 0.03
EF5	28.71 ± 2.0	Irregular and aggregated	56.12 ± 1.8	0.17 ± 0.03
EF6	28.05±0.08	Spherical	71.21 ± 1.0	0.18 ± 0.05
EF7	29.17 ± 1.8	Spherical	70.23 ± 2.2	0.23 ± 0.03
EF8	30.67 ± 1.6	Irregular and aggregated	37.76 ± 1.8	0.33 ± 0.05
EF9	29.80 ± 1.4	Spherical	45.18 ± 1.4	0.48 ± 0.03
EF10	30.84 ± 1.5	Spherical	42.65 ± 2.4	0.53 ± 0.04
EF11	32.54 ± 1.6	Irregular and aggregated	45.82 ± 2.5	0.67 ± 0.03
EF12	31.60 ± 1.2	Spherical	73.12 ± 1.4	0.83 ± 0.06
EF13	31.89 ± 2.2	Spherical	70.67 ± 2.4	0.96 ± 0.03

Table 3: Kinetic study of in vitro release data

Batch no.	Zero-Order (r^2)	First-Order (r^2)	Higuchi (r^2)	Korsmeyer-Peppas (n)
EF6	0.897	0.884	0.838	3.489

Table 1: Formulation of Satranidazole microspheres

Batch no.	Drug:Polymer Ratio	Polymeric solution concentration (%w/v)	Name of surfactant	Surfactant concentration(%w/v)	Stirring speed (rpm)
EF1	1: 1	10	Span-80	0.5	1200
EF2	1: 2	10	Span-80	0.5	1200
EF3	1: 2	10	Span-80	1.0	1400
EF4	1: 2	15	Span-80	1.0	1400
EF5	1: 3	10	Span-80	0.5	1200
EF6	1: 3	10	Span-80	1.0	1400
EF7	1: 3	15	Span-80	1.0	1400
EF8	1: 4	10	Span-80	0.5	1200
EF9	1: 4	10	Span-80	1.0	1400
EF10	1: 4	15	Span-80	1.0	1400
EF11	1:5	10	Span-80	0.5	1200
EF12	1:5	10	Span-80	1.0	1400
EF13	1:5	15	Span-80	1.0	1400

during preparation of microspheres (Table 2). The result also indicates that batch no. EF4, EF6, EF7, EF12 and EF13 have adequate drug loading efficiency; hence they were evaluated for in vitro dissolution study.

Swellability of different microspheres was determined, but no significant swelling was observed, which ensure better resistance of Eudragit microspheres in the upper GI tract to swelling and preventing subsequent drug release at the nontarget site[7] (Table 2).

In vitro satranidazole release study of Eudragit microspheres was performed in pH progression medium (pH 1.2 to pH 7.4) at 37ºC ± 0.5ºC. The results showed that the rate of release of drug from Eudragit microspheres was mainly influenced by polymer concentration. The comparative study of in vitro release of drug shows the effect of polymer on the drug release (Fig.4). As the amount of the polymer increases the extent of drug release decreases. This could be attributed to an increase in the density of the polymer matrix and the diffusional path length that the drug has to traverse. The cumulative percentage drug release from Eudragit based microspheres showed the desired rate, as there was no measurable drug release observed up to 2 hours in SGF (pH 1.2) and no drug release occurred below the pH of polymer solubility while at pH 7.4, the significant drug release was observed. Satranidazole release from Eudragit-based microspheres followed the order EF4 > EF6 > EF7> EF12> EF13 (Fig. 3). On the basis of drug release, the formulation EF6 is suitable for colonic delivery of satranidazole.

Table 3 shows data analysis of release profile of optimized and selected formulation EF6 according to different kinetic models. The kinetic treatment reflected that release data of EF6 showed r^2 value of 0.9508, which is close to 1, indicating that release of drug follows zero order kinetics, indicating that the concentration was nearly independent of drug release. Further Korsmeyer and Peppas equation resulted into the value of n= 2.821, which appears to indicate Super Case II transport[9, 10].

The results of stability study of optimized formulation of satranidazole microspheres (EF6) revealed that there was no significant change in size, shape drug content, entrapment efficiency and dissolution profile. Thus, formulation was stable at different conditions of temperature and humidity.

CONCLUSION

From the results of present study, it can be concluded that Eudragit L100 based satranidazole microspheres offer a high degree of protection from premature drug release in simulated upper GIT conditions and deliver most of the drug load in the colon and allow drug release to occur at the desired site. Thus, spherical Eudragit based satranidazole microspheres are a potential system for colon delivery of satranidazole for chemotherapy of amoebic infection.

ACKNOWLEDGEMENT

The authors acknowledge the kind help received from Alkem Laboratories Mumbai, India, for gift samples of satranidazole. The authors gratefully acknowledge The Management, Department of Pharmacy, Raj Kumar Goyal Institute of Technology, Delhi-Meerut Road, Ghaziabad (India) for providing facilities to carry out this research.

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Source of support: Nil, Conflict of interest: None Declared