AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Formulation and Evaluation of Tinidazole Microspheres by Using Eudragit S100, HPMC 6000 and HPC 1000 Polymers

Mohammed Hafeez Khan[1]*, Mohammed Sarddam[1], Mohammed Mujeeb Ali[1], Mohammed Younus[1], Shaik Almas[1], P.Sireesha[1]

1. AZAD College Of Pharmacy,Moinabad,R.R.District,Telangana,India-501504.

ABSTRACT

The aim of the present study is to prepare Tinidazole microspheres by using different polymers. Microspheres were prepared by solvent evaporation method by using various polymers. Formulation is optimized on the basis of acceptable microspheres properties and *in-vitro* release. In order to obtain best optimized product, 6 different formulations were developed. Different polymers like Eudragit S100, HPMC 6000 and HPC 1000 were taken as variables. Particle size analysis, Shape and Surface Morphology, Flow properties, Degree of swelling, Drug entrapment efficacy, Invitro drug release study were studied as response variables. The different physical properties showed best comparable results with drug. But higher percentage of drug release was observed when the formulation contained Eudragit S 1000 in 1:1 ratio(f3) compared to other formulations. The formulation contained Eudragit was selected as optimized product.

Keywords: Tinidazole, HPMC, HPC, Eudragit.

*Corresponding Author Email: ramacognacy@gmail.com

Received 13 May 2015, Accepted 25 May 2015

Please cite this article as: Khan MH *et al*., Formulation and Evaluation of Tinidazole Microspheres by Using Eudragit S100, HPMC 6000 and HPC 1000 Polymers. American Journal of PharmTech Research 2015.

INTRODUCTION

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm). Microspheres are sometimes referred to as microparticles[1]. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Microspheres are characteristically free flowing powders[2,3] consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm[2]. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. One such approach is using microspheres as carriers[4,5] for drugs. Tinidazole is a member of the group of drugs known as Nitroimidazoles. It is an antiparasitic drug[6] used against protozoal infections. It is an antiprotozoal agent. The nitro group of tinidazole is reduced by cell extracts of *Trichomonas*. The free nitro radical generated as a result of this reduction may be responsible for the antiprotozoal activity. The aim of the present study is to prepare Tinidazole microspheres by using Eudragit S100, HPMC 6000, and HPC 1000 polymers.

MATERIAL AND METHOD

Drug & Exceipient Profile

Drugs: Tinidazole.

Polymers: Polymethacrylates, Hydroxypropyl Cellulose, Hydroxy Propyl Methyl Cellulose.

PREFORMULATION STUDIES

Identification of Pure Drug:

Identification of Tinidazole was examined by FT-IR and was compared with the reference spectrum of Tinidazole.

Solubility Analysis:

Solubility analysis was done to select suitable solvents/ solvent systems to dissolve the drug, polymer as well as various excipients used for the formulation of microspheres.

Melting point determination:

Melting point of Tinidazole was determined by using Thiele's tube apparatus. Melting point of a drug sample is a first indication of purity of the sample. The presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range. Melting point of Tinidazole was determined by using Thiele's tube apparatus.

Compatibility studies:

Compatibility of the Tinidazole with polymer used to formulate microspheres was established by FT-IR. Spectral analysis of Tinidazole with other formulation.

Determination of λ_{max}[33]:

10 mg of Tinidazole was dissolved in methanol and diluted to 100 ml with the same solvent. 1 ml of this solution was diluted to 10 ml with the methanol and examined between 220 – 350 nm. The maximum obtained in the graph was considered as λ_{max} for the pure drug. The solution showed an absorption maximum at 310 nm.

Standard Calibration Curve for Tinidazole

In Distilled Water[34]:

The standard solution of Tinidazole was prepared in distilled water. It was necessary to warm on water bath to accelerate the dissolution process. Accurately weighed 100 mg tinidazole was dissolved in distilled water to get the stock solution of 200 µg/ml. From this stock solution, aliquots of 0.25, 0.5, 1.0, 1.25, 1.5 and 1.75 ml were withdrawn and volume was made up to 10 ml with distilled water to get concentrations in the range of 5 to 35 µg/ml. The absorbance of these solutions was measured at 318 nm by UV- Visible Spectrophotometer. A graph of concentration Vs absorbance was plotted. The standard solution of Tinidazole was prepared in distilled water. It was necessary to warm on water bath to accelerate the dissolution process.

In simulated gastric fluid (acidic buffer) pH 1.2:

Weighed quantity of Tinidazole (100mg) was dissolved in pH1.2 buffer and the volume was made up to 100ml with the same medium. From this sock solution serial dilutions were made to obtain the solutions in concentration ranging from 5-35µg/ml. the absorbance of the solution was measured at 318nm.

In phosphate buffer pH 7.4:

Weighed quantity of Tinidazole (100mg) was dissolved in pH 7.4 buffer and the volume was made up to 100ml with the same medium. From this sock solution serial dilutions were made to obtain the solutions in concentration ranging from 5-35µg/ml. the absorbance of the solution was measured at 318nm.

Preparation Microspheres:

The microspheres were prepared by emulsion dehydration technique. polymer and Tinidazole were dissolved in 20 ml of distilled water and stirred overnight to solubilize completely. This drug-polymer solution was dispersed in 50 ml isooctane containing 1.25% wt/vol Span 80 and stirred at 1000 rpm continuously to obtain stable water/oil (w/o) emulsion. The solution was rapidly

cooled to 15°C and then 50 ml of acetone was added in order to dehydrate the droplets. This system was maintained under mechanical agitation with propeller stirrer at 1000 rpm at 25°C for 30 min to allow the complete solvent evaporation. The microspheres were dried overnight and kept in an airtight container for further studies. Microspheres were prepared using different polymers. The microspheres were prepared by emulsion dehydration technique.

Table 1: Formulation Plan

Batch Code	Polymer	Drug: Polymer Ratio
F-1	HPMC 6000	1:1
F-2	HPMC 6000	1:1.5
F-3	polymethacrylates	1:1
F-4	polymethacrylates	1:1.5
F-5	HPC 1000	1:1
F-6	HPC 1000	1:1.5

Percentage Yield:

The percentage yield of the prepared microspheres was determined.

CHARACTERIZATION OF THE MICROSPHERES

Particle Size:

Determination of average particle size of Tinidazole microspheres was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of microspheres was spread on a clean glass slide and average size of 100 microspheres was determined in each batch.

Surface Morphology:

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface.SEM studies were carried out by using JEOL JSMT-330A scanning microscope (Japan). The samples of SEM were prepared by lightly spinkling the microspheres powder on a double adhesive tape, which was stuck on an aluminium stub. The stubs were then coated with gold to thickness of about 300ű using a sputter coater. The photomicrographs were taken with the help pf SEM analyzer.

Flow properties:

Flow properties of microspheres were studied by measuring the angle of repose of the formulation by employing fixed funnel method. Tinidazole microspheres were weighed and passed through the funnel, which was kept at a height of ‘h’ from the horizontal surface. The passed microspheres formed a pile of a height ‘h’ above the horizontal surface and the radius ‘r’ of the pile was measured and the angle of repose was determined for all the batches by using this $\tan\theta = h / r$ formula.

Drug Entrapment Efficiency:

Twenty milligrams of microspheres were accurately weighed. They were added to 5 ml of ethanol. After the microspheres dissolved completely, 5 ml of phosphate buffer (pH 7.4) was added to this solution and mixed thoroughly. The resulting solution was analyzed for Tinidazole content by measuring absorbance in a UV-spectrophotometer at 318 nm using phosphate buffer (pH 7.4) and ethanol mixture (1:1) as blank.

***In Vitro* Drug Release:**

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 filled into tea bags. The tea bags were tied using thread with paddle and loaded into the basket of the dissolution apparatus. 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 upto 8 h. The samples were withdrawn from the dissolution medium at various time intervals. 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.

Stability Studies:

Information on the stability of drug substance is an integral part of the systemic approach to stability evaluation.

Procedure

All the six formulations F-1 to F-6 were divided in to 3 sample sets and stored at;

- 4°C in refrigerator
- 30º C ± 2º C/65% RH ± 5% RH in humidity control oven (GINKYA IM 3500 series).
- Ambient temperature and humidity.

Two parameter's namely residual percentage drug content and *in-vitro* release studies of a selected formulation (F-3) were carried out at the end of the month by following the procedure as discussed in methodology section.

RESULTS AND DISSCUSSION

Preformulation Studies

Identification of Pure Drug:

Identification of Tinidazole was examined by FT-IR and was compared with the reference spectrum of Tinidazole.

Solubility Analysis

Tinidazole sample was found to be soluble in acetone and in dichloromethane; sparingly soluble in methyl alcohol.

Melting point determination:

The melting point of the obtained drug sample was found to be 128^0C which is the reported range of 125^0C-128^0C. It complies with USP standards thus indicating the purity of the drug soluble.

Compatibility studies:

From the FT-IR spectra of the pure drug and the combination spectra of drug with the polymers, it was observed that all the characteristics peaks of Tinidazole were present in the combination spectra thus indicating the compatibility of the drug with the polymers used. The individual spectra of the pure drug Tinidazole, polymer Pectin, Eudragit S100 as well as the combination of the drug and polymers are shown in the Figures 8,9, 10 and 11 respectively. it was observed that all the characteristics peaks of Tinidazole were present in the combination spectra thus indicating the compatibility of the drug with the polymers used.

Determination of λ_{max} :

The absorption spectrum of pure was scanned between 200- 400 nm with 10µg concentration prepared in methanol. The λ_{max} of pure drug Tinidazole was found to be 318 nm.

Standard Calibration Curve For Tinidazole

Table 2: Standard Calibration Curve of Tinidazole in PBS pH 7.4 and Acidic Buffer pH 1.2

Concentration (µg/ml)	Absorbance	
	pH 1.2	pH 7.4
5	0.053	0.090
10	0.096	0.180
15	0.139	0.276
20	0.189	0.366
25	0.235	0.460
30	0.278	0.550

Parameters of Microspheres

Table 3: Percentage Yield, Particle Size, Flow properties and Drug Entrapment Efficiency of Microspheres

Formulation	% Yield	Particle Size (µm)	Angle of Repose	Encapsulation Efficiency (%)
F-1	72.6	182.70	20^0 11	63.85
F-2	76.5	200.55	22^0 36	65.29
F-3	79.8	224.00	25^0 51	66.70
F-4	83.7	230.02	27^0 75	68.90
F-5	85.5	240.73	28^0 34	75.38
F-6	88.9	246.47	33^0 66	76.00

In Vitro Drug Release

In vitro Tinidazole release study of Eudragit microspheres was performed in pH progression medium (pH 1.2 to pH 7.4) at 37ºC ± 0.5ºC. The *in vitro* release profile obtained for all six formulations, F-1 to F-6. The cumulative percent drug release after 8 h was found to be 92.30 %, 82.00%, 76.90 %, 67.83 %, 66.78 % and 62.58 % for F-1 to F-6 respectively, where as cumulative percent drug release of pure drug Tinidazole was 93.21 % within 8 hrs. The results showed that 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 h in SGF (pH1.2) and no drug release occurred below the pH of polymer solubility while at pH 7.4, the significant drug release was observed.

CONCLUSION

The present study concluded that Tinidazole Microspheres have been formulated and developed by using fusion technique, in order to obtain best optimized product, 6 different formulations were developed. For 6 formulations the different physical properties showed best comparable with reference product. But higher percentage of drug release was observed when the formulation contained Eudragit S100 when compared with other formulations. The formulation F3 has shown drug release NLT 75% in 30min accordance with the USP dissolution criteria for solid dispersions. The results suggest that formulation F3 is best formulation.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of Dr. ROSHAN.S (Principal, AZAD college of Pharmacy, moinabad) for his help in carrying out the experiments.

REFERENCES

1. Liu L, Fishman M, Kost J, Hicks KB. Pectin based systems for colon specific drug delivery

via oral route. Biomaterials 2003; 24:3333-43.

2. Abdul B, Bloor J. Perspectives on colonic drug delivery, business briefing. Pharmtech 2003:185-90.
3. Cheng G, Jou MJ, Sun J, Hao XU, He YX. Time- and ph dependent colon- specific drug delivery for orally administered diclofenac sodium and 5-amino salicylic acid. World J Gastroenterol 2004; 10(12):1769-74.
4. Krishnaiah YSR, Satyanarayana S, editors. Advances in controlled and novel drug delivery. 1st ed New Delhi: CBS Publishers and Distributors 2001; p.89-119.
5. Toratora Gradowski. Principles of anatomy and physiology. 10th ed New York: John Wiley & Sons 2002; p. 866-73.
6. RamPrasad YV, Krishnaiah YSR, Satyanarayana S. Trends in colonic drug delivery: a review. Ind Drugs 1995;33(1):1-10.
7. Kumar R, Patil MB, Patil RS, Paschapur SM. Polysaccharides based colon specific drug delivery: a review. Int J Pharm Tech Res 2009;1(2):334-46.
8. Maestrelli F, Cirri M, Corti G, Mennini N, Mura P. Development of enteric- coated calcium pectinate microspheres intended for colonic drug delivery. Eur J Pharm Biopharm 2008;69:508–18.
9. Asghar LFA, Chandran S. Multiparticulate formulation approach to colon specific drug delivery: current perspectives. J Pharm PharmSci2006;9(3):327-38.
10. Rubenstein A. Colonic drug delivery. Drug Discov Today Technol 2005; 2(1):33-7.