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FORMULATION DEVELOPMENT AND *IN-VITRO* EVALUATION OF BI-LAYER TABLET OF SUSTAINED RELEASE (S.R) FLURBIPROFEN AND IMMEDIATE RELEASE DOXYCYCLINE

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ABSTRACT

The purpose of this study was to formulate and characterize bi-layer tablet of doxycycline immediate release and flurbiprofen sustained release. Bi-layer tablets were formulated by wet granulation method. Ethyl cellulose and HPMC K 100 were used as polymer for sustained release. Five immediate release formulations were developed with varying excipients. Bi-layer tablets were evaluated by pre formulation and post formulation parameters as stated by USP. Dissolution was conducted in 0.1N HCl and 6.8 phosphate buffer and resulting data were analyzed statistically by one way ANOVA and drug release kinetics was studied using various release kinetic models. Data from pre formulation confirmed the purity of doxycycline hyclate and flurbiprofen. FTIR spectroscopy confirmed the absence of incompatibility between drugs and excipients. Dissolution profile in 0.1N HCl and 6.8 phosphate buffer was according to USP guidelines. The regression coefficient (R^2) values from kinetic analysis showed that release followed Higuchi model indicating release mechanism followed diffusion transport. Results of one way ANOVA confirmed that there is no statistically significant difference between drug dissolution of all formulations($p > 0.05$). Bi-layer tablet was successfully formulated and it is a suitable approach to increase patient compliance and decrease cost of therapy.

Keywords: Flurbiprofen, Doxycycline hyclate, Bi-layer tablet, FTIR spectroscopy, Sustained release

INTRODUCTION

Bi-layer tablets are those having two different drugs in single formulation, which may be pharmaceutically incompatible but have synergistic effects. So that patient compliance can be increased by decreasing the number of doses and reducing the cost of the therapy (Panchal et al., 2012).

Bi-layer tablets have been widely used for modified release action, in which we can manipulate the total surface area by sandwiching with one or two actives or by some excipients in order to attain erodible or swellable barriers of modified release. Most wide application and reason to develop bi-layer tablet is to give pharmaceutical incompatible APIs in combination (Deshpande et al., 2011).

Flurbiprofen (2-(2-flurobiphenyl-4yl) is propionic acid derivative, non-steroidal anti-inflammatory drug having good analgesic, anti-inflammatory and antipyretic activities. Clinically it is used for osteo-arthritis, rheumatoid arthritis, periodontic pain, degenerative joint disease, acute musculoskeletal disorders, lower back pain and other similar conditions. They impart their action by inhibiting the synthesis of prostaglandins involved in pain and inflammation (Kumar et al., 2004).

Doxycycline hyclate ((4S, 4aR, 5S, 5aR, 6R, 12aS)-4-(dimethylamino)-3,5,10,11,12a-pentahydroxy-6-methyl-1,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide; etanol;dihydrochloride) is a broad spectrum antibiotic belonging to the class of tetracyclines. It is clinically used in bacterial infections, especially in urinary tract infections, respiratory tract infections,

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periodontics (gum disease) and in other bacterial infections. Doxycycline is also used in rheumatoid arthritis in combination with some other drugs. Doxycycline hyclate is a synthetic antibiotic derived from oxytetracycline and inhibits the bacterial protein synthesis by binding to 30s ribosomal unit (Jantratid et al., 2010).
Both of these drugs have synergistic effect, and especially in destructive diseases like rheumatoid arthritis, chronic periodontics, and refractory periodontal disease. It is assumed that sub antimicrobial dose of doxycycline and flurbiprofen reduces the mammalian collagenase and other metalloproteinase. It is also assumed that combination of these two drugs helps to uptake doxycycline based matrix metalloprotein inhibitors in the inflammatory lesion so efficacy of both of the drugs is enhanced (Golub et al., 2008; Sgolastra et al., 2011).
The present work was aimed to study the development and in-vitro evaluation of Bi-layer tablet of sustained release flurbiprofen and immediate release doxycycline hyclate by wet granulation method using different excipients and varying excipients ratio, which will increases the patient compliance and decrease the cost of therapy especially in destructive diseases like rheumatoid arthritis, chronic periodontics, and refractory periodontal disease.

MATERIALS AND METHODS

Chemicals

Flurbiprofen and doxycycline hyclate were gift from Caraway Pharmaceuticals Pvt. Ltd. Islamabad, Pakistan. Ethyl cellulose, HPMC k 100M (Research grade were purchased from local market, Primogel, starch, PVP-k-30, microcrystalline cellulose, Aerosil, lactose, Talcum and magnesium stearate (commercial grade), sodium hydroxide (NaOH), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), acetone, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium acetate, glacial acetic acid were obtained from the research lab.

Formulation of bi-layer tablets

Five different formulations with varying excipients were prepared as stated in Table 1 and Table 2. For this purpose, weighed amount of drugs and excipients were sieved through mesh#20 separately. Active pharmaceutical ingredients were mixed with lactose separately. Then PVP K30 dissolved in IPA was added in mixtures.

Table I: Composition of sustained release flurbiprofen layer

Ingredient	Amount, (%)
Flurbiprofen	200mg (66.60%)
Ethyl cellulose	20.0mg (6.66%)
HPMC-K-100	23.7mg (7.90%)
PVP-K30	12.0mg (4.00%)
Primogel	8.0mg (2.60%)
Lactose	28.0mg (9.30%)
Mg. Stearate	6.0mg (2.00%)
Talc	2.3mg (0.76%)

Table II: Composition of immediate release doxycycline hyclate layer

INGREDIENTS	D1(MG)	D2(MG)	D3(MG)	D4(MG)	D5(MG)
DOXYCYCLINE	100(40%)	100(40%)	100(40%)	100(40%)	100(40%)
PRIMOGEL	5(2%)	5(2%)	5(2%)	5(2%)	5(2%)
STARCH	50(20%)	40(16%)	30(12%)	35(14%)	45(18%)
TALCUM	-	-	2(0.8%)	3(1.2%)	2.5(1%)
PVP K30	10(4%)	20(8%)	10(4%)	12(4.8%)	14(5.6%)
AEROSIL	8(3.2%)	8(3.2%)	8(3.2%)	8(3.2%)	8(3.2%)
MICROCRYSTALLINE CELLULOSE	70(28%)	55(22%)	50(20%)	50(20%)	50(20%)
MAGNESIUM STEARATE	5(2%)	5(2%)	5(2%)	5(2%)	5(2%)
LACTOSE	2(0.8%)	17(6.8%)	35(14%)	22(8.8%)	5.5(2.2%)
CMC	-	-	5(2%)	10(4%)	15(6%)
TOTAL	250	250	250	250	250

Wet masses were sieved through mesh # 20 and then dried at 60°C for 2-3 h. Then dried granules were sieved again and rests of excipients were added except magnesium stearate and talcum. Mixing was done until uniform

mixing was achieved. At the end lubrication was added and final mixing was done. The most critical step was the compression in bi-layer tablets. For research purpose, bi-layer tablets can be compressed on a single punch machine. In this project we preferred single punch machine in which weighed amount of granules of first layer tablets that was of flurbiprofen was initially filled in dye and was pre compressed, then granules of second layer which were of immediate layer of doxycycline were added in dye and final compression was done. Bi-layer tablets were prepared successfully. To relate it with industrial scale and check flow properties of our granules, these were also compressed on a bi-layer tablet machine at Caraway Pharmaceuticals (Pvt) Ltd, and bi-layer tablets were also manufactured successfully.

***In-vitro* characterization**
All physical and chemical parameters were measured. Complete pre formulation and post formulation parameters were measured as stated by USP-NF.

Fourier transformed infrared (FTIR) spectroscopy
The compatibility of flurbiprofen, doxycycline hyclate and excipients were studied through FTIR spectroscopic analysis (BRUKUR- FTIR spectrophotometer). Spectrum was recorded between 4000 to 400cm^{-1}.

UV Spectroscopy
For assay UV spectroscopy was used. Solutions of drugs were prepared in 0.1N NaOH and absorption at respective lambda max of each drug was calculated. Method was calibrated and validated.

***In-vitro* release studies**
USP apparatus II was used for *In-vitro* release studies of dissolution test. First apparatus was filled with 900ml of 0.1 N HCl, single tablet was put in each basket of the dissolution apparatus. Paddle of the apparatus was rotated at 50 rpm for the first 2 h. After that 0.1N HCl was replaced by phosphate buffer 6.8 pH. Paddle was continuously rotated at 50 rpm for up to 12 h. Samples for immediate release layer were collected at the interval of 5, 10, 15, 20, 30, 45, 60 and 90 min and for sustained release layer at the interval of 2, 4, 6, 8, 10 and 12 h. Samples were analyzed at 247 nm for flurbiprofen and 375 nm for doxycycline hyclate by using UV spectrophotometer.

Release kinetics
Different methodsincluding model dependent and model independent approaches were used to analyze dissolution data and to study release pattern of drug from dosage form as well as to check variation among the data of five formulations formulated as well as to compare with the dissolution of brand leader. Three different approaches are common to analyze dissolution data and check the parameters mentioned above.

Statistical analysis
We used analysis of variance ANOVA and results were interpreted. The level of significance was set at $p < 0.05$ (Soni et al., 2010).

Determination of difference (f1) and similarity (f2) factor
Difference factor (f1) and similarity factor (f2) was applied by using equation of difference and similarity factor. Doxycycline dissolution results were compared with brand leader vibramycin results in three different dissolution mediums. Sustained released tablet of flurbiprofen is not available in market so all formulations were compared with the formulation having the most suitable results i.e. F3. Best formulations were pointed out which were closer to the standard. Difference factor of 0-15 ensures minor difference between two products. Similarity factor of 50-100 ensure sameness of two products (Dash et al., 2010).

Model dependent approach
Dissolution data were analyzed using various kinetic models: Zero order, First order, Higuchi model and Hixon-Crowell to determine the release kinetics of the formulations.

RESULTS

Pre formulation and post formulation results
All pre formulation parameters were applied. Bulk density and tapped density are two of the most important studies to be done before the development of formulation. Bulk density, angle of repose, compressibility index, Hausner's ratio, moisture content and tapped density were checked by adopting the standard methods as described in USP. Results are shown in Table 3. These values were in acceptable range.
In this project, extensive post formulation studies were done and we tried to cover each and every aspect of *in-vitro* evaluation of tablets. Physical test and assay results are mentioned in Table 4.

Infrared spectra
FTIR spectra of flurbiprofen and doxycycline hyclate showed the same absorption pattern as the combination of drugs and excipients of formulations.

***In-vitro* dissolution**
The cumulative release of drug in acidic and buffer media reveals that immediate layer of doxycycline release its

Table III: Pre formulation results of granules of Doxycycline hyclate and flurbiprofen

Code	Bulk density (g/cm³)	Tapped density (g/cm³)	Angle of Repose (θ)	Compressibility Index (%)	Hausner's ratio	Moisture Content (%)
D1	0.351	0.344	26.00	11.32	1.032	0.55
D2	0.381	0.320	29.00	13.67	1.045	0.59
D3	0.360	0.391	26.00	11.99	1.075	0.47
D4	0.340	0.300	27.00	12.98	1.056	0.61
D5	0.362	0.349	28.00	13.49	1.067	0.66
F1	0.383	0.650	31.42	16.03	1.224	0.38

Table IV: Post formulation results of bi-layer tablets

Code	Thickness (mm) ± S.D	Hardness (kg/cm2) ± S.D	Friability (%)± S.D	Avg.Weight (mg) ± S.D	Assay (%)± S.D	
					Doxycycline	Flurbiprofen
F1	3.190±0.010	7.16± 0.28	0.690±0.010	549.5±2.32	98.5±0.51	100.8±0.76
F2	3.173±0.005	5.50± 0.50	0.077±0.020	551.4±2.31	98.2±0.46	99.1±0.26
F3	3.170±0.010	8.34± 0.57	0.540±0.005	550.0±2.90	98.9±0.85	99.5±0.50
F4	3.200±0.011	5.16 ±0.28	0.730±0.020	550.4±2.01	98.4±0.53	100.8±0.28
F5	3.190±0.011	7.00± 0.50	0.590±0.006	549.3±1.33	99.4±0.36	100.2±0.61

content in acidic media before 120 minutes while almost 40% flurbiprofen was released in acidic medium. In 6.8 phosphate buffer almost 90% flurbiprofen was released in 10 h at it was sustained release layer. Thus, the results were desirable as stated in Table 4, 5 and 6.

Release kinetic data

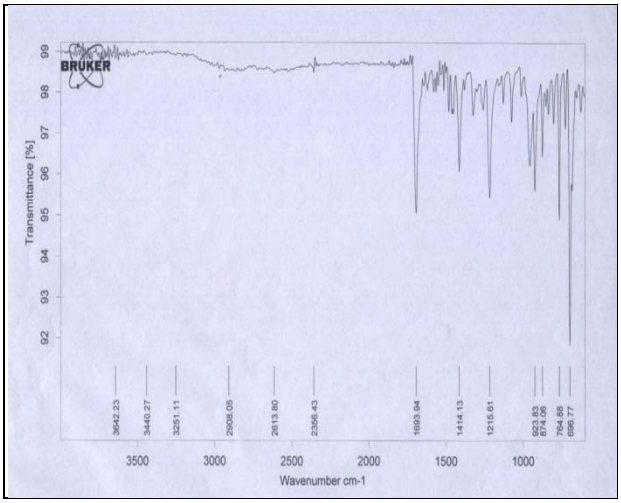
When cumulative release was subjected to kinetic modeling, all the formulations followed Higuchi model. Among all these four models kinetic release profile was best explained by Higuchi model having R^2 value > 0.96 for all five formulations and release of drug from the profile was through diffusion mechanism and kinetic release profile was best explained by Higuchi model as stated in Table 7, 8. 9 and 10

Difference and similarity factor

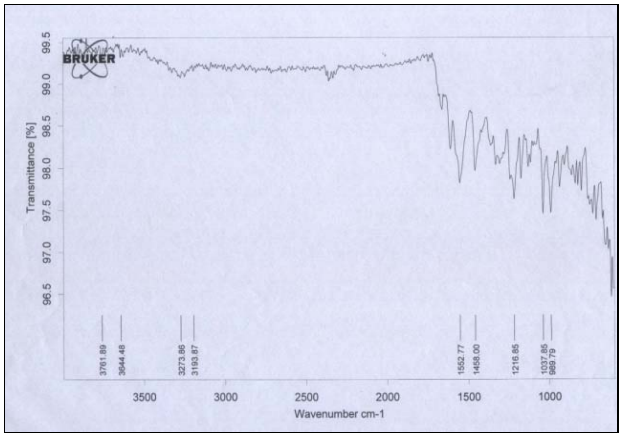
Difference and similarity factor was calculated using standard equations to determine the best formulation which is closest in its dissolution profile to the standard. It was observed that F2 behaved to be the best formulation in 0.1 N HCl, while F3 formulation was found to be the best in 6.8 pH phosphate buffer. Overall F3 formulation passed the requirements of both *f*1 and *f*2 tests in both media. The results are given in the Table 11, 12, 13 and 14.

Statistical evaluation

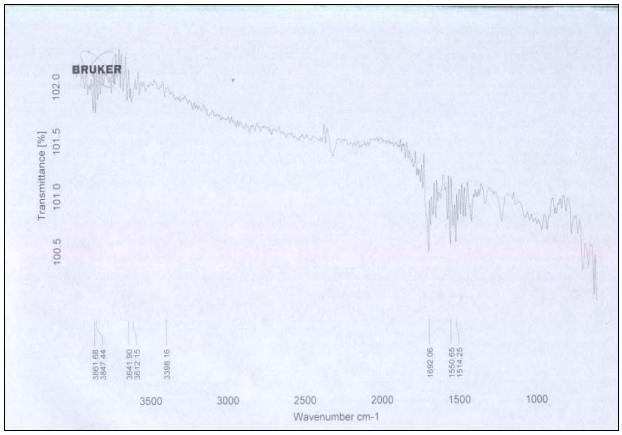
One way ANOVA was used to determine statistically significant difference between dissolution profile of



FTIR spectrum of flurbiprofen



FTIR Spectrum of pure doxycycline hyclate



FTIR Spectrum of formulation F3

different formulations of doxycycline and flurbiprofen in 0.1 N HCl and 6.8 pH phosphate buffer. There was no significant difference ($P > 0.05$) between drug dissolution of all formulations studied in both mediums. The data of one way ANOVA is given in the Table 15.

Table 4: Dissolution profile of doxycycline hyclate and flurbiprofen in 0.1N HCl.

Code	0 min		10min		15min		30 min		60 min		90 min	
	A	B	A	B	A	B	A	B	A	B	A	B
F1	0	0	39.4	8.01	41	13.10	93	26.10	99	31.20	99	40.30
F2	0	0	39.6	6.20	50.01	10.00	99	22.40	99.97	34.00	99	42.10
F3	0	0	42	7.80	44.48	12.40	97.78	29.20	98	33.50	99.63	45.20
F4	0	0	48	5.90	56.2	12.10	99	21.70	98.90	37.10	99.01	43.60
F5	0	0	49.96	7.10	50.5	14.10	96.60	30.03	99.97	39.30	99.80	46.10

Table 5: Dissolution profile of doxycycline hyclate in 6.8 phosphate buffer

Code	Percentage of Drug Release (Doxycycline hyclate)				
	0	0.5 hr	1 hr	1.5 hr	2 hr
F1	0	52.10	91.80	95.8	97.90
F2	0	63.20	78.20	97.9	100.02
F3	0	80.30	92.60	98.3	99.10
F4	0	78.20	90.10	97.8	100.10
F5	0	73.50	84.40	94.9	100.06

Table 6: Dissolution profile of flurbiprofen in 6.8 phosphate buffer

Code	Percentage of Drug Release (Flurbiprofen)									
	0	0.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	24 hr
F1	0	23.60	48.00	63.80	67.09	79.40	93.50	95.20	97.10	99.97
F2	0	43.30	52.60	53.80	55.90	79.70	81.50	86.30	99.97	100.60
F3	0	44.60	53.60	57.20	58.80	86.40	84.50	91.50	97.30	100.20
F4	0	27.00	59.00	60.40	64.40	76.50	87.04	91.50	92.70	97.00
F5	0	24.50	58.90	63.20	72.10	73.34	82.40	85.69	92.50	97.10

Table 7: Kinetic study of drug release profile of doxycycline hyclate in 0.1N HCl

Code	Zero order kinetic model		First order kinetic Model		Higuchi model		Hixson Crowell cube root law	
	K°	R²	K°	R²	K°	R²	K°	R²
F1	4.016	0.977	1.19295	0.3540	51.52	0.997	-0.033	0.787
F2	4.168	0.968	0.84280	0.2910	53.64	0.995	-0.038	0.584
F3	4.092	0.972	1.01790	0.3240	52.58	0.996	-0.038	0.725
F4	4.195	0.964	0.78302	0.3170	54.05	0.993	-0.330	0.602
F5	4.150	0.970	0.88660	0.3007	53.37	0.995	-0.042	0.757

Table 8: Kinetic study of drug release profile of flurbiprofen in 0.1N HCl

Code	Zero order kinetic model		First order kinetic Model		Higuchi model		Hixson Crowell cube root law	
	K°	R²	K°	R²	K°	R²	K°	R²
F1	1.325	0.991	7.396	0.890	16.84	0.989	-0.007	0.928
F2	1.319	0.986	7.406	0.878	16.65	0.997	-0.008	0.969
F3	1.447	0.990	7.111	0.881	18.37	0.990	-0.008	0.920
F4	1.387	0.986	7.249	0.875	17.52	0.997	-0.008	0.966
F5	1.566	0.993	6.837	0.875	19.90	0.992	-0.009	0.914

Table 9: Kinetic study of drug release profile of doxycycline hyclate in 6.8 phosphate buffer

Code Doxycycline	Zero order kinetic model		First order kinetic Model		Higuchi model		Hixson Crowell cube root law	
	K°	R²	K°	R²	K°	R²	K°	R²
F1	122.3	0.970	14.244	0.753	266.1	0.993	-1.448	0.846
F2	116.1	0.985	28.694	0.570	251.3	0.999	-2.493	0.962
F3	123.7	0.971	11.217	0.667	268.9	0.993	-1.132	0.987
F4	122.9	0.974	12.862	0.562	267.1	0.995	-2.234	0.979
F5	119.7	0.980	20.429	0.568	259.5	0.997	-2.340	0.960

Code Flurbiprofen	Zero order kinetic model		First order kinetic Model		Higuchi model		Hixson Crowell cube root law	
	K°	R²	K°	R²	K°	R²	K°	R²
F1	28.570	0.874	12.026	0.483	166.80	0.965	-0.162	0.901
F2	26.850	0.884	15.971	0.537	156.00	0.967	-0.168	0.819
F3	27.770	0.878	13.857	0.514	161.80	0.966	-0.153	0.944
F4	27.490	0.872	14.550	0.573	160.80	0.966	-0.109	0.821
F5	27.170	0.870	15.238	0.616	159.40	0.969	-0.105	0.858

DISCUSSION

Bi-layer tablet of sustained release flurbiprofen and immediate release doxycycline hyclate were successfully formulated. All pre formulation and post formulation parameters were within the acceptable limits as stated by USP. FTIR spectra of drugs and formulation showed almost similar pattern of absorption predicts that there was compatibility between drugs and excipients.

Sustained release layer showed satisfactory results as it released drug in 6.8 phosphate buffer media and released less amount of drug in acidic media, while immediate layer released almost all drug in acidic medium which was desirable. Release kinetics of all formulations showed that Higuchi model best explains the release of drugs from formulation and drug release was through diffusion mechanism as explained by Higuchi model.

Difference and similarity factor test was done and results indicate that except F1 all formulations are more similar and less different to standard. F2 and F5 also deviated to some extent in 6.8 phosphate buffer.

Table 11: Difference and similarity factor for doxycycline in 0.1N HCl

Doxycycline	F1 vs. S	F2 vs. S	F3 vs. S	F4 vs. S	F5 vs. S
f 1	17.56	3.36	7.16	6.06	5.16
f 2	39.63	74.95	58.10	60.20	62.83

Table 12: Differece and similarity factor for flurbiprofen in 0.1N HCl

Flurbiprofen	F1 vs. F3	F2 vs. F3	F4 vs. F3	F5 vs. F3
f 1	6.79	7.98	7.18	8.53
f 2	61.84	57.38	56.91	59.20

Table 13: Difference and similarity factor for flurbiprofen in 6.8 phosphate bufffer

Doxycycline	F1 vs. S	F2 vs. S	F3 vs. S	F4 vs. S	F5 vs. S
f 1	14.32	14.45	3.76	4.89	8.59
f 2	35.17	40.33	67.97	64.18	52.71

Table 14: Difference and similarity factor for flurbiprofen in 6.8 phosphate buffer

Doxycycline	F1 vs. S	F2 vs. S	F3 vs. S	F4 vs. S	F5 vs. S
f 1	14.32	14.45	3.76	4.89	8.59
f 2	35.17	40.33	67.97	64.18	52.71

Table 15: One way ANOVA results

Medium	Flurbiprofen		Doxycycline	
	F	P-Value	F	P-Value
0.1 N HCl	0.064	0.991	0.033	0.997
6.8 pH phosphate buffer	0.02	0.99	0.255	0.899

CONCLUSION

All five formulations were within the official limits as stated by international pharmacopoeias, but the best formulation which gave better results was F3. Kinetic release studies of formulation in all three media were better explained by Higuchi model which indicated that release of drug from formulation is through diffusion mechanism.

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