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Formulation and Evaluation of Microparticles of Metronidazole

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Abstract
In the present study it was aimed to formulate delayed release Metronidazole microparticles, which will have enteric as well as sustained release properties. For the preparation of Metronidazole microparticles CAP, HPMCP, Eudragit L-100 and Eudragit S-100 were used as coating materials. Among the prepared microparticles the best formulation is reported by *in-vitro* release studies. Microparticles of Metronidazole were prepared using cellulose acetate phthalate as the retardant polymer by emulsion-solvent evaporation method. The microparticles formed were collected by filtration. They were evaluated for morphology, melting point, size distribution, drug content and percentage drug entrapped, flow property, in-vitro drug release and comparative drug release studies with commercial dosage forms. Drug content and percentage of drug entrapment were found to good in all the batches, as the entrapment values were not less than 85%. All batches of microparticles were found to have enteric release property as it was expected. The drug release from all batches of microparticles was found to be concentration dependent (first order release kinetics).The mechanism of drug release was found to be erosion as it was revealed by $(1-M_t/M)^{1/3}$ versus time plots. Comparative drug release study revealed that the formulated product (microparticles) have more sustained effect than the marketed product.
Key words:- Emulsion solvent evaporation, Erosion, Sustained effect, Microparticles

Introduction

Metronidazole belongs to Nitroimidazole derivative, which is widely used in the long term therapy for trichomoniasis, amoebiasis and giardiasis. Since its biological half life is about 8 hours; multiple daily dosing (approximately 3-4 times/day) is necessary for the maintenance of its therapeutic effect throughout the day. Delayed release systems are those that are intended to release the drug after some time (or) after the system (dosage form) has passed through one part of the G.I.T. to another. Examples of delayed release systems include repeat-action tablets, capsules and enteric-coated dosage form where timed release is achieved by a barrier coating. An enteric coating is a coating system that resists disintegration (or) dissolution in gastric medium but disintegrates (or) dissolves in intestinal fluids. [1, 2]

Enteric coating polymers should resist dissolution at pH values below 4.0; but begin to dissolve at pH 5.0 (or) above and become readily at pH 7.0 in the gastrointestinal tract. The coating must have low water permeability, compatibility with a broad spectrum of drugs, and a low tendency to be hydrolysed in a humid and a high temperature environment. In addition, the enteric coating system must be environmentally safe and acceptable. The most effective enteric materials are long-chain polymers with ionizable carboxyl groups. In the low pH environment of the gastric fluid, the carboxyl group remains unionized so that the polymeric coating will remain insoluble. But the polymeric coating disintegrates (or) dissolves in the higher pH intestinal environment to allow the release of the drug contents in the small intestine.[3]

Materials and Methods

Metronidazole was obtained as a gift sample from the Halmak pharmaceuticals Pvt. Ltd, Secunderabad. Cellulose Acetate phthalate, Hydroxy propyl methylcellulose phthalate Eudragit L-100, Eudragit S-100 and Cyclohexane L.R obtained commercially from S.S.R Enterprises, Tirupati, Andhra Pradesh, India.

Preparation of microparticles

Delayed release microparticles containing Metronidazole was prepared by emulsion-solvent evaporation method. The drug (1g) was dispersed in 10% w/v solution of cellulose acetate phthalate (in acetone: Ethanol 8:2 solvent mixture). The resulting solution was passed slowly at constant rate to 100 ml of liquid paraffin in a 250 ml beaker under stirring at 1000 rpm to disperse

the added mixture as fine droplets. The system was stirred for 4 hours to evaporate the solvent at room temperature and to form microparticles of Metronidazole. The prepared microparticles were collected by filtration and washed with cyclohexane to remove adhering liquid paraffin. They are dried at room temperature, sieved and kept in well closed containers. [4, 5] (Table 1)

Evaluation of microparticles

Morphology

For surface characteristics, microcapsules were vacuum dried with gold vapours and scanned under a Hitachi Gold scanning Electron Microscope. Photographs of SEM analysis were shown in Fig No. 1 and 2. [6, 7]

Size Analysis

Size analysis of all the batches of prepared microparticles were carried out using a set of standard sieves ranging from 10-100 meshes. The microparticles were passed through the set of sieves and the amount retained on each sieve was weighed. The arithmetic average diameter was determined by dividing the total weight size by 100. [8] (Fig no.3)

Flow Property

Flow property of the prepared microparticles was examined by measuring the angle of repose by fixed funnel method. A funnel was fixed to a stand in such a way that the tip of the funnel was at a height of 6cm from the surface. The microparticles of 30/40-mesh size were passed through the funnel, so that they form a conical heap on the surface. The height (h) and radius (r) of heap were measured and the angle of repose was calculated.

Drug Content Analysis

50 mg of microparticles of 30/40 mesh size was accurately weighed and was dissolved in 20 ml of cyclohexane. This was made up to 50ml with 0.1N Hydrochloric acid. The solution was filtered to remove the precipitated polymer. From the filtrate 5ml was taken and made up to 100ml with 0.1 N Hydrochloric acid. Spectrophotometric measurement was taken at 277nm. The concentration was obtained from the standard curve and the drug content of each batch was calculated. [9]

Drug Release Study

In-vitro drug release profile of Metronidazole from microparticles was examined in pH 1.2 buffer from 0 to 2 hours and in pH 7.4 buffer from 2 to 12 hours by rotating basket method specified in USP XXI at 100 rpm using 900ml of test fluid maintained at 37°C ± 1°C. Microparticles retained on sieve no. 40 were taken for drug release study. Microparticles equivalent to 200mg of Metronidazole was accurately weighed and placed in the 40-mesh baskets. The baskets were rotated at about 100 rpm. At suitable intervals 1ml aliquot was removed, the same volume of fresh test fluid was added to the test medium to maintain the original volume. The drawn 1ml samples were made up to 10ml with pH 1.2 buffer for first two hours and with pH 7.4 buffer for remaining 10 hours. Absorbance of the resulting solution was measured at 277 nm. Concentration of the drug was calculated from the standard curve. [10, 11] (Table 2)

Results and Discussion

The Metronidazole microparticles were prepared by emulsion solvent evaporation technique. Cellulose acetate phthalate was used as the main retardant polymer for pH dependant release of Metronidazole from microparticles. Scanning electron micrographs of the prepared microparticles reveals that they are discrete and spherical in shape. The microparticles showed the same melting point as that of the pure sample of Metronidazole. Microparticles were tested for the drug content uniformity. The drug content was found to be good and the drug was found to be encapsulated above 85% in almost all the batches of microparticles, which shows that there is no wastage of drug

Table No. 1: Composition of different batches of delayed release microparticles

Batch code	Drug (gms)	Liquid Paraffin (ml)	Solvent Acetone: Ethanol	CAP (gms)	HPMCP (gms)	E_L-100 (gms)	E_S-100 (gms)
B I	1	100	8:2	1	--	--	--
B II	1	100	8:2	0.8	0.2	--	--
B III	1	100	8:2	0.8	--	0.2	--
B IV	1	100	8:2	0.8	--	--	0.2
B V	1	100	8:2	0.8	0.1	0.1	--
B VI	1	100	8:2	0.8	--	0.1	0.1
B VII	1	100	8:2	0.8	0.1	--	0.1
B VIII	1	100	8:2	0.8	0.0660	0.0660	0.0660

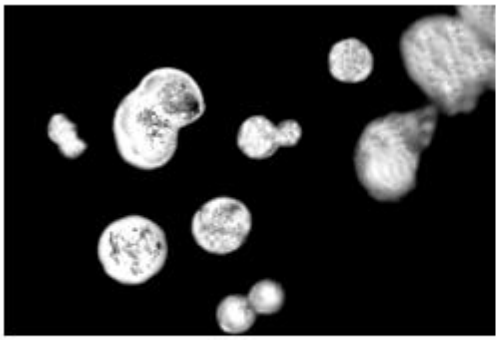

Fig no. 1: A group of microparticles: batch code – VI

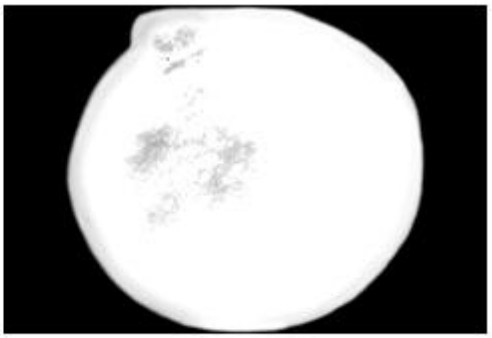

Fig no. 2: Close view of single microparticle batch code – VII

Table No. 2: *In vitro* drug release data of Metronidazole from Microparticles prepared from cap, HPMC and E_s 100 containing 200mg Metronidazole; ***Batch code*** - **VII**

Time in hours	Amount of drug released (mg)	Percentage of drug released	Percentage of drug remaining	Log percentage of drug remaining	$\left(1-\frac{Mt}{M}\right)^{1/3}$
1	3.00	1.50	98.50	1.9934	0.9949
2	6.26	3.13	96.87	1.9861	0.9894
3	8.42	4.21	95.79	1.9813	0.9857
4	24.76	12.38	87.62	1.9426	0.9569
5	56.54	28.27	71.73	1.8557	0.8951
6	63.04	31.52	68.48	1.8355	0.8814
7	92.92	46.46	53.54	1.7286	0.8120
8	115.42	57.71	45.29	1.6262	0.7506
9	128.84	64.42	35.58	1.5512	0.7086
10	164.46	82.23	17.77	1.2496	0.5622
11	189.52	94.76	5.24	0.7193	0.3742
12	199.74	99.87	0.13	0.6380	0.1091

Table No. 3: Dissolution data for prepared Microparticles of Batch VII containing 200mg of Metronidazole

Time in hours	Amount of drug released (mg)	Percentage of drug released	Cumulative percentage of drug released
1	3.24	1.62	1.62
2	7.04	3.52	3.52
3	9.22	4.61	4.61
4	26.56	13.28	13.28
5	58.34	29.17	29.18
6	69.06	34.53	34.56
7	99.28	49.64	49.67
8	113.52	56.76	56.81
9	133.04	66.52	66.58
10	156.70	78.35	78.42
11	169.29	89.62	89.70
12	197.46	98.73	98.82

Table No. 4: Dissolution data for film coated marketed formulation: Flagyl (200mg) tablet

Time in hours	Amount of drug released (mg)	Percentage of drug released	Cumulative percentage of drug released
1	8.72	4.36	4.36
2	19.06	9.53	9.53
3	69.52	34.76	34.77
4	126.64	63.32	63.35
5	152.82	76.41	76.48
6	189.14	94.57	94.65
7	202.16	101.23	101.33

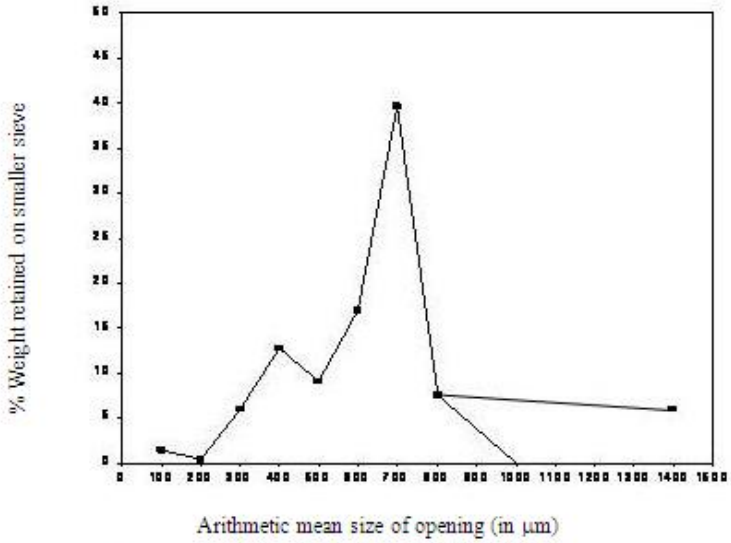

Fig no. 3: Size Distribution analysis of microparticles of Batch-VII

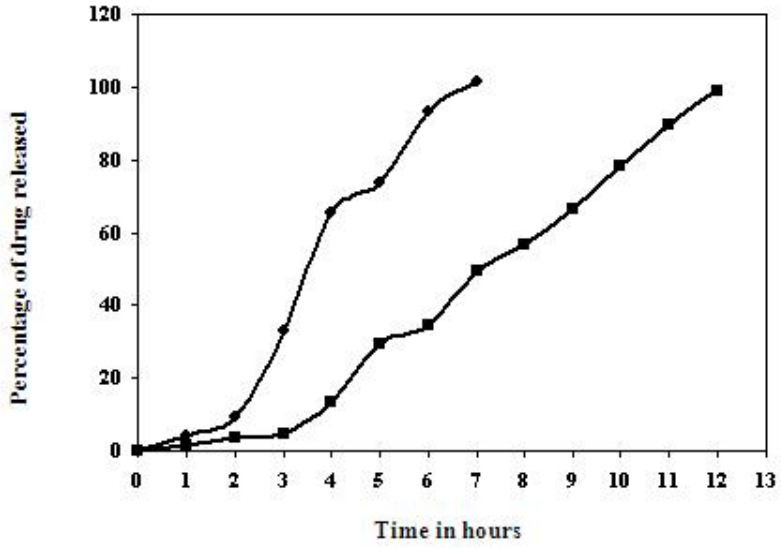

Fig no. 4: Comparative drug release studies

and hence this method is economical regarding encapsulation efficiency.

The dissolution studies were performed for all the bathes of Metronidazole microparticles in pH 1.2 buffer for the first 2 hours and in phosphate buffer of pH 7.4 for the remaining 10 hours. Out of all the batches, the microparticles having CAP, HPMCP, and E_S-100 (Batch-VII) is meeting both enteric as well as sustained drug release pattern. The results showed that the drug release in the acidic medium for the first 2 hours was found to be negligible. The drug release in the remaining period in phosphate buffer started to release the drug. The percentage of drug release was found to be 99.87%. (Batch-VII). The kinetics of drug release was found to be linear when *log* percentage of drug remaining was plotted against time. This indicates that the drug release followed first order kinetics and the mechanism of drug release was erosion. The drug release from the microparticles containing 200mg equivalent of Metronidazole was compared with that of film coated marketed sample, Flagyl tablet containing 200mg of Metronidazole. The sustained release for the marketed sample is about for 7 hours. The sustained release of the best formulation (Batch -VII) containing CAP, HPMCP and Eudragit S-100 microparticles are more than that of commercial film coated tablet. (Flagyl) (Table 3, 4) (Fig no.4)

Conclusion

The results of this study demonstrate that delayed release microparticles of Metronidazole could be prepared by emulsion solvent evaporation technique by using the polymers like CAP, HPMCP, E_L-100 and E_S-100. The microparticles prepared using CAP, HPMCP, E_L-100 and E_S-100 showed little amount of brittleness. This defect can be rectified by adding the plasticizer like castor oil, tween and span. The correct percentage of plasticizer to be added can be determined. Comparative drug release study revealed that the formulated product (microparticles) have more sustained effect than the marketed product.

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