

STUDIES ON COLON TARGETED DRUG DELIVERY SYSTEM FOR SECNIDAZOLE AND ITS β -CYCLODEXTRIN COMPLEX TO TREAT AMOEBIASIS

Hangargekar Sachin R.^{1*}, Shyale Somashekar S², Sameer Shafi¹, Nagoba Shivappa N.³

¹Shivlingeshwar College of Pharmacy, Hasegaon, Latur, Maharashtra, India

²V. L. College of Pharmacy, Raichur, Karnataka, India

³Karpagam University, Coimbatore, Tamilnadu, India

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ABSTRACT

The present investigation was planned to formulate colon targeted tablets of Secnidazole using xanthan gum as matrix carriers. Also, it was planned to improve the solubility of Secnidazole by forming inclusion complex with β -cyclodextrin. Different formulations were prepared by changing the concentrations of matrix carriers and β -cyclodextrin. Initially, granules were prepared and evaluated for various rheological properties like bulk density, tapped density, compressibility index and angle of repose and for compression characteristics like hardness, thickness and disintegration time by using standard techniques. *In vitro* release was conducted for all the formulations in USP XXIV for 24 h in different pH resembling different portions of gastro intestinal tract. Formulations containing 25% w/w xanthan gum showed maximum drug release in colonic environment. These formulations were complexed with β -cyclodextrin. After complexation of Secnidazole with β -cyclodextrin, Secnidazole dissolution is significantly increased in colonic environment. The colon targeted matrix tablet of Secnidazole showed no change and significant difference in physical appearance, drug content or in dissolution pattern after stability studies conducted at 40°C / 75 % RH for 180 days.

KEYWORDS: Amoebiasis, Secnidazole, Colonic disease, β -Cyclodextrin, Targeted drug delivery, Xanthan gum.

*Corresponding author

Sachin R. Hangargekar, Department of Pharmaceutics, Shivlingeshwar College of Pharmacy, Hasegaon, Latur, Maharashtra, India E-mail: hangargekar.s@gmail.com

INTRODUCTION

Protozoal infections are common among people in the under developed tropical and subtropical countries where sanitary conditions, hygienic practices and control of the vectors of transmission are inadequate. Amoebiasis is caused by *Entamoeba histolytica*, named for its lytic action on tissues¹. The disease can be acute or chronic with patients showing varying degrees of illness, from no symptoms to mild diarrhea and fulminating dysentery². From the last few decades, a great deal of research work has been devoted to the development of the site specific drug delivery systems which offer several benefits over the traditional drug therapies. The therapeutic advantages of targeting the drug to the diseased organ include delivery of the drug in its intact form as close as possible to the target site, reduced incidence of adverse side effects etc³. The colon, as a site for drug delivery, offers distinct advantages on account of a near neutral pH, a

much longer transit time, relatively low proteolytic enzyme activity and a much greater responsiveness to absorption enhancers. Various diseases of colon such as ulcerative colitis, Crohn's disease, carcinoma and infections require local therapy. So the development of locally acting colon targeted drug delivery systems may revolutionize the treatment of colonic diseases^{4,5,6}. Successful colonic drug delivery requires careful consideration of a number of factors, including the properties of the drug, the type of delivery system and its interaction with the healthy or diseased gut. The objective of the present study is to develop and characterize colon targeted oral tablets using different concentrations of xanthan gum as a matrix carrier for producing local action. Secnidazole is the drug proposed to be used for the present study. It is used in the treatment of amoebiasis, giardiasis and trichomoniasis. Chemically it is 5- nitroimidazole derivative. The main

interest in such dosage form was to target the drug to the colon by ensuring minimal amount of drug release in the physiological environment of the upper GI tract. In the present study simultaneous attempt has been made to enhance the oral bioavailability of Secnidazole by enhancing its solubility by forming inclusion complex with β -cyclodextrin⁷.

MATERIAL AND METHODS

Secnidazole was obtained as a Complimentary sample by Aarati Drugs Ltd, Mumbai. β -Cyclodextrin and Xanthan gum was obtained by Danmed pharmaceuticals Pvt Ltd, Hyderabad. All other chemicals used were of analytical grade.

Development of Formulations

Secnidazole matrix tablets were prepared by wet granulation using different excipients and different concentrations of polymer like xanthan gum and sodium CMC. Sodium CMC was used as diluents in all formulations. Xanthan gum was used as matrix carrier. Starch paste (15 % w/w) was used as binder in all formulations. All the powders were weighed and grounded to fineness in a clean and dry mortar and pestle. The powder blend was then passed through a sieve 120. The powder was then kneaded with a clean and dry pestle using distilled water as granulating fluid. The wet mass was then passed through a mesh 16. The particles were allowed to dry for 2-3 h in an oven at 40°C. The dried granules were then passed through a mesh 18. Fine particles to an extent of 20 % w/w were blended thoroughly with the particles of 16/18 and then to this powder blend was added talc and magnesium stearate at 2:1 ratio and used for further processing. Totally 6 formulations (F1 to F6) were developed and studied. Also, the dried granules of Secnidazole were characterized for rheological properties. After adding lubricant (talc) and anti-adherent (magnesium stearate) to the granule bed, subsequent blending the granules were compressed into tablets on a pilot press machine using 13 mm diameter, flat faced punches at a pressure of approximately 5 kgs/cm².

Characterization

Evaluation of Pre-compression Characteristics of Granule Bed

Granules prepared by wet granulation technology were evaluated for various rheological properties like bulk density, compressibility index, flow properties (angle of repose) by using standard procedures. All studies were carried out in triplicate (n=3) and average values were reported.

Bulk Density

Bulk density was determined (bulk density apparatus, Konark instruments, India) by taking the dried granules in a measuring cylinder and measures the volume and weight of the total granules.

Bulk density = total volume/total weight

Compressibility Index

Compressibility index was determined by placing the dried granules in a measuring cylinder and the volume (V₀) was noticed before tapping and after tapping's again volume (V) was noticed.

Compressibility index = $(1 - V / V_0) \times 100$

Where, V₀ = volume of powder/granules before tapping.

V = volume of powder/granules after tapping's.

Angle of Repose

Angle of repose was determined by measuring the height, radius of the heap of the granules. A cut system funnel was fixed to a stand and bottom of the funnel was fixed at a height of 3cm from the plane. Granules were placed in funnel and allowed to flow freely and measured the height and radius of the heap of granules. Similar studies were carried out after incorporating lubricants / glidants.

$\tan \theta = h / r$

Where, h = height of heap of granules

r = radius of heap of granules

Evaluation of Compression Characteristics of Tablets

Tablets were evaluated for their thickness, weight uniformity, hardness, and disintegration time and dissolution profiles by using standard procedures⁸.

Thickness- The tablets were evaluated for their thickness using a micrometer (Mitutoyo, Japan). Average of three readings were taken and the results were tabulated (n = 3).

Weight Variation- Ten tablets were taken and weighed individually. Average weight was calculated standard deviation and percent coefficient of variance was computed.

Hardness Test- Prepared tablets were evaluated for their hardness by using Pfizer hardness tester.

Disintegration Time- Disintegration test was performed by using 0.1N HCl at 37°C by using USP disintegration apparatus.

Drug Content Uniformity

From each batch three randomly selected tablets were weighed accurately and powdered in a glass mortar with pestle. Powder equivalent to 250 mg of drug was transferred into 100 ml volumetric flask containing little amount of distilled water; the remaining volume was made up to 100 ml with distilled water. Shake it for 24 h and filter the solution, make up desired dilutions and

analyzed for drug content at λ max 320.5 nm against a blank tablets prepared in similar manner excluding drug.

In vitro Dissolution Studies

Colon targeted tablets of Secnidazole were evaluated with the aid of USP XXIV type I (basket) apparatus⁹. The tablets were put in the jars containing 900 ml of dissolution medium and the medium was stirred at 100 rpm and the temperature of the medium was maintained at 37 ± 0.5 °C. For the first 2 h the jars are filled with buffer of pH 1.2. After 2 h the jar assembly was lifted and the dissolution fluid was replaced with phosphate buffer pH 7.4 for a period of 5 h. Further once again the jar assembly was lifted and the dissolution fluid was replaced with buffer of pH 6.8 for a period of 24 h. Samples of 1 ml were collected at predetermined time intervals for 24 h. All the studies were carried out in triplicate (n = 3).

Stability Studies

The stability experiments were conducted to investigate the influence of temperature and relative humidity on the drug content of different formulations. The formulations were exposed to a temperature at 40°C and also a relative humidity of 75 % RH. The sample was removed from the oven at the end of 24 h and analyzed for drug content every day for 180 days¹⁰. Average of triplicate readings was taken. The observations were tabulated.

Statistical Analysis

The *in vitro* data was subjected to regression analysis by least squares method. The standard deviation was calculated and reported. In vitro Data was analyzed by ANOVA, a value of $p < 0.05$ was considered to be statically significant^{11,12}.

RESULTS

The value of U.V. absorbance maxima 320.5nm obtained during this study corroborates with literature value 320 nm. Melting point was found to 74°C which corroborates with the literature. Solubility of Secnidazole was found to be 30.30 mg/ml in distilled water, 4.39 mg/ml in methanol and the solubility of Secnidazole β -cyclodextrin complex in water is 163.55 mg/ml. The complexation of secnidazole with β -Cyclodextrin was confirmed by XRD diffractograms and the complex showed that the secnidazole forms inclusion complex to a lesser extent with kneading method in solid state. Bulk density of powder bed formulations is in the range 0.38 gm/ml to 0.62 gm/cc which was found to be less than 1 for all formulations. During the studies compressibility index was found to be varying between 4.76 % and 12.68 %. Angle of Repose ($^{\circ}$) was found to be in the range of 29.08⁰ and 31.04⁰ before incorporating glidants. Similar studies conducted showed reduced angle of repose

varying between 27.04⁰ and 29.79⁰ after incorporating glidants for formulations containing talc: magnesium stearate ratio at 2:1. The thicknesses of the tablets were found to be uniform between 4.02 ± 0.01 mm to 5.41 ± 0.01 mm. The drug content of all tablet formulations was in the range of 96.14 % to 98.52 %. The *in vitro* dissolution study was conducted using USP XXIV dissolution testing apparatus method I using basket at 100 rpm in pH 1.2, pH 7.4, pH 6.8 buffer solution at a constant temperature of 37 ± 0.5 °C for a period of 24 h. The data was also subjected to regression analysis by least squares method (r) to assess the linearity of the curve. *In vitro* release data obtained was statistically analyzed by ANOVA and a value of $p < 0.05$ was considered to be significant. At the end of 24 h, F1 released 88.32 % (r = 0.974) within 8 h; F2 released 79.98 % (r = 0.950); F3 released 75.57 % (r = 0.942); F4 released 71.64 % (r = 0.928); the drug released from the β -cyclodextrin complex was as follows F5 released 95.16 % (r = 0.979); F6 released 97.09 % (r = 0.964). Stability studies were conducted according to ICH guidelines for region IV at 40° C / 75 % RH indicates that, there is no significant change in the drug content and dissolution profile for a period of 180 days.

DISCUSSION

The analytical method was found to be accurate and precise. The bulk density of all formulations is less than 1. Carr's Compressibility index was found to be less than 15 % for all powder beds indicating that the powder bed is easily compressible. Angle of repose of the powder beds was found to be less than 29° hence were regarded to be easily flowing. Hardness of the tablet varied between 4.24 ± 0.10 Kg/cm² to 4.68 ± 0.27 Kg/cm² indicating good binding and satisfactory strength of tablets to with stand stress during transportation and displacement. The drug content was found to be fairly uniform, reproducible and consistent in all the formulations. All the experiments were repeated for at least 3 times and average was computed and the data have been tabulated. The data obtained were subjected to regression analysis (r), and ANOVA was performed and a value of $p < 0.05$ was considered statistically significant. Data was plotted according to zero order release kinetics.

Stability studies were conducted according to ICH guidelines region IV at 40°C/75 % RH indicates that there is no significant change in drug content and dissolution profile for a period of 180 days. The results of the *in vitro* study has indicated that, the colon targeted tablets containing 25% w/w of xanthan gum showed maximum drug release in colonic environment as

compared to other formulations so these formulations were selected for complexation with β -cyclodextrin to improve the dissolution rate as it is the rate limiting step. The results showed improvement in the release rate of these formulations. A one third dose of the secnidazole- β -cyclodextrin complex would release more percentage of drugs in colonic environment than the drug alone. Thus, study of pre-compression, compression characteristics, *in vitro* release and stability studies concluded the objectives of the investigation.

It was concluded that dissolution of secnidazole can be effectively increased by forming inclusion complex with β -cyclodextrin. Xanthan gum at 25 % w/w in a tablet would target the tablet to colon.

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Table 1: Composition of all Formulations

Formulation Code →	F1	F2	F3	F4	F5	F6
Ingredients (mg)	SC 50	SX 25	SX 30	SX 35	SBC 50	SBX 25
Secnidazole	50	250	250	250	50	50
Secnidazole β -cyclodextrin complex (equivalent to 50 mg drug)	-----	-----	----	-----	306	306
Starch Paste	90	90	90	90	97.5	97.5
Xanthan gum	----	150	180	210	----	162.5
Carboxy methyl cellulose sodium	451	101	71	41	168.5	6
Talc and Mg stearate(2:1) 1.5%w/w	9	9	9	9	28	28
Total tablet weight (mg)	600	600	600	600	650	650

SC50- Secnidazole conventional tablet, **SX25-** Secnidazole with 25% xanthan gum colon tablet, **SX30-** Secnidazole with 30% xanthan gum colon tablet **SX35-**Secnidazole with 35% xanthan gum colon tablet **SBC50-**Secnidazole β -cyclodextrin complex conventional tablet **SBX25-**Secnidazole β -cyclodextrin complex with 25% xanthan gum colon tablet.

Table 2: Evaluation of Rheological Characteristics of Granule Bed Compression of Colon Targeted Tablets of Secnidazole and Secnidazole β -Cyclodextrin (n=3).

Formulation Code	Formulation Name	Compressibility index %	Bulk density gm/ml	Tapped density gm/ml	Angle of repose (° θ)	
					Before adding glidants	After adding Glidants
F1	SC 50	12.13	0.38	0.43	31.04	28.48
F2	SX 25	12.24	0.61	0.67	30.46	27.04
F3	SX 30	12.28	0.62	0.68	30.26	28.54
F4	SX 35	12.63	0.61	0.70	29.08	27.21
F5	SBC 50	12.68	0.38	0.49	30.25	29.79
F6	SBX 25	4.76	0.48	0.48	29.63	28.02

Table 3: Physical Characteristics of Colon Targeted Tablets of Secnidazole and Secnidazole-β Cyclodextrin (n=3).

Formula-tion Code	Formula-tion Name	Weight (mg±SD)	Hardness (kg/cm ²)	Thickness (mm)	Drug content (mg)	% Friability	Disinte-gra-tion Time (h)
F1	SC 50	594 ± 6.63	4.52	4.02	48.90	0.66	0.75 h
F2	SX 25	598.4 ± 7.33	4.64	4.26	240.37	0.98	ND
F3	SX 30	601.6 ± 6.18	4.32	4.21	246.32	0.68	ND
F4	SX 35	595 ± 2.02	4.68	4.21	243.57	0.45	ND
F5	SBC 50*	644 ± 4.89	4.24	5.36	48.96	0.60	ND
F6	SBX 25*	652 ± 4.01	4.38	5.41	48.84	0.68	ND

ND= Not Disintegrated.

* 650 mg tablet weight.

Table 4: *In vitro* Drug Release Data of Colon Targeted Tablets of Secnidazole and Secnidazole-β Cyclodextrin.

Sr No.	Time (h)	Percentage cumulative drug release					
		F1	F2	F3	F4	F5	F6
1	0	0	0	0	0	0	0
2	0.01	0.34 ± 0.00	0	0	0	0.34 ± 0.00	0
3	0.05	0.79 ± 0.10	0	0	0	0.78 ± 0.10	0
4	0.08	1.57 ± 0.10	0	0	0	1.91 ± 0.10	0
5	0.17	3.60 ± 0.33	0	0	0	4.04 ± 0.25	0
6	0.25	6.97 ± 0.87	0	0	0	8.18 ± 0.53	0
7	0.5	12.47 ± 0.34	0	0	0	12.78 ± 0.19	0
8	0.75	19.44 ± 0.50	0	0	0	19.62 ± 0.67	0
9	1	24.83 ± 0.16	0.14 ± 0.16	0.09 ± 0.10	0.07 ± 0.00	25.44 ± 0.34	0.45 ± 0.10
10	1.5	32.25 ± 0.16	0.43 ± 0.38	0.25 ± 0.10	0.32 ± 0.41	32.84 ± 0.16	1.35 ± 0.10
11	2	40.45 ± 0.53	0.85 ± 0.44	0.51 ± 0.16	0.61 ± 0.10	42.37 ± 0.41	2.48 ± 0.10
12	2.5	43.71 ± 0.91	1.21 ± 0.10	0.60 ± 0.10	1.04 ± 0.10	43.94 ± 0.91	3.15 ± 0.16
13	3	49.55 ± 0.34	1.71 ± 0.10	1.14 ± 0.16	1.35 ± 0.19	50.44 ± 0.25	4.16 ± 0.16
14	4	59.89 ± 0.62	2.31 ± 0.10	1.72 ± 0.10	1.76 ± 0.16	60.30 ± 0.25	6.08 ± 0.25
15	5	72.14 ± 0.83	3.41 ± 0.16	2.14 ± 0.94	2.17 ± 0.16	72.63 ± 0.25	8.44 ± 0.57
16	6	75.96 ± 0.10	4.91 ± 0.16	4.04 ± 0.91	2.41 ± 0.10	78.01 ± 0.44	8.89 ± 0.10
17	7	81.69 ± 0.33	7.18 ± 0.16	5.98 ± 0.66	3.32 ± 0.19	84.18 ± 0.53	10.80 ± 0.53
18	8	88.32 ± 0.50	9.99 ± 0.16	8.34 ± 0.19	5.03 ± 0.50	95.16 ± 0.78	12.71 ± 0.34
19	9	-	13.56 ± 0.16	11.18 ± 0.19	7.24 ± 0.19	-	18.45 ± 0.16
20	10	-	17.88 ± 0.00	14.83 ± 0.25	11.07 ± 0.25	-	24.75 ± 0.10
21	11	-	22.81 ± 0.16	18.72 ± 0.59	16.76 ± 0.16	-	31.95 ± 0.10
22	12	-	29.49 ± 0.34	25.54 ± 0.59	23.33 ± 0.16	-	40.39 ± 0.16

23	13	-	37.90 ± 0.25	33.46 ± 0.69	30.72 ± 0.53	-	42.53 ± 0.25
24	14	-	47.11 ± 0.25	42.32 ± 0.25	39.36 ± 0.25	-	53.78 ± 0.91
25	15	-	57.21 ± 0.25	51.82 ± 0.16	48.50 ± 0.16	-	66.60 ± 0.29
26	18	-	68.05 ± 0.16	63.71 ± 0.25	59.19 ± 0.66	-	80.78 ± 0.16
27	24	-	79.98 ± 0.16	75.57 ± 0.25	71.64 ± 0.44	-	97.09 ± 0.50

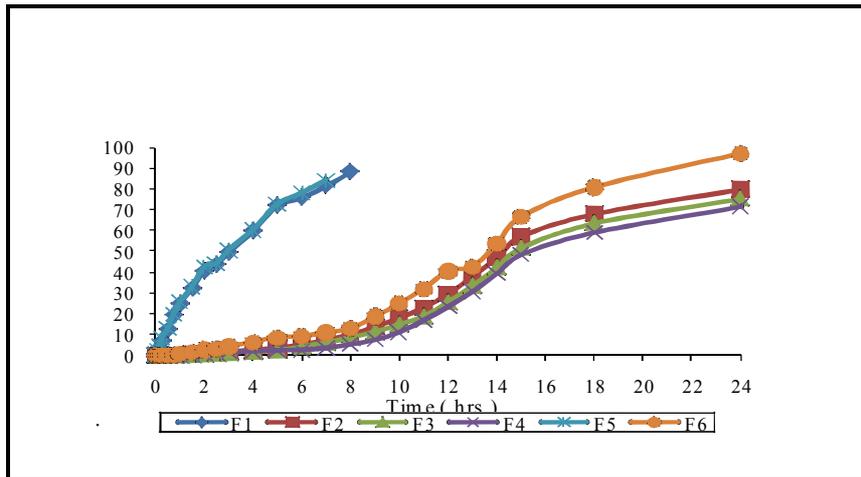
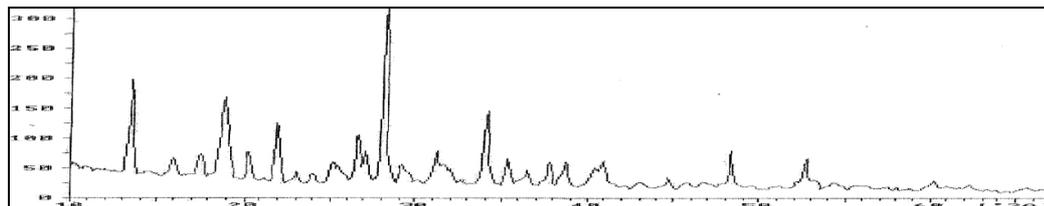
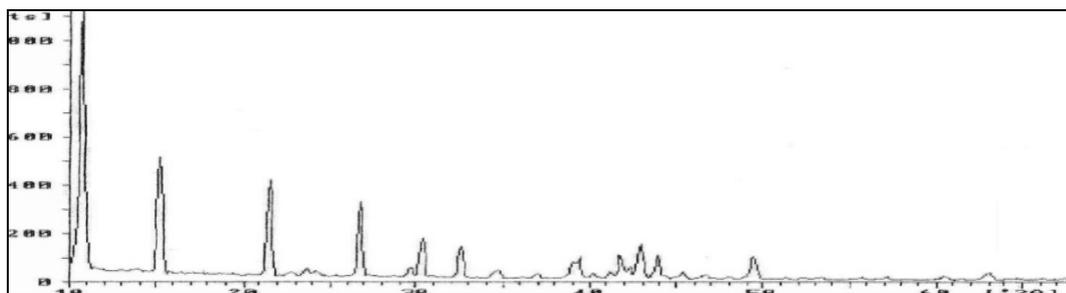


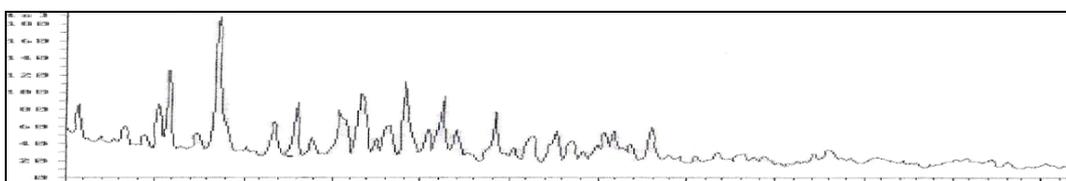
Figure 1: *In vitro* Drug Release From All Formulations



A) Number of Peaks = 43



B) Number of Peaks = 39



C) Number of Peaks = 34

Figure 2: Powder X-ray Diffraction Patterns of A) Secnidazole B) -Cyclodextrin C) Secnidazole- -Cyclodextrin Complex by Kneading Method

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