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DESIGN, FORMULATION DEVELOPMENT AND *IN VITRO* EVALUATION OF MUCOADHESIVE TABLETS OF CLOTRIMAZOLE-β CYCLODEXTRIN COMPLEXES

Naga Prasad Arepalliy[*1], Malleswara Rao Pillaboina[2], K. Navya Sree[3], K. L. N. Mallikharjunarao[4], K. V. V. S. Ujwala[5], M. Manjula[6]

[*1]Scientist II, Santha Biotechnics Ltd, A Sanofi Company, Medchal, Hyderabad, A.P, India.
[2]Department of Pharmaceutics, A.M.Reddy Memorial College of Pharmacy, Petlurivaripalem, Narasaraopeta, Guntur, A.P, India.
[3]Department of Pharmacology, Jawaharlal Nehru Technological University, Kakinada, East Godavari, A.P, India.
[4]Department of Biotechnology, AKRG College of Pharmacy, West Godavari, A.P, India.
[5]Department of Pharmacology, Giet School of pharmacy, East godavari, A.P, India.
[6]Department of Pharmacology, Jawaharlal Nehru Technological University, Vijaya Nagaram, A.P, India.

ABSTRACT

Clotrimazole is an antifungal agent widely used as a first line treatment for oral candidiasis. Clotrimazole is a poorly water soluble drug, so solubility is main constraints for its oral bioavailability. An attempt has been made to increase its solubility by complexation with β-cyclodextrin and then formulating mucoadhesive tablets of best formulation with β-cyclodextrin. The phase solubility analysis indicated the formation of 1:1 molar inclusion complexes. The inclusion complexes prepared by different methods viz. Physical mixture, Kneading and Solvent evaporation methods. The prepared complexes were characterized using DSC. Mucoadhesive tablet formulations were prepared by direct compression technique using four mucoadhesive polymers namely Carbopol 974P, HPMC K15M and HPMC K4M. The entire formulation blend was evaluated Precompression studies and prepared mucoadhesive tablets were evaluated for post compression studies and the results were found to be within the limits. The compatibility studies were performed which resulted in no interactions between drug and excipients.

KEY WORDS

Clotrimazole, β-cyclodextrin**,** HPMC, Carbopol 974P and Mucoadhesive tablet.

Author of correspondence:
Naga Prasad Arepalliy,
Scientist II, Santha Biotechnics Ltd,
A Sanofi Company, Medchal,
Hyderabad, Andhra Pradesh, India.

Email: nagaprasad79@gmail.com.

INTRODUCTION

Mucoadhesion as a new strategy to improve the efficacy of various drug delivery system. Potential of mucoadhesive polymers was shown in ocular, nasal, vaginal and buccal drug delivery systems leading to a significantly prolonged residence time of sustained release delivery systems on these mucosal membranes[1]. In addition, the development of oral

mucoadhesive delivery systems was always of great interest as delivery systems capable of adhering to certain gastrointestinal (GI) segments would offer various advantages. However, mucoadhesive drug delivery systems have so far not reached their full potential in oral drug delivery, because the adhesion of drug delivery systems in the GI tract is insufficient to provide a prolonged residence time of delivery systems in the stomach or small intestine. The conventional dosage forms stays in the stomach for 0.5-3 hours and passes to small intestines from where it gets absorbed within 3-6 hours. It is therefore difficult to adjust release retardation and stomach retention for longer period of time. Some antibiotics produce effect depending on concentration at the site of bacterial infection. The bioavailability of active ingredients which are not completely absorbed decreases because part of the dose is lost, so frequent administration of dosage form is required. In the present investigation an attempt has been made to develop controlled release mucoadhesive tablets containing an antifungal agent, clotrimazole to release the drug unidirectionallly in buccal cavity for extended periods of time for improvement in bioavailability, to reduce the dosing frequency and to improve the patient compliance for an effective and safe therapy of oral candidiasis. Since the drug has poor absorption window orally and highly lipophilic, therefore it was planned to improve its solubility, by forming inclusion complex with β- cyclodextrin for optimized formulation and to study the effect of hydrophilic additives like HPMC K4M, HPMC K15M and Carbopol on release rate of the drug.

MATERIALS AND METHOD[2-7]

Materials

Clotrimazole was procured by Biochem Pharmaceutical Daman India, HPMC K4M, HPMC K15M and remaining excipients β-Cyclodextrin, Lactose, Magnesium Stearate, Talc were obtained from Richer Pharmaceuticals Pvt Ltd, Hyderabad. All materials used were of analytical grade.

Method

Phase solubility studies

Phase solubility studies were carried out at room temperature (25°) in triplicate according to the method reported by Higuchi and Conners. Excess amount of drug was added to distilled water containing various concentrations of β -CD (0-15 mM) in a series of stoppered conical flasks and shaken for 48 h on a rotary flask shaker. The suspensions were filtered through Whatman No. 1 filter paper and assayed for clotrimazole using a UV/Vis spectrophotometer (Shimadzu, UV 1601) at 262 nm against blanks prepared using same concentration of β -CD in distilled water.

Preparation and characterization of clotrimazole β-cyclodextrin complex

Physical mixture

1:1 molar ratio of clotrimazole, β-CD were transferred to a mortar and mixed for about one hour with constant trituration, passed through sieve no. 80 and stored in a desiccators over fused calcium chloride (Table No.4).

Kneading method

Clotrimazole with β-CD in 1:1 molar ratio was taken. First cyclodextrin is added to the mortar, small quantity of 50 % ethanol is added while triturating to get slurry like consistency. Then slowly drug is incorporated into the slurry and trituration is further continued for one hour. Slurry is then air dried for 24 h, pulverized and passed through sieve no. 80 and stored in desiccators over fused calcium chloride.

Solvent evaporation Method

Drug and cyclodextrin in 1:1 molar ratio are dissolved in a common solvent to get a clear solution. Mixed the both solutions than the clear solution was kept for stirring on a magnetic stirrer till all the solvent got evaporated. The mass obtained was dried at 50°C and further sieved no. 80 or 100 sieve.

Evaluation of Clotrimazole Inclusion Complexes[8-17]

Solubility study

The solubility of both the drug and clotrimazole-β-cyclodextrin inclusion complex was determined in distilled water, methanol, ethanol and phosphate buffer pH 6.8 according to the method proposed by Die C H *et. al.*[8]. Triplicate readings were taken and average was calculated.

Drug Content Estimation

Inclusion complexes prepared by above methods were assayed for clotrimazole content by dissolving a specific amount of the complexes (Drug Equivalent to 100mg) in methanol and analyzing for the clotrimazole content spectrophotometrically at 262 nm on a spectrophotometer.

Differential scanning calorimetry

DSC was used to confirm the formation of inclusion complex. It was conducted by using Perkin Elmer DSC7, USA apparatus at a heating rate of 10/min over a 30-300^{0}C or 30-225^{0}C temperature range (Figure No.2).

***In vitro* dissolution profile**

Dissolution studies were carried out by USP Paddle method at 37 ± 0.5°C, taking 500 ml of simulated saliva solution pH 6.75 as dissolution medium. Speed of rotation of the paddle was set at 100 rpm for 12 hrs. Absorbance was measured at 262 nm in a Shimadzu UV- Spectrophotometer.

Compression of clotrimazole and β-cyclodextrin inclusion complexes into mucoadhesive tablets by direct compression method

After elucidation of best inclusion complex of drug with β-cyclodextrin which shows that the most satisfactory *in vitro* dissolution criteria and better solubility criteria, the particular complex was formulated as mucoadhesive tablets of clotrimazole β-cyclodextrin Inclusion Complex by mixing it with selected excipients. In this present study HPMC K4M. HPMC K15M, Carbapol selected as mucoadhesive polymers, Lactose selected as diluent, Talc as glidant and Magnesium stearate selected as lubricant. The prepared inclusion complex of drug and excipients were passed through sieve (#60) and mixed thoroughly. The drug and excipients mixture was finally compressed after lubricating with magnesium stearate for 10 min.

Evaluation of Precompression Characteristics of Tablet

Blend Precompression parameters

The prepared tablet blend was evaluated for Precompression parameters like Bulk density, Tapped density, compressibility index, Angle of repose and Hausner's ratio to know the flowability of blend (Table No.5).

Evaluation of prepared tablets

The prepared tablets were evaluated for parameters like hardness, thickness, friability, drug content uniformity, weight variation, Mucoadhesive strength and Force, Swelling Study, *in vitro* disintegration and *in vitro* drug release studies.

Mathematical Modeling of Dissolution Data

The release data obtained were fitted into various mathematical models to know which mathematical model was best to fit the obtained release profile (Table No.8). The parameters; the time exponent (n), the release rate constant (k), the regression coefficient (R2), were determined for Korsmeyer-Peppas equation to know the release mechanism. The various models studied were

1. Zero order
2. First order
3. Higuchi model and
4. Peppas model

The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows,

1. Cumulative % of drug released Vs Time (zero order kinetic models)
2. Log cumulative percent drug remaining to be absorbed Vs Time (First order model)
3. Cumulative % of drug released Vs Square root of time (Higuchi model)
4. Log % drug release Vs Log time (Peppas model).

RESULTS AND DISCUSSION

Dissolution, assay and Physical appearance studies of selected formulation F12 and of Clotrimazole complexes prepared by Kneading method (CK) were carried out after subjecting the formulation for

stability study (Table No.2, Table No.9 and 10. From the data, the formulation is found to be stable under the conditions mentioned before since there was no significant change in the percentage amount of drug content (Table No.2 and 3). In the present work, complexation of Clotrimazole with β-cyclodextrin was tried in an attempt to improve its solubility and dissolution rate, the phase solubility studies revealed a linear relationship between the aqueous drug solubility with increasing in β-CD concentration shown in Table No.1, 6, 7 and Figure No.1.

Table No.1: Solubility of Clotrimazole and Clotrimazole β-CD inclusion complex

S.No	Water (mg/ml)		Ethanol (mg/ml)	Methanol (mg/ml)	Phosphate buffer pH 6.8 (mg/ml)
1	Drug	Drug : β-CD inclusion complex(1:1M)	Drug	Drug	Drug
2	0.082	0.726	92.87	103.4	0.118

Table No.2: Drug content Estimation

S.No	Complexation Method	Drug: Cyclodextrin Ratio	Complex Code	Amount of drug present in 100mg Equivalent powder	%Drug content
1	Physical Mixture	1:1	CP	100.5	100.5
2	Kneading Method	1:1	CK	101.4	101.4
3	Solvent evaporation method	1:1	CS	99.85	99.85

Table No.3: Comparison of *in vitro* dissolution data of all complexes with respect to pure drug dissolution

S.No	Time (min)	% CDR			
		Pure Drug	CP	CK	CS
1	0	0	0	0	0
2	10	6.52322	39.6516	53.6543	47.8483
3	20	20.5479	58.4357	74.1461	56.0450
4	30	25	63.5587	82.3429	77.2199
5	40	26.0274	78.2445	85.4166	79.2691
6	50	27.7397	81.3183	90.8811	82.6844
7	60	28.0821	83.3674	93.9549	87.4658

Table No.4: Formulations composition of CL tablet of F 1 to F 13

S.No	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
1	Clotrimazole	-	-	-	-	-	-	-	-	-	-	-	-	100
2	(CL : β-CD)	346	346	346	346	346	346	346	346	346	346	346	346	346
3	HPMC K4M	60	80	100	-	-	-	95	85	70	-	-	-	-
4	HPMC K15M	-	-	-	60	80	100	-	-	-	90	80	70	70
5	Carbopol	-	-	-	-	-	-	10	20	30	10	20	30	30
6	Talc % W/W	6	6	6	6	6	6	6	6	6	6	6	6	6
7	Magnesium Stearate	3	3	3	3	3	3	3	3	3	3	3	3	3
8	Lactose	85	65	45	85	65	45	40	40	45	45	45	45	291

* Total weight of tablet – 500 mg **Note:** All ingredients are taken in mg

Table No.5: Evaluation of Pre-Compression Parameters

S.No	Formulation code	Evaluation of Precompression parameters				
		Angle of repose* (θ)± SD	Bulk density* (g/cc)±SD	Tapped density* (g/cc) ± SD	Carr's index* (%) ±SD	Hausner's Ratio*± SD
1	F1	29.56±0.27128	0.410±0.002887	0.483±0.006928	16.40±0.687047	1.195±0.009815
2	F2	28.46±0.178979	0.426±0.005774	0.505±0.008083	15.68±0.207846	1.185±0.002887
3	F3	28.15±0.17979	0.43±0.005196	0.508±0.007506	15.4±0.225167	1.181±0.002887
4	F4	27.52±0.18752	0.427±0.00774	0.492±0.00750	13.19±0.15011	1.151±0.002309
5	F5	28.56±0.178979	0.442±0.005774	0.527±0.00866	16.06±0.277128	1.191±0.00346
6	F6	27.63±0.184752	0.41±0.004677	0.481±0.006928	14.75±0.14433	1.172±0.002309
7	F7	30.32±0.219393	0.421±0.00519	0.508±0.00750	17.17±0.196299	1.206±0.00288
8	F8	29.17±0.121244	0.403±0.00461	0.485±0.00692	16.76±0.23094	1.195±0.00866
9	F9	29.1±0.121244	0.426±0.00519	0.508±0.00346	16.14±0.45033	1.192±0.006351
10	F10	28.46±0.178979	0.426±0.005774	0.505±0.008083	15.68±0.207846	1.185±0.00288
11	F11	28.15±0.17897	0.43±0.00519	0.508±0.00750	15.4±0.225167	1.181±0.00288
12	F12	27.63±0.184752	0.41±0.004677	0.481±0.006928	14.75±0.144338	1.172±0.002309
13	F13	29.1±0.121244	0.426±0.00519	0.508±0.00346	16.14±0.45033	1.192±0.00635

Table No.6: Evaluation of Post Compression parameters

S.No	Formulation Code	Hardness (Kg/cm^2)	Thickness (cm)	% Friability	Weight variation (mg)	% Drug content	MucoadhesiveStrength (gm)
1	F1	6.5 ±0.158	4.2 ±0.004	0.56	498 ±1.24	100.34	36.65±2.24
2	F2	6.6 ±0.876	4.2 ±0.005	0.62	504 ±1..54	100.58	37.42±2.53
3	F3	6.5 ±0.956	4.3 ±0.005	0.45	502 ±2.25	99.75	38.75±1.86
4	F4	7.4 ±0.545	4.3 ±0.008	0.48	502 ±2.6	99.89	37.12±1.89
5	F5	6.9 ±0.234	4.2 ±0.004	0.58	501 ±2.05	100.5	37.54±1.98
6	F6	6.8 ±0.230	4.4 ±0.008	0.57	499 ±1.18	100.2	39.88±1.75
7	F7	7.1 ±0.142	4.3 ±0.007	0.49	498 ±1.90	99.8	41.52±2.54
8	F8	7.1 ±154	4.4 ±0.009	0.55	502 ±1.89	100.2	42.55±2.12
9	F9	6.9 ±0.355	4.4 ±0.007	0.61	501 ±1.78	100.1	44.89±2.09
10	F10	6.8 ±0.267	4.3 ±0.007	0.48	498 ±1.85	99.75	42.76±1.95
11	F11	6.7 ±0.325	4.3 ±0.091	0.58	504 ±1.55	99.98	44.95±1.78
12	F12	6.7 ±0.348	4,4 ±0.089	0.49	503 ±1.83	100.2	46.68±1.89
13	F13	6.5 ±0.225	4.5 ±0.07	0.61	499 ±2.99	99.79	40.74±2.45

Table No.7: *In vitro* drug release for all tablets formulation

S.No	Time(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1	12.25	13.78	15.20	14.24	16.73	16.78	13.56	15.14	15.24	17.28	17.16	16.46	15.72
3	2	25.50	26.88	29.31	26.31	30.23	33.42	27.48	30.25	34.39	32.24	35.35	40.67	20.55
4	3	40.72	42.35	44.36	43.54	45.65	50.21	43.21	44.49	46.25	53.36	57.48	61.56	24.28
5	4	53.29	55.43	56.65	56.49	58.92	60.27	56.67	57.28	60.38	67.34	71.62	72.48	27.84
6	5	63.75	64.98	66.45	68.18	69.31	71.29	66.92	68.18	69.75	74.48	76.95	75.25	29.78
7	6	63.89	65.86	67.91	69.46	72.23	74.78	68.21	70.45	73.36	76.78	78.49	81.64	30.14
8	7	64.45	66.9	69.16	70.83	73.48	76.47	70.12	71.26	75.38	78.61	80.36	83.76	31.45
9	8	65.68	67.21	71.85	72.26	74.90	77.23	72.84	73.97	77.87	79.08	82.54	85.42	31.85
10	9	66.54	68.87	72.43	73.48	75.37	79.98	74.49	75.69	79.28	83.03	84.34	88.29	32.5
11	10	68.85	72.65	75.82	74.21	77.19	80.42	76.83	77.25	82.14	85.78	87.67	91.23	34.75
12	11	69.29	74.35	78.34	75.42	80.25	82.87	77.26	78.92	83.08	86.37	91.68	94.56	38.85
13	12	73.55	77.85	80.24	76.29	82.93	84.22	78.87	80.69	84.45	87.62	92.85	96.74	44.55

Table No.8: Curve fitting data analysis for all tablet formulations

S.No	Formulation Code	Zero Order	First Order	Higuchi	Korsemeyer Peppas	
		R^2	R^2	R^2	Slope(n)	R^2
1	F1	0.790	0.873	0.867	0.697	0.894
2	F2	0.813	0.912	0.893	0.652	0.904
3	F3	0.824	0.935	0.910	0.633	0.916
4	F4	0.785	0.872	0.862	0.658	0.895
5	F5	0.810	0.923	0.895	0.617	0.915
6	F6	0.801	0.927	0.892	0.610	0.905
7	F7	0.816	0.920	0.897	0.677	0.907
8	F8	0.815	0.925	0.900	0.639	0.912
9	F9	0.820	0.946	0.909	0.640	0.908
10	F10	0.792	0.940	0.879	0.620	0.894
11	F11	0.793	0.963	0.881	0.620	0.883
12	F12	0.802	0.976	0.894	0.625	0.871
13	F13	0.825	0.870	0.927	0.361	0.957

Table No.9: Stability data of optimized formulation BK2 stored at Room temperature and at 40^0C for six

S.No	Time	Stability data of CK stored at Room temperature		Stability data of CK stored at 40^0C	
		Physical appearance (Colour)	% Drug Content	Physical appearance (Colour)	% Drug Content
1	First day	White	100.45	White	99.85
2	1^{st} week	White	99.98	White	100.46
3	3^{rd} week	White	101.6	White	101.45
4	6^{th} week	White	100.79	White	100.87

Table No.10: Stability data of optimized formulation F12 stored at Room temperature and at 40^{0}C for six weeks

S.No	Time	Stability data of CK stored at Room temperature		Stability data of CK stored at 40^{0}C	
		Physical appearance (Colour)	% Drug Content	Physical appearance (Colour)	% Drug Content
1	First day	White	99.82.45	White	101.5
2	1st week	White	100.26	White	99.94
3	3rd week	White	101.3	White	100.52
4	6th week	White	100.84	White	101.34

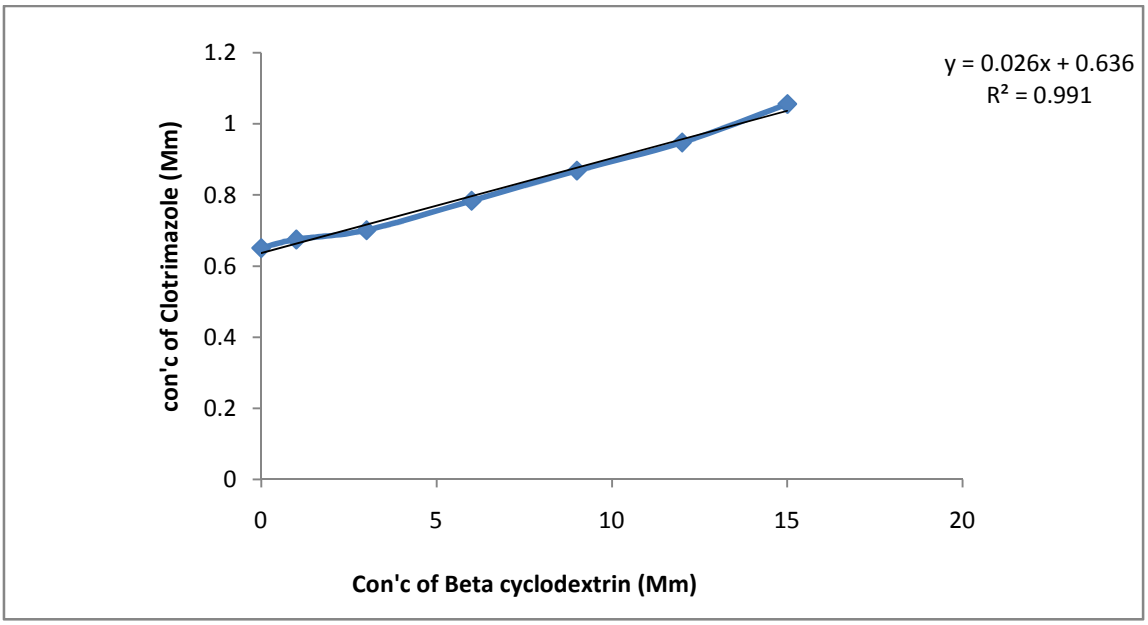

Figure No.1: Phase solubility studies

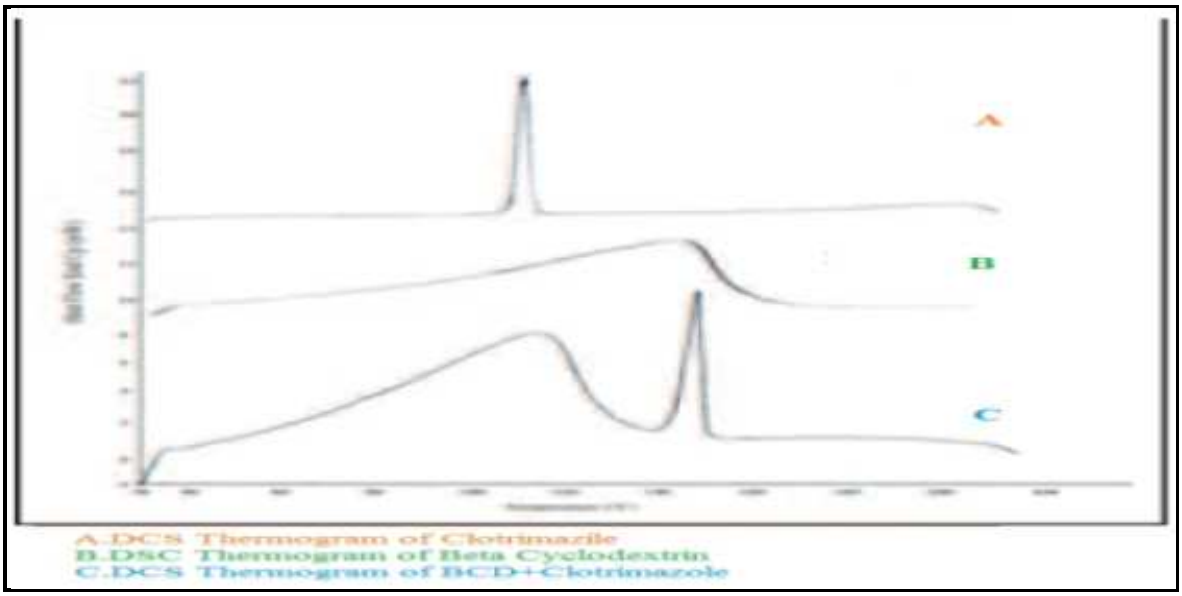

Figure No 2: DSC Studies

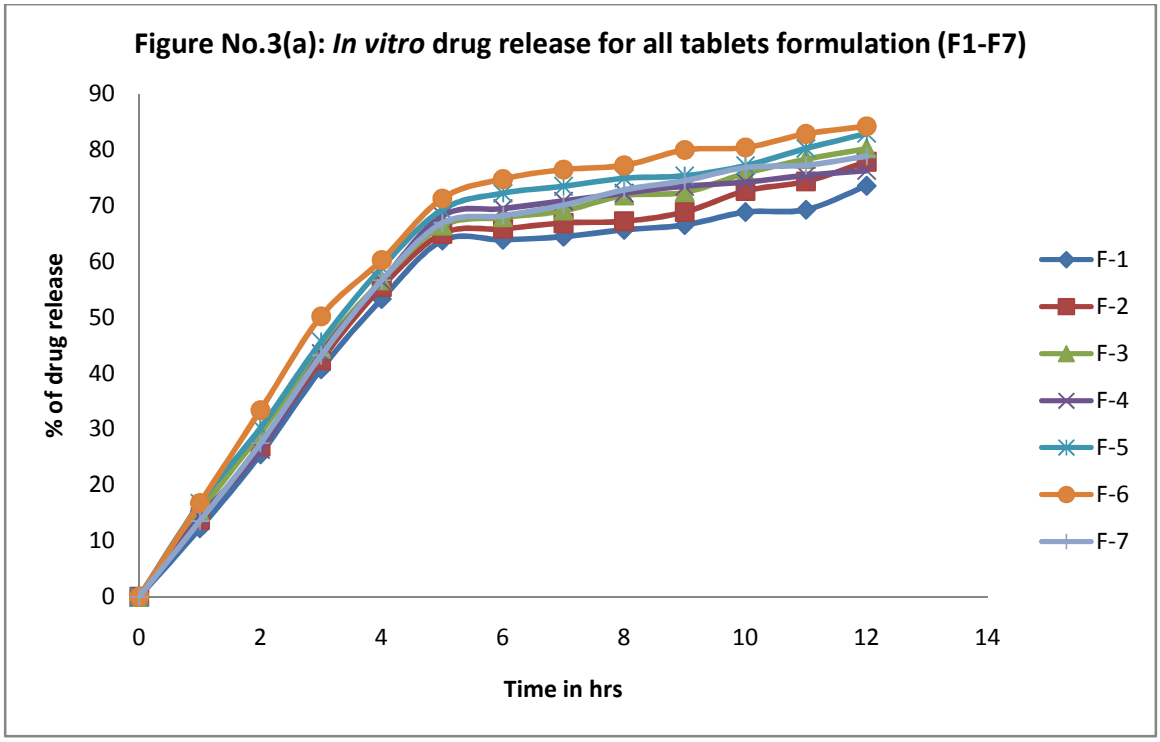

Figure No.3 (a): *In vitro* drug release for all tablets formulation (F1-F7)

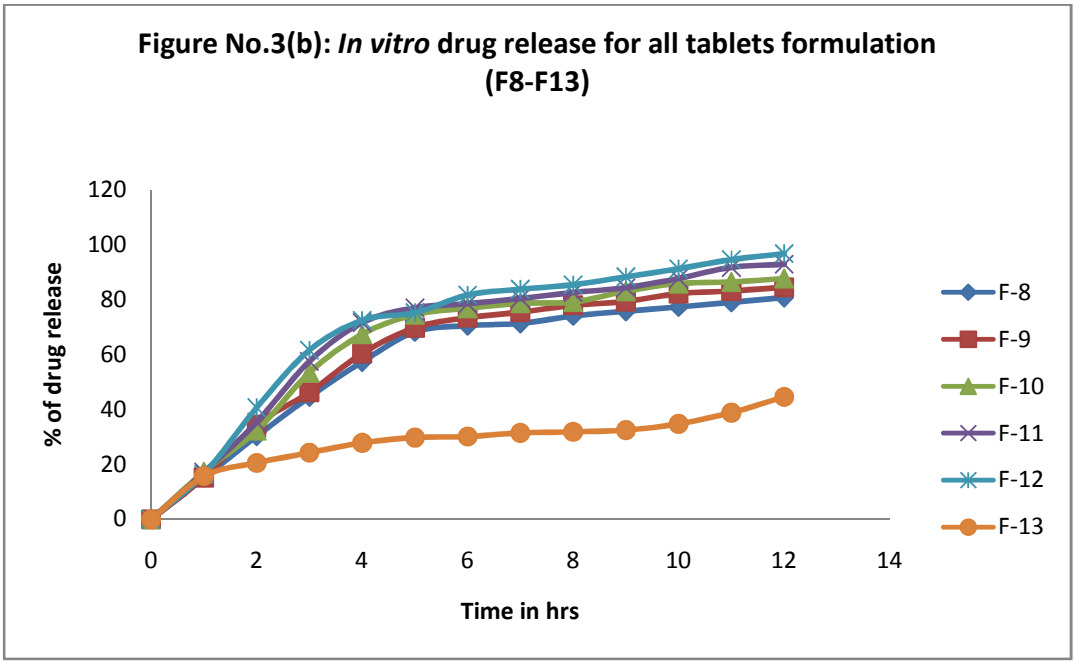

Figure No.3 (b): *In vitro* drug release for all tablets formulation (F8-F13)

CONCLUSION

The present investigation aimed at successful development and optimization of mucoadhesive tablets of clotrimazole. Based on the in vitro dissolution studies, it was found that formulation F12 (complexing the clotrimazole with β-cyclodextrin (1:1 molar ratio) by Kneading method) showed maximum drug release in 12 hrs. Stability studies were performed for formulation F12 and CK Complexes as per ICH guidelines. The formulations showed no significant variations in the drug content and they were stable for specified time period. Finally it was concluded that β-CD can be used to improve the oral bioavailability of Clotrimazole and Carbopol, HPMC K4M, HPMC K15M polymers can be successfully used in the formulation on mucoadhesive tablets of Clotrimazole.

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