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FORMULATION AND EVALUATION OF BILAYER TABLET CONTAINING METRONIDAZOLE AS AN IMMEDIATE RELEASE LAYER AND NORFLOXACIN AS A SUSTAIN RELEASE LAYER

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ABSTRACT:
The aim of this work is to formulate Sustained bilayer tablets of Norfloxacin (NFX) and Metronidazole (MET) by wet granulation by using different ratios of Xanthan gum and Carbopol. Metronidazole is a synthetic antibacterial, anti-amoebic and anti protozoal agent. Norfloxacin used as antibacterial agent. The Combination of MET with NFX is expected to be improving effectiveness of therapy in comparison with individual drugs. Xanthan gum and Carbopol were selected because they were reported to give sustained release and naturally occurring. The MET and NFX bi-layer sustained release tablets were prepared by wet granulation method by using different polymers such as xanthan gum and carbopol as sustained release polymers and along with Poly vinyl pyrrolidine K 30 (PVP K30) as binding agent. The NFX Sustained release and MET immediate release was evaluated for morphological characteristic, physical characteristic, chemical characteristic and stability. The results obtained were satisfactory and within specified limits as per Pharmacopoeias. MET immediate layer released 98% to 99% within 2 hours. But NFX sustained layer 99.5% drug released up to 12 hours in F8 formulation. So, F8 was showed good results compare to other formulations. Formulation F8 fulfills our objective of formulating a bilayer tablet of sustained release of NFX and immediate release layer of MET.

Key words: *Sustained bilayer tablets, Norfloxacin, Metronidazole, Xanthan gum, Carbopol.*

INTRODUCTION:

In the recent times, bi-layer tablets are gaining importance in the design of oral controlled drug delivery systems. Bi-layer tablets are novel drug delivery systems where combination of two or more drugs in a single unit[1]. They are preferred for the following reasons: to co-administer two different drugs in the same dosage form, to minimize physical and chemical incompatibilities, for staged drug release, IR and SR in the same tablet, for chronic condition requiring repeated dosing. In the present study a combination drug therapy is recommended for treatment of amoebiasis and other colonic infections to allow medications of different mechanism of action to complement each other and together effectively combat the microbes at lower than maximum doses of each[2,8]. The rational for combination therapy is to encourage the use of lower doses of drug to minimize dose dependent side effects and adverse reactions.

MET is a highly bitter drug, used in the treatment of intestinal protozoal infection like a moebiasis, giardiasis, trichomonas vaginitis[3] etc. These drugs are to be delivered to the colon for their effective action against E. histolytica wherein the trophozoites reside in the lumen of the caecum and large intestine and adhere to the colonic mucus and epithelial layers. But the pharmacokinetic profile of metronidazole indicates that the drug is completely and promptly absorbed after oral administration reaching a concentration in plasma of about 10 µg/ml approximately 1 hr after a single 500 mg dose. The administration of this drug in conventional tablet dosage form provides minimal amount of metronidazole for local action in the colon, still resulting in the relief of amoebiasis, but with unwanted systemic effects [4].

Norfloxacin, l-ethyl-6-fluoro-1,4-di-hydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid, is a synthetic antibacterial fluoroquinolone [5]. Quinolones belongs to synthetic class of antimicrobial agents with potent antimicrobial activity which are effective orally and parentally for a wide variety of infectious diseases [6]. It is active on both actively dividing as well as dormant bacteria by inhibiting bacterial DNA gyrase. It is effective in the treatment of urinary tract infections, gonococcal urethritis and infectious diarrhea [7].

The objective of the study is to design and evaluate bilayer tablets of MET and NFX using polymers such as Xanthan gum and Carbopol. And to carry out the Pre and post compressional parameters for the powder blend of bilayered tablets as well as final finished dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents:

Table 1 shows the chemical/reagent and drugs and their source. And table 2 shows the source of instruments used for this study.

Table 1: Source of chemicals and ingredients

S. No.	Ingredients/chemicals/solvents	Manufacturer /supplier
1	Norfloxacin	Medrich Labs, Bangalore
2	Metronidazole	Aurobindo Pharma, Hyderabad
3	Xanthan gum	Colorcon Asia private limited
4	Carbopol	Colorcon Asia private limited
5	PVP K30	BASF corporation
6	Iso propyl alcohol	Qualigens fine chemicals
7	Stearic acid	DMV- Fonterra excipients
8	Starch	DMV- Fonterra excipients
9	Talc	Signet chemical corporation pvt ltd
10	Di potassium hydrogen phosphate	S.D. fine chemicals

All the chemicals were of AR grade.

Table 2: Manufacturers of Instruments/Apparatus

S.NO	INSTRUMENT/APPARATUS	MANUFACTURER
1	Tablet pilot press, 9 stations	Chamunda pharma pvt Ltd, Ahmedabad. (Model PP-1).
2	Friability test apparatus	Singhala scientific industries, Ambala.
3	Monsanto hardness tester	Singhala scientific industries, Ambala.
4	Dissolution test apparatus (USP Type II),	Electro lab, Mumbai.(Model: TDT-08L)
5	UV Spectrophotometer	Schimadzu
6	FTIR	Schimadzu
7	Digital vernier caliper	Absolute Digimate, industrial gin stores, Hyderabad
8	Digital balance	LCGC Chromatographic solution, Hyderabad
9	pH Meter	Singhala scientific industries, Ambala.
12	Glass wares	--

Methods:

Formulation of tablets:

Sustained release Bilayer metronidazole and Norfloxacin tablets were prepared by wet granulation method. Various batches were prepared by changing the ratio of Xanthan gum and Carbopol to identify the most effective formulation. Norfloxacin and polymer mixture were prepared by homogeneously mixing with Xanthan gum, Carbopol, Starch dried, PVP, Talc and Stearic acid in a glass mortar for 15 minutes. The powder was screened through a 60 µm sieve to get uniform granules.

Simultaneously Metronidazole and polymer mixture were prepared by homogeneously mixing with Starch (Dried) and PVP, Talc and Stearic acid in a glass mortar for 15 mins. The powder was dried for 15 mins in hot air oven and screened through 60 µm sieve to get uniform granules. Metronidazole and Norfloxacin granules were then compressed using an 8 mm diameter die in a 9-station rotary punching machine (Ahmadabad, India). The upper punch was raised and the Metronidazole granules were compressed, again Norfloxacin granules were placed on the 1st compressed metronidazole layer. The two layers were then compressed into a bilayer tablet. Each tablet weighed 600 mg. Table 3 summarizes the formulas to prepare bylayer immediate release metronidazole and sustain release Norfloxacin.

Table 3: Composition of immediate release layer of metronidazole and sustain release Norfloxacin bylayer tablets

Formulation codes		F1	F2	F3	F4	F5	F6	F7	F8	F9
INGREDIENTS	**Immediate Release layer (mg)**									
	Metronidazole	250	250	250	250	250	250	250	250	250
	Starch Dried	28	28	28	28	28	28	28	28	28
	PVP	15	15	15	15	15	15	15	15	15
	Stearic acid	5	5	5	5	5	5	5	5	5
	Talc	2	2	2	2	2	2	2	2	2
	Sustained Release layer (mg)									
	Norfloxacin	150	150	150	150	150	150	150	150	150
	Xanthan gum	50	75	100	-	-	-	50	50	25
	Carbopol	-	-	-	50	75	100	25	50	50
	Starch Dried	83	58	33	83	58	33	58	33	58
	PVP	10	10	10	10	10	10	10	10	10
	Stearic acid	5	5	5	5	5	5	5	5	5
	Talc	2	2	2	2	2	2	2	2	2
	IPA	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
	Total Weight (mg)	**600**	**600**	**600**	**600**	**600**	**600**	**600**	**600**	**600**

Evaluation of Granules Flow Properties [1, 8]:

The prepared granules were evaluated for parameters like bulk density, taped density, Carr index, Angle of repose, and Hausner's ratio, loss on drying. The results are as in table 4 and 5.

Evaluation of tablets [1, 8]:

The prepared formulas were subjected to following tests:

Thickness

The thickness of the each tablet was measured by using vernier caliper and the average thickness was calculated.

Weight variation

Formulated tablets were tested for weight uniformity, 20 Tablets were weighed collectively and individually. From the collective weight, average weight was calculated. The percent weight variation was calculated by using the following formula.

Hardness

The hardness of Tablets was measured by Monsanto hardness tester. The hardness was measured in terms of kg/cm2.

Friability

The Roche friability test apparatus was used to determine the friability of the Tablets. Twenty pre-weighed Tablets were placed in the apparatus and operated for 100 revolutions and then the Tablets were reweighed. The percentage friability was calculated according to the following formula.

Drug Content

For drug content, the one tablet was crushed and transferred to 1000 ml volumetric flask and add small quantity of acetic acid then make up to 1000 ml with pH 6.8 phosphate buffer. The solution was filtered through Whatman filter paper (0.45µm pore size), Metronidazole and Norfloxacin were analyzed at 320nm and 279nm respectively using double beam UV/Visible spectrophotometer after suitable dilution. The content of drug was calculated from standard curve.

In-vitro drug release study

The USP type II rotating paddle method was used to study the drug release from the bilayer tablet. The dissolution medium consisted of 900 ml of phosphate buffer pH 1.2 for two hours. The release study was performed at 37 ± 0.50 C, with a rotation speed of 50 rpm. The disk was placed at the bottom of the dissolution vessel. After that dissolution medium was changed to phosphate buffer pH 6.8 for a period of 10 hrs. Aliquots (5ml each) were withdrawn at regular time intervals and replaced with fresh medium to maintain sink conditions. The samples were filtered, with appropriate dilutions with phosphate buffer pH 6.8 and Metronidazole and Norfloxacin were analyzed at 320nm and 279nm respectively using double beam UV/Visible spectrophotometer after suitable dilution. The content of drug was calculated from standard curve.

Stability studies:

The formulation F8 was selected and the stability studies were carried out at accelerated condition of 40±2 0C, 75±5 % RH conditions, stored in desiccators, the tablets were packed in amber colour screw cap container and kept in above said condition for period of three months. The tablets were analyzed periodically for their physical appearance and in-vitro drug release.

Kinetic Studies

The following plots were made: cumulative % drug release vs. time (zero order kinetic model); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (higuchi model). The regression coefficient R2 value nearer to 1 indicates the model best fits the release mechanism.

RESULTS AND DISCUSSION:

Drug excipient compatibility by FTIR Spectroscopy:

FTIR study demonstrates that no change in the individual peaks of MET and NFX and physical mixture of both drugs. It showed that drugs have no incompatibility problem. FTIR spectras were represented in Figure 1-4.

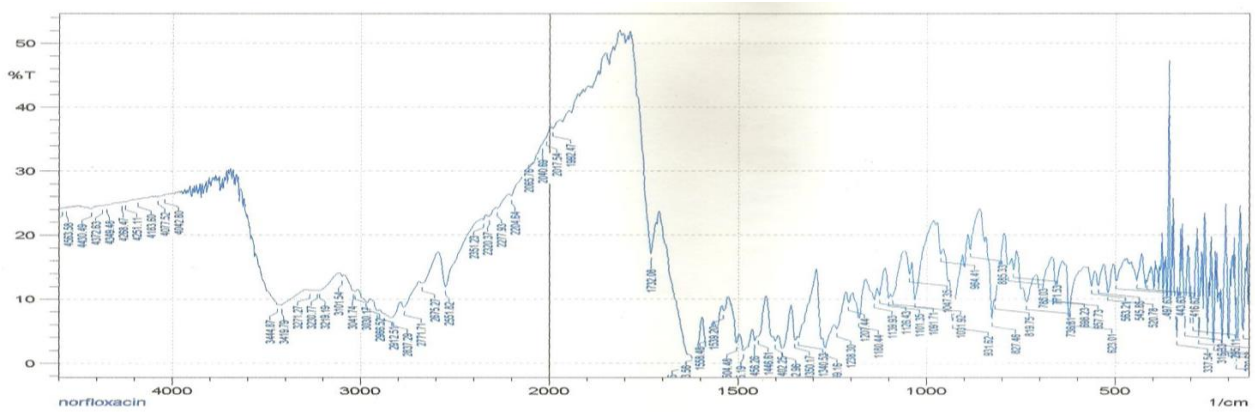

Figure 1: FTIR Spectrum of Norfloxacin

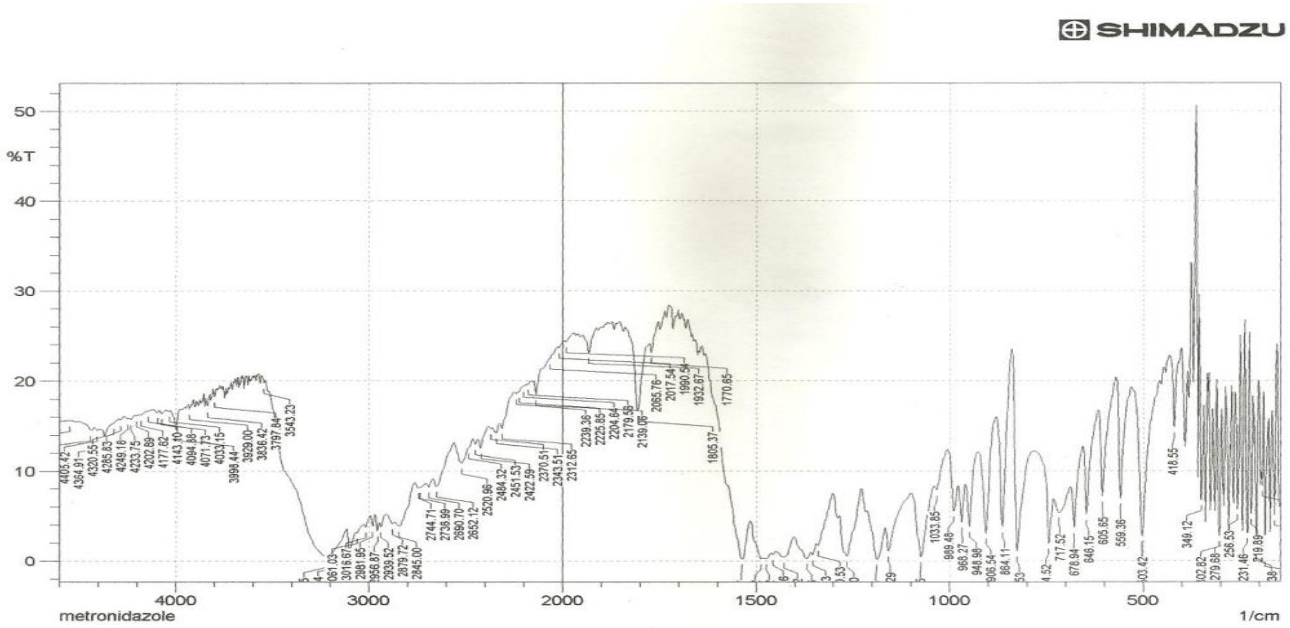

Figure 2: FTIR Spectrum of Metronidazole

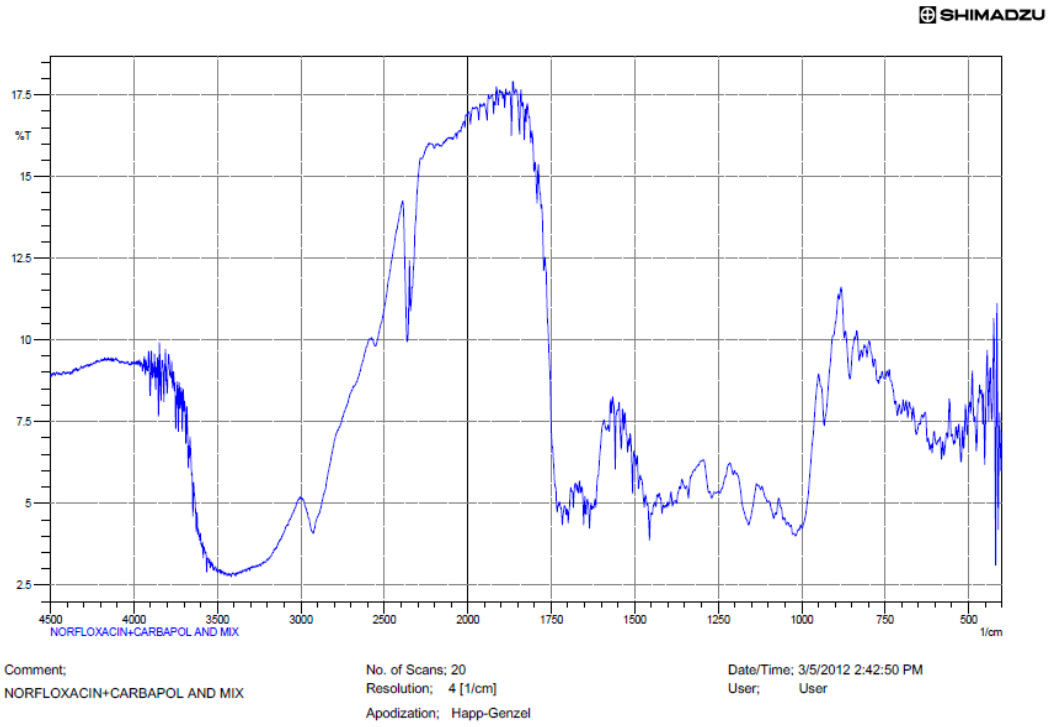

Figure 3: FTIR Spectrum of Norfloxacin with Polymer mixture

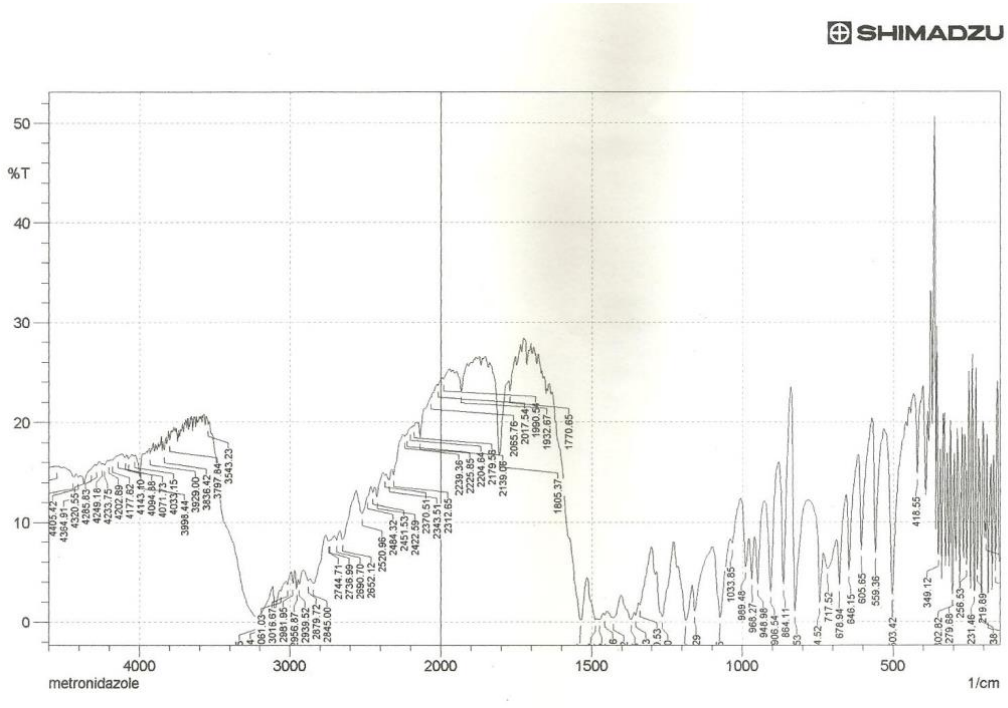

Figure 4: FTIR Spectrum of Metronidazole with Polymer mixture

Evaluation of Granules:

The sustained release layer and immediate release layer granules were evaluated for bulk density, tapped density, angle of repose, compressibility index, hausner's ratio, loss on drying of the all formulations i.e. F1 to F9. The results were found to be within the limits of standard specifications. The granules were shows good flow properties. The results were tabulated in table 4 and 5.

Table no 4: Results of evaluation of immediate release layer granules

Formulations	Bulk Density (gm/ml) (Mean±SD)	Tapped Density (gm/ml) (Mean±SD)	Carr's index (%) (Mean±SD)	Hausner ratio (Mean±SD)	Angle of Repose (θ) (Mean±SD)
F1	0.557 ± 0.0055	0.642 ± 0.0022	13.23 ± 0.16	1.15 ± 0.0044	29.53 ± 0.066
F2	0.548 ± 0.0036	0.632 ± 0.0021	13.29 ± 0.22	1.15 ± 0.0037	32.25 ± 0.023
F3	0.564 ± 0.0022	0.643 ± 0.0051	12.33 ± 0.89	1.14 ± 0.0029	30.23 ± 0.026
F4	0.561 ± 0.0057	0.647 ± 0.0028	13.29 ± 0.12	1.15 ± 0.0023	29.45 ± 0.057
F5	0.579 ± 0.0010	0.645 ± 0.0075	10.25 ± 0.6	1.11 ± 0.0024	31.47 ± 0.026
F6	0.580 ± 0.0022	0.658 ± 0.0011	11.85 ± 0.88	1.13 ±0.0084	30.40 ± 0.034
F7	0.542 ± 0.0010	0.629 ± 0.0023	13.83 ± 0.32	1.16 ± 0.001	33.26.00 ± 0.060
F8	0.544 ± 0.0089	0.618 ± 0.0098	11.97 ± 0.73	1.13 ± 0.0027	31.44 ± 0.026
F9	0.522 ± 0.0090	0.599 ± 0.0009	12.85 ± 0.29	1.14 ± 0.0020	34.27 ± 0.019

Table no 5: Results of evaluation of sustain release layer granules

Formulations	Bulk Density (gm/ml)(Mean±SD)	Tapped Density (gm/ml)(Mean±SD)	Carr's index (%)(Mean±SD)	Hausner ratio (Mean±SD)	Angle of Repose (θ) (Mean±SD)
F1	0.557 ± 0.0055	0.642 ± 0.0022	08.75 ± 0.16	1.09±0.0044	29.00 ± 0.066
F2	0.548 ± 0.0036	0.678 ± 0.0021	09.65 ± 0.22	1.13±0.0037	27.00 ± 0.023
F3	0.574 ± 0.0022	0.633 ± 0.0051	07.33 ± 0.89	1.11±0.0029	26.00 ± 0.026
F4	0.601 ± 0.0057	0.657 ± 0.0028	08.98 ± 0.12	1.15±0.0023	28.00 ± 0.057
F5	0.599 ± 0.0010	0.645 ± 0.0075	11.75 ± 0.6	1.18±0.0024	29.00 ± 0.026
F6	0.600 ± 0.0022	0.698 ± 0.0011	13.89 ± 0.88	1.55±0.0084	28.00 ± 0.034
F7	0.512 ± 0.0010	0.599 ± 0.0023	12.64 ± 0.32	1.19 ± 0.001	29.00 ± 0.060
F8	0.644 ± 0.0089	0.693 ± 0.0098	15.98 ± 0.73	1.18±0.0027	27.00 ± 0.026
F9	0.522 ± 0.0090	0.599 ± 0.0009	13.89 ± 0.29	1.17±0.0020	28.00 ± 0.019

Evaluation of Tablets:

The prepared bi-layer sustained release tablets were evaluated for physico-chemical properties like description, thickness, weight variation, hardness, friability, disintegration time for immediate layer, drug content.

Description

The prepared bi-layer tablets were oval in shape, sustained released was in off white colour, immediate released was in white colour.

Weight variation and Thickness

The weight variation of bi-layer tablets was found to be in the range of 591 ± 9.19 mg to 604 ± 5.32 mg and the thickness of bi-layer tablets was found to be in the range of 6.9 ± 0.02 to 7.0 ± 0.04 mm for all the formulations (F1 TO F9). The results were tabulated in table 6.

Hardness and friability

Hardness was affecting the release of drug from formulations. The hardness of bi-layer tablets was found to be in the range of 5.1 ± 0.31 kp to 6.3 ± 0.52 kp and friability of bi-layer tablets was found in the range of 0.34 ± 0.025% to 0.74 ± 0.043% for all the formulations (F1 TO F9). The results were tabulated in table 6.

Drug content

Drug content of bi-layer tablets was estimated by assay using UV spectrophotometer. The amount of metronidazole was found to be in the range of 98.99% to 101.45% and the amount of norfloxacin was found to be in the range of 98.76 % to 101.3 % for all the formulations (F1 - F9). The results were tabulated in table 6.

Table no 6: Results of Physiochemical properties of bilayer tablets

Formulation Code	Thickness (mm)± SD	Weight Variation (mg)± SD	Hardness (Kg/cm^2) ± SD	Friability (%) ± SD	Drug Content(%)	
					Metronidazole	Norfloxacin
F1	6.9 ± 0.03	601 ± 8.55	5.7 ± 0.15	0.34 ± 0.025	99.89	100.4
F2	7.0 ± 0.01	595 ± 7.94	5.6 ± 0.25	0.42 ± 0.03	101.45	98.90
F3	6.9 ± 0.02	593 ± 9.81	5.1 ± 0.31	0.35 ± 0.042	99.32	99.56
F4	7.0 ± 0.02	604 ± 5.32	5.3 ± 0.21	0.43 ± 0.036	100.25	99.23
F5	7.0 ± 0.03	591 ± 9.19	6.1 ± 0.24	0.52 ± 0.023	98.99	101.3
F6	7.0 ± 0.04	594 ± 5.9	5.9 ± 0.45	0.63 ± 0.053	99.78	98.98
F7	6.9 ± 0.05	599 ± 6.3	6.3 ± 0.52	0.48 ± 0.06	100.5	99.45
F8	7.0 ± 0.04	602 ± 4.9	5.4 ± 0.63	0.59 ± 0.07	99.86	100.6
F9	6.9 ± 0.06	596 ± 6.8	5.7 ± 0.74	0.74 ± 0.043	101.3	98.76

In vitro Dissolution Study

Based on the in-vitro release profile Metronidazole immediate layer released 98% to 99% within 2 hours. But Norfloxacin sustained layer 99.5% drug released up to 12 hours in F8 formulation. So, F8 was showed good results compare to other formulations. This was achieved by increasing the polymer concentration by combining two polymers such as which release the drug in a controlled rate at regular time intervals in appropriate concentrations as per the limits. Hence formulation F8 was selected for further stability studies. Invitro drug release patterns were shown in figure 5.

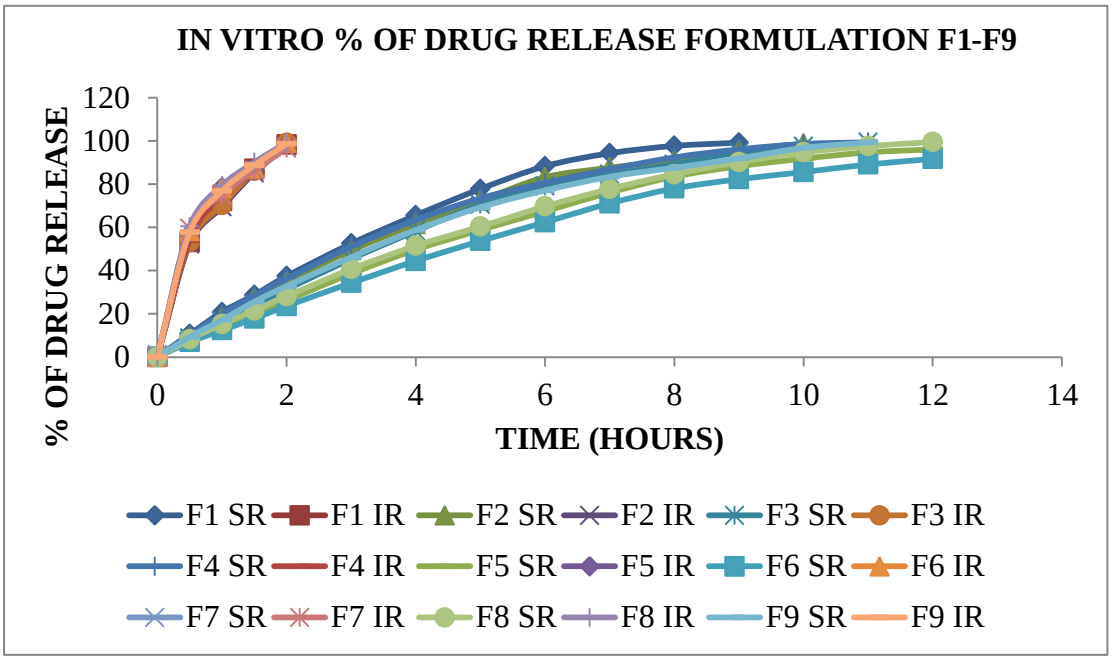

Figure 5: In Vitro % of Drug Release of formulations F1-F9.

Stability Study

The stability studies of formulation F8 were carried out at accelerated condition of 40±2 ºC, 75±5 % RH conditions. The tablets were withdrawn at every one month and evaluate the tablet parameters like Appearance, drug content and *in-vitro* drug release at end of 3rd month. There is no change in physical parameters and the drug content was reduced but it is within the standard specifications .There is no significant change in the *In-vitro* drug release. The results were tabulated in table 7. According to three months data, the shelf of stability sample (F8) was calculated.

Table 7: Stability data of Bilayer tablets (F8)

Parameters		**INITIAL**	**1st month**	**2nd month**	**3rd month**
Physical appearance		White round shape	No Change	No Change	No change
Drug content (%)	Metronidazole	100.5	99.4	98.35	98.05
	Norfloxacin	99.9	98.6	97.8	97.8
Drug release (%)	Metronidazole	99.2	98.4	97.5	96.5
	Norfloxacin	99.5	98.6	96.8	96.5

CONCLUSION:

The Metronidazole and Norfloxacin bi-layer sustained release tablets were prepared by wet granulation method by using different polymers such as xanthan gum and carbopol as sustained release polymers and along with Poly vinyl pyrrolidine K 30 (PVP K30) as binding agent. The Norfloxacin Sustained release and Metronidazole immediate release was evaluated for morphological characteristic, physical characteristic, chemical characteristic and stability. The results obtained were satisfactory and within specified limits as per Pharmacopoeias

Metronidazole immediate layer released 98% to 99% within 2hours. But Norfloxacin sustained layer 99.5% drug released up to 12 hours in F8 formulation. So, F8 was showed good results compare to other

formulations. Formulation F8 fulfills our objective of formulating a bilayer tablet of sustained release of Norfloxacin and immediate release layer of Metronidazole.

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