



Development and Evaluation of Colon targeted tablets of Praziquantel and its β -Cyclodextrin complex to treat Schistosomiasis

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Abstract

The present investigation was planned to formulate colon targeted tablets of praziquantel using xanthan gum and guar gum as matrix carriers. Also, it was planned to improve the solubility of praziquantel by forming inclusion complex with β -CD. Different formulations were prepared by changing the concentrations of matrix carriers and β -CD. Initially, granules were prepared and evaluated for various rheological properties like bulk density, true density, compressibility index and angle of repose and for compressional 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 40% w/w xanthan gum and guar gum showed maximum drug release in colonic environment. These formulations were complexed with β -CD. After complexation of praziquantel with β -CD, praziquantel dissolution is significantly increased in colonic environment. Stability studies at 40°C / 75 % RH was planned to be conducted for 90 days.

Keywords : *Schistosomiasis, Praziquantel, Colonic disorders, β -cyclodextrin, Targeted drug delivery.*

Introduction

The major disorders of the colon are inflammatory bowel disease, colon cancer, crohn's disease and schistosomiasis. Most of the conventional drug delivery systems are failed to treat such diseases as the drugs do not reach the site of action in appropriate concentrations. Thus, an effective and safe therapy of these colonic disorders, using site-specific drug delivery systems is a challenging task to the pharmaceutical technologists. Colonic delivery can be accomplished by oral or rectal administration¹. Rectal dosage forms such as suppositories and enemas are not always effective since a high variability in the distribution of these forms is observed. Suppositories are only effective in the rectum because of the confined spread and enema solutions can only offer topical treatment to the sigmoid and descending colon. Therefore, oral administration is preferred, but for this purpose, many physiological barriers have to be overcome. Absorption or degradation of the active ingredient in the upper part of the GI tract is the major obstacle and must be

circumvented for successful colonic drug delivery. Schistosomiasis or bilharzia is a parasitic disease caused by several species of flatworm². Schistosomiasis is endemic in 74 tropical developing countries. Some 600 million people are at risk of becoming infected. It is estimated that 200 million people are already infected. Schistosomiasis mainly affects adult workers in rural areas, employed either in agriculture or the freshwater fishing sector. In many areas, a high proportion of children between the ages of 10 and 14 are infected. Urinary schistosomiasis affects 66 million children throughout 54 countries. Praziquantel³⁻⁴ is one of the drugs used in the treatment of schistosomiasis whose half life is 0.8-1.5 h and insoluble in water and the oral bioavailability of praziquantel is relatively very small. The aim of the present study is to develop and characterize the matrix tablet of praziquantel using matrix carriers like xanthan gum and guar gum for colonic delivery of praziquantel. In the present study, simultaneously an attempt has made to enhance

the oral bioavailability of praziquantel by enhancing its solubility by forming inclusion complex with β CD⁵.

Materials and Methods

Praziquantel Complimentary sample from Cipla Ltd, Mumbai, β -Cyclodextrin Complimentary sample from Danmed pharmaceuticals Pvt Ltd, Hyderabad, Microcrystalline cellulose Himedia laboratories, Pvt. Ltd, Mumbai, Xanthan gum Danmed pharmaceuticals, Hyderabad. Guar gum Sigma Aldrich, USA. Himedia laboratories, Pvt. Ltd, Mumbai, Starch I.P, Talc, Magnesium Stearate were from S.d. Fine chemicals limited Mumbai. All other chemicals used were of analytical grade.

Experimental Section

Development of Formulations

Praziquantel matrix tablets were prepared by wet granulation using different excipients/polymer like xanthan gum, guar gum, and micro crystalline cellulose (MCC). MCC was used a diluent in all formulations. Xanthan gum, Guar gum were used as matrix carriers. 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 20. Fine particles to an extent of 20 % w/w were blended thoroughly with the particles of 16/20 and then to this powder blend was added talc and magnesium stearate at 2:1 ratio and used for further processing.

Totally 9 formulations (F1 to F9) were developed and studied. Also, the dried granules of praziquantel 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

a. Evaluation of pre-compression characteristics of granule bed⁶

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.

b. 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.

$$\text{Bulk density} = \text{total volume} / \text{total weight}$$

c. Compressibility index

Compressibility index was determined by placing the dried granules in a measuring cylinder and the volume (V₀) was noticed before tapping. After 100 tappings again volume (V) was noticed.

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

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

V = volume of powder/granules after 100 tappings.

d. 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 3 cm 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

e. Evaluation of compression characteristics of tablets

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

- 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 uniformity- 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 150 mg of drug was transferred into 100 ml volumetric flask containing ethanol water (1:3), the remaining volume was made up to 100 ml with methanol. Shake it for 24 hr and filter the solution, make up desired dilutions and analyzed for drug content at λ max 263.5 nm, against a blank tablet prepared in the similar manner excluding drug.

In vitro dissolution studies⁷

Colon targeted tablets of praziquantel were evaluated with the aid of USP type 1 (basket) apparatus. The tablet was 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 hours 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 9.0 for a period of 24 h. Samples of 1 mL were collected at predetermined time intervals for 24 hr. All the studies were carried out in triplicate (n = 3).

Stability studies⁸

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 of 40°C and also a relative humidity of 75 % RH. The sample was removed from the oven at the end of 24 hours and analyzed for drug content every day for 90 days. Average of triplicate readings was taken. The observations were tabulated.

Statistical analysis^{9,10}

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.

Results

The value of U.V. absorbance maxima 263.5 obtained during this study corroborates with literature value 263 nm. Melting point was found to 140°C which corroborates with the literature. Solubility of praziquantel was found

to be 0.3 mg/ml in distilled water, 647.6 mg/ml in ethanol and 80.29 mg/ml in acetone and the solubility of drug- β -CD complex in water is 2.01 mg/ml. The complexation of praziquantel with β -CD was confirmed by DSC and XRD indicates that, inclusion complex shows a stronger interaction between praziquantel and β -CD in solid state. Bulk density of powder bed formulations is in the range 0.4 gm/ml to 0.485 gm/cc which was found to be less than 1 for all formulations.

During the studies compressibility index was found to be varying between 7.8 % and 12.86 %. Repose of angle ($^{\circ}$) was found to be in the range of 29.06 and 31.45 $^{\circ}$ before incorporating glidants. Similar studies conducted showed reduced angle of repose varying between 24.99 $^{\circ}$ and 27 $^{\circ}$ after incorporating glidants for formulations containing talc: magnesium stearate ratio at 2:1. The thickness of the tablets was found to be uniform, between 2.856 \pm 0.02 mm to 2.57 \pm 0.46 mm. The drug content of all tablet formulations was in the range of 95.87 % to 99.14 %. The *in vitro* dissolution study was conducted using USP 24 dissolution testing

apparatus method I using a basket at 100 rpm in pH 1.2, pH 7.4, pH 9.0 solution at a constant temperature of 37 \pm 0.5 $^{\circ}$ C for a period of 12 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 88.13 0 % (r = 0.811) within 3 h ; F2 released 62.69 % (r = 0.911); F3 released 66.8 % (r = 0.911); F4 released 36.41 % (r = 0.902); F5 released 69.36 % (r = 0.911); F6 released 62.99 % (r = 0.911); F7 released 45.03 % (r = 0.956); F8 released 72.2 % (r = 0.903); F9 released 66.78 % (r = 0.913); F10 released 50.41% (r = 0.903); The drug released from the β -cyclodextrin complex was as follows F11 released 66.20 % (r = 0.903); F12 released 71.4 % (r = 0.956). F13 released 79.55 % (r = 0.969). Stability studies were conducted according ICH guidelines for region IV at 40 $^{\circ}$ C / 75 % RH indicates that there is no significant change in the drug content for a period of 3 months.

Table 1: Composition of all formulations

Ingredients (mg)	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8 ^s	F 9 ^s
Praziquantel	150	150	150	150	150	150	150	-	-
β -CD complex equivalent to 50 mg drug	-	-	-	-	-	-	-	181.65	181.65
Starch Paste	60	60	60	60	60	60	60	90	90
Xanthan gum	-	80	120	160	-	-	-	160	-
Guar gum	-	-	-	-	80	120	160		160
Talc % w/w	8	8	8	8	8	8	8	12	12
Magnesium stearate% w/w	4	4	4	4	4	4	4	6	6
MCC	178	98	52	18	98	52	18	160.35	160.35
Total tablet weight (mg)	400	400	400	400	400	400	400	600	600

Table 2. Evaluation of rheological characteristics of Colon targeted tablets of praziquantel or praziquantel- β cyclodextrin (n=3).

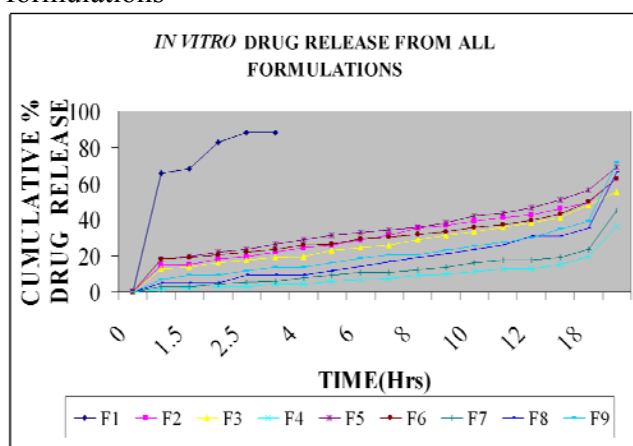
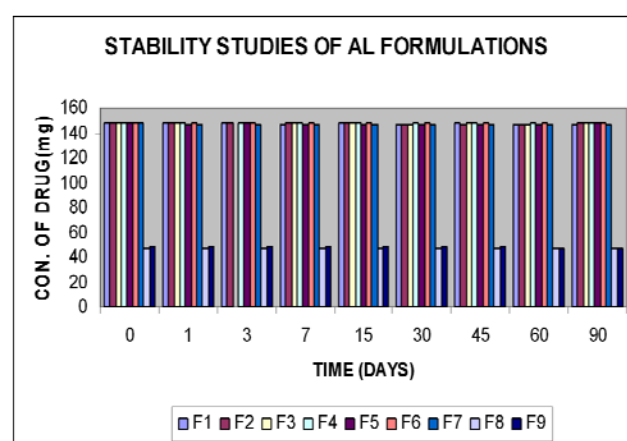
Formulation code	Compressibility index %	Bulk density gm/ml	True density gm/ml	Angle of repose (° C)	
				Before adding glidants	After adding glidants
F1	7.80	0.460	0.500	30.27	25.00
F2	9.36	0.434	0.476	30.23	24.99
F3	10.21	0.444	0.50	30.96	26.56
F4	12.86	0.413	0.475	30.91	25.44
F5	12.68	0.416	0.476	29.37	25.9
F6	11.79	0.480	0.568	29.06	26.65
F7	11.23	0.426	0.466	30.41	25.15
F8	10.01	0.439	0.483	31.28	26.00
F9	12.84	0.445	0.506	30.70	26.77

Table 3. Physical Characteristics of Colon Targeted tablets containing praziquantel or praziquantel- β Cyclodextrin (n=3)

Formula code	Weight (mg)	Hardness (kg/cm ²)	Thickness (mm)	Drug content (mg)	% Fraibility	Disintegration Time
F 1	394.6±2.54	3.9±0.141	2.856± 0.02	147.61±0.441	0.76	0.5 h
F 2	404.65±2.04	4.08±0.19	2.89± 0.0141	147.8± 0.237	0.419	ND
F 3	401.95± 2.44	3.92±0.166	2.92± 0.024	147.77±0.103	0.322	ND
F 4	406.1±2.1	3.22±0.246	3.27± 0.046	148.41±0.424	0.942	ND
F 5	404.2±3.05	3.28±0.214	3.02± 0.036	147.74±0.362	0.321	ND
F 6	406.3±2.74	3.22±0.13	3.016± 3.02	148.36±0.391	0.368	ND
F 7	401.5±2.87	3.88±0.203	3.34±1.05	147.5±0.408	0.88	ND
F 8 ^s	591.4± 2.72	4.16±0.14	5.21±.053	47.61±0.388	0.98	ND
F 9 ^s	591.2±2.86	4.26±0.174	5.20±0.044	48.85±0.373	0.952	ND

ND = not disintegrated

§ = 600 mg tablet weight

Fig 1. In vitro drug release from all formulations**Fig 2.** Stability studies of all formulations

Tab 4: *In vitro* release data of praziquantel from the formulations F1- F9

S. No	Time (h)	Cumulative % drug release								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	0	0	0.0	0	0	0	0	0	0	0
2	1	65.84 ±6.37	14.52 ±1.06	12.95 ±1.08	1.51 ±0.01	18.29 ±2.15	18.21 ±0	3.05 ±1.07	4.73 ±0	6.91 ±3.26
3	1.5	68.19 ±3.22	15.30 ±0.01	13.71 ±0	2.27 ±1.07	19.8 ±0	18.97 ±1.07	3.05 ±2.15	4.73 ±0	9.21 ±0
4	2	82.69 ±3.16	18.35 ±0.00	16.0 ±1.08	3.03 ±2.15	22.10 ±1.08	20.49 ±1.07	4.57 ±1.07	4.73 ±0	9.2 ±0
5	2.5	88.11 ±1.57	19.8 ±0.00	17.52 ±1.08	3.03 ±0	23.63 ±1.08	22.01 ±1.07	5.34 ±1.07	9.45 ±0	11.52 ±3.26
6	3	88.14 ±1.6	22.18 ±1.08	19.05 ±1.08	4.55 ±1.08	26.67 ±1.08	23.53 ±1.07	6.10 ±1.07	9.46 ±0	13.83 ±0
7	4	-	24.47 ±0.01	19.81 ±3.15	4.55 ±0	28.96 ±0	25.80 ±0	7.63 ±2.15	9.46 ±0	13.83 ±0
8	5	-	26.01 ±0.00	22.86 ±0	6.07 ±2.15	31.25 ±1.08	26.56 ±1.07	9.16 ±1.07	11.82 ±3.34	16.13 ±3.26
9	6	-	29.06 ±0.00	24.38 ±2.15	6.85 ±1.11	32.77 ±1.08	29.60 ±1.07	10.68 ±1.07	14.19 ±0	18.44 ±0
10	7	-	31.36 ±1.08	25.91 ±0	7.58 ±0	34.30 ±1.08	30.36 ±1.07	10.68 ±2.15	16.55 ±3.34	20.74 ±3.26
11	8	-	35.1 ±0.01	28.95 ±0	9.10 ±0	35.82 ±1.08	31.88 ±1.07	12.21 ±0.12	18.92 ±0	20.74 ±3.26
12	9	-	36.73 ±0.00	31.24 ±1.08	9.86 ±1.07	38.11 ±2.15	33.40 ±2.14	13.74 ±1.07	21.28 ±3.34	23.05±0
13	10	-	39.02 ±1.08	33.53 ±0	11.38 ±1.08	41.92 ±1.08	35.67 ±3.22	16.032 ±3.02	23.65 ±0	25.35 ±3.26
14	11	-	41.31 ±0.01	35.81 ±1.08	12.89 ±1.08	43.45 ±1.08	37.19 ±4.29	17.56 ±2.15	26.01 ±3.34	27.66 ±0
15	12	-	42.85 ±2.05	38.10 ±0	12.9 ±1.08	46.49 ±1.08	39.47 ±5.36	17.56 ±1.07	30.74 ±3.35	29.96 ±3.26
16	15	-	45.9 ±0.00	41.15 ±1.08	15.17 ±0	51.07 ±1.08	43.26 ±6.43	19.08 ±1.07	30.75 ±3.35	34.57 ±3.26
17	18	-	49.7 ±1.08	48.0 ±1.08	19.72 ±2.14	56.40 ±0	50.09 ±4.29	23.66 ±1.07	35.47 ±3.35	39.18 ±3.26
18	24	-	62.69 ±0.01	55.62 ±1.08	36.40 ±2.15	69.35 ±1.08	62.99 ±1.07	45.02 ±2.15	66.2 ±0	71.43 ±3.26

Table 5. Data of Stability studies of Praziquantel tablet formulations at 40°C / 75% RH

Times (Days)	Formulation code								
	F1 DC (mg)	F2 DC (mg)	F3 DC (mg)	F4 DC (mg)	F5 DC (mg)	F6 DC (mg)	F7 DC (mg)	F8 DC (mg)	F9 DC (mg)
0	147.61	147.8	147.77	148.41	147.74	148.36	147.5	47.61	48.85
1	147.5	147.75	147.5	148	147.25	148	147.	47	48
3	147.5	147.75	147.2.5	148	147.5	148	146.75	47.5	48.25
7	147.25	147.75	147.5	147.75	147.25	148	147.25	47.25	48.5
15	147.5	147.5	147.77	147.9	147.25	147.5	147	47	48.25
30	147.25	146.75	147.25	148	147	148	147	46.75	48.25
45	147.5	147	147.5	148.25	146.75	148.25	146.75	47.5	48
60	147	147.25	147	148	147	147.75	147	47.25	47.8
90	147	147.5	147.75	147.75	147.5	148.25	147.25	47	47.5

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 27° hence were regarded to be easily flowing. Hardness of the tablet varied between 3.16 ± 0.101 Kg/cm² to 4.08 ± 0.19 Kg/cm² indicating good binding and satisfactory strength of tablets to withstand 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 for a period of 3 months. The results of the *in vitro* study has indicated that, the colon targeted tablets containing 40% w/w of xanthan gum, guar gum, eudragit RSPO showed maximum drug release in colonic environment 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 praziquantel- β CD complex would release more percentage of drug 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 praziquantel can be effectively increased by forming inclusion complex with β CD. Xanthan gum and Guar gum at 40 % w/w in a tablet would target the tablet to colon.

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