Formulation and *in vitro* evaluation of colchicines and naproxen sodium sustain release tablets in combination for treatment of gout

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Abstract: Present investigation concern with combination of two drugs for the treatment of gout. One of these drug (naproxen sodium) is pain killer which is sustain their action within the body for 12 hours and the other drug (colchicine) is anti-gout, which release as conventional dosage. After oral administration naproxen will act as sustain release dosage and increase patient compliance about six batches of tablet were developed and evaluate .For the sustain release action polymers Methocel K4M and HPMCK15were used. These polymers were used in combination used with other inactive ingredients. Two methods were used for proration of final tablets. In 1st method only naproxen sodium granules were prepared which are sustained released. In second method these granules were mixed with colchicines powder and other all inactive ingredients. This method is easy and cost effective characterization of pallets and final tablets were performed. Final tablets were evaluated for all tests like appearance, friability, dissolution, hardness, assay, weight variation and in-vitro release study performed. The results obtained were satisfactory and complies with USP specification. Formulation containing combination of Methocel K4M and HPMC K15 showed good sustain release profile for 12 hours.

Keywords: Methocel K4M and HPMC K15, naproxen, colchicine and sustain release.

INTRODUCTION

Combination therapy is new technique for the treatment of the disease. (Connor, Rafter, & Rodgers, 2004) Sustain release technology is relatively modern field and combination of two drug which have different release profile is also new concept of dosage form (Mehmood). The basic purpose of this research is to achieve a steady state blood level for both drugs. In this research the design of two different release profiles was critical thing and was important element to complete this research. The success of a therapy depends on selection of the appropriate delivery system as much as drug itself. Colchicines drug is specifically indicated for the treatment and relief of severe pain in attacks of acute gouty arthritis. (Schlesinger, 2004) It is also prescribing for regular use between attacks as a prophylactic measure,(Schlesinger, Schumacher, Catton, & Maxwell, 2006) and is very effective in aborting an attack when taken at the first sign of articular discomfort. The usual dose of this drug to relieve or abort an attack is 1 to 1.2mg (Mehta, Deshmukh, Seth, & Banty, 2011). Naproxen sodium also prescribe for the treatment of rheumatoid arthritis, osteoarthritis, (Bombardier *et al*., 2000) ankylosing spondylitis, bursitis, tendinitis and acute gout (Schlesinger, 2004). It also prescribes in the relief of mild to moderate pain and is very useful in the treatment of primary dysmenorrheal. (Chan, FUCHS, & POWELL, 1983) The recommended initial dose of (naproxen sodium) tablets in adults is 500mg once daily.(Reddy, Mutalik, & Reddy, 2003).

MATERIALS AND METHOD

Materials

Colchicines and naproxen sodium were obtained as a gift sample from Ameer & Adnan pharmaceuticals Lahore Pakistan. Methocel K4M and HPMC K15were also purchased (The Dow Chemical *Company USA).* Lactose (Merck, KgaA, and Darmstadt, Germany), kolidon 30 (ISP Technologies, Inc. Wayne, NJ), Mag stearate, aerosol, avicel102 (FMC Corporation, USA). Double distilled water was used throughout the research. Other materials used for analysis were also purchased from local market but these were all of analytical grade (Merck & Co., Inc.).

Instrumentation

UV-Visible double beam spectrophotometer with matched quartz cells (1cm) Model: Evolution 201Make: Thermo Scientific, 81 Wyman Street Waltham, Massachusetts, US.FTIR (Model No.7600).

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Materials used polymer suspension

Methocil K4M	220mg
Propylene glycol	70ml
Water	150ml

Materials used in pigment suspension

HPMC K15	4.5g
Titanium dioxide	2.4g
Talcum	10g
Water	100ml

Process parameters for coating

Temperature of inlet air	60°C
Temperature of outlet air	38°C
Temperature of product	37°C
Air flow	70m³/h
Diameter of nozzle	0.8mm
Approximately spray rate.	11g/min
Spraying time for spraying	55 min
Pressure	1.0bar
Drying time	45°C/ 5min
Weight of coat	2mg film former/cm²

Preparation of Naproxen sustained-release (SR) granules

a) Formulation

The formulation is designed for 500mg of pure naproxen granules each tablet contains 550mg naproxen sodium equivalent to 500mg naproxen base. Grains prepared by wet granulation technique using isopropyl alcohol. Dry granules coated with polymer solution.

b) Method of Preparation for the all suspensions

Polymer suspension

Add Methocel K4M mentioned quantity in propylene glycol and then mix this into given quantity of water with continue stirring.

Pigment suspension

Add HPMC K15 in the given quantity of RO water. Dissolved titanium dioxide and talcum with continuous stirring and homogenize the mixture in a corundum disk mill.

Spray suspension

Incorporate the above pigment suspension into the polymer suspension with vigorously stirring. The prepared suspension must be stirred during the spraying process to prevent settling of the particles.

c) Coating

Granules are start to coat by the coating machine or coating pan. The suspension is spray continuously onto the fluidized, pre-heated naproxen granules from the top.

Method

Tablets were prepared by direct compression technique.

Procedure

Mix Naproxen Sodium granules and colchicines after passing it through 20 SS mesh for 30-45 minutes in horizontal mixer.

Add kolidon 30 in this mixture as binder.

Add this to the content of the mixer and Knead for 15-20 minutes.

Load this granular powder in horizontal mixer and add Avicel PH 102 after sieving SS mesh # 40 mix for 30 minutes then add Mg. Stearate, & Aerosil after screening mesh # 40 and mix for 10 minutes.

Shift the material in containers containing Poly bags with proper identification, to the in process quarantine.

When the batch is passed for compression, shift it to compression section when required.

After the compression is completed intimation for release for coating is given to QA. After getting sampled shift the tablets to the in process quarantine.

When passed for coating, shift it to coating section when required.

After the batch is coated give intimation to QA for release of batch for blistering and shift the coated tablets in airtight containers to in process quarantine.

After the batch is approved for blistering, shift the batch to blistering section if required.

Important study; compatibility evaluation was carried out using infra-red spectra. Infrared spectrum of formulated granules and drug alone were recorded and observed between 400nm and 5000nm. Infra -red spectrum of pure drug was also run individually.

Compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR) spectrums of drug naproxen and colchicine individual and with mixture were obtained for compatibility study. The range of scanning was from 400 to 5000cm-1(Elzein, Nasser-Eddine, Delaite, Bistac, & Dumas, 2004)

Drug content (Naproxen sodium)

Raw material Assay

Reference solution

Take 40mg of naproxen in a 100ml volumetric flask and dissolve it in 0.1N sodium hydroxide. Make volume up to mark with the 0.1N sodium hydroxide. Dilute 5ml of the solution to 100ml with the same solvent.

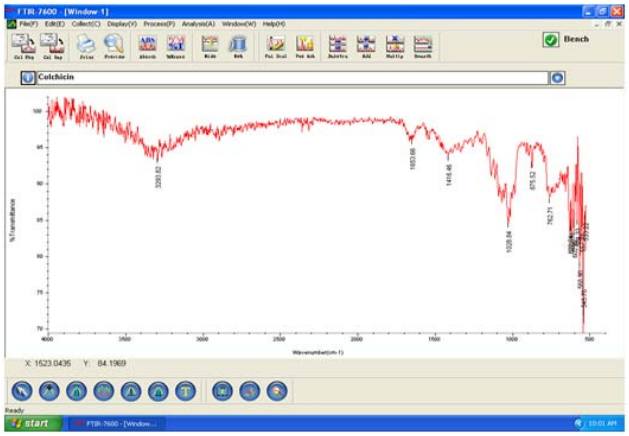

Fig. 1: FTIR of colchicines

Sample preparation

Transfer sample equivalent to 40mg of naproxen to100 ml volumetric flask. Make volume up to mark with 0.1N sodium hydroxide. Dilute 5ml of the solution to 100ml with the same solvent.

Procedure

Determine the absorbance of sample and Ref solution of 1 cm cell on a U.V/Visible spectrophotometer at 331nm using 0.1N sodium hydroxide as a blank and compare absorbance of sample.

Contents of naproxen

% age = Absorbance of Sample /Absorbance of standard x 100

Limit: USP 90% - 110 %

Drug content of colchicines

Raw material Assay

Carry out the following procedure in dim light. Measure quantity of the powder 0.5 mg of Colchicines and add 10 ml of absolute ethanol and shake for 20 minutes. Centrifuge and wash the residue with ethanol. Now again dilute the combined extract and again wash 50ml with ethanol and determine the absorbance of the resulting solution at the maximum at 350 nm. Calculate the content of $C_{22}H_{25}NO_6$ taking 440 as the value of A (1%, 1cm) at the maximum at 350 nm. (Mahrous, Abdel-Khalek, & Abdel-Hamid, 1984).

Evaluation of tablets

Thickness

By using the venire caliper determine the thickness of several tablets, 3 tablets were used from each formulation and take the average of these 3 tablets (Fell & Newton, 1970).

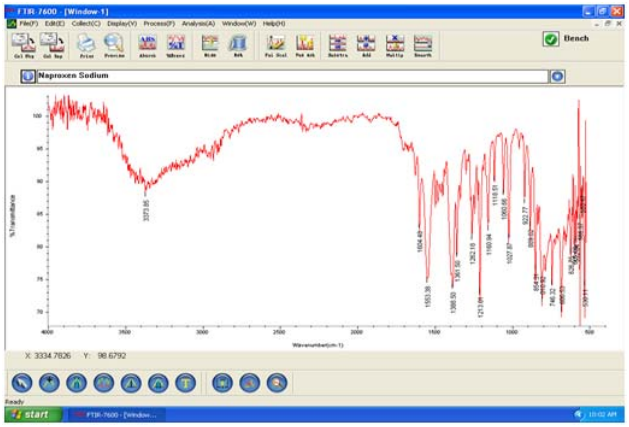

Fig. 2: FTIR of Naproxen

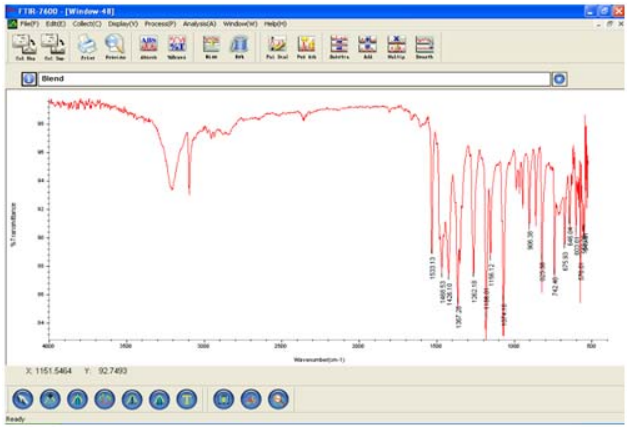

Fig. 3: FTIR of Blend

Weight variation test for tablets

To verify weight variation test, 10 tablets manage from each developed formulation and weighed using an electronic balance (srtorius MA45), and the test was carried out by the official method (Dunnett & Crisafio, 1955).

Hardness and friability

Determine the friability and hardness of the tablets according to the official method, calculate 6 tablets from each of formulation and measure the hardness (Monsanto tester) and friability (Roche friabilator labsonic electronics, Lahore), respectively (Abdelbary *et al*., 2005)

Uniformity content of colchicines

ASSAY

Count, evaluate and powder 20 tablets. Perform the following procedure in dim light. Measure quantity of the powder containing 0.5mg of Colchicines and add 10 ml of ethanol and shake for 20 minutes. Centrifuge and wash the residue with ethanol. Now dilute the combined extract and again wash 50ml with ethanol and determine the absorbance of the resulting solution at the maximum at 350 nm. Calculate the content of $C_{22}H_{25}NO_6$ taking 440 as the value of A (1%, 1cm) at the maximum at 350 nm(Orr & Sallam, 1978).

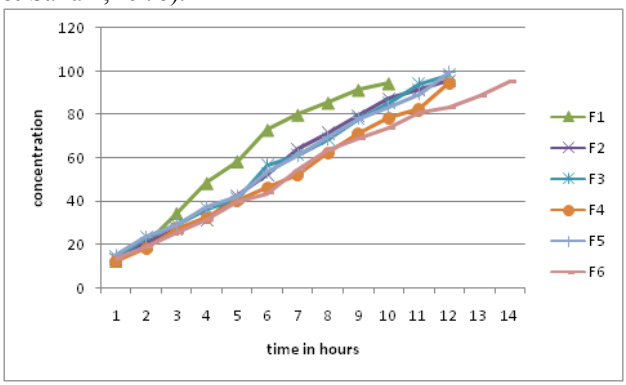

Fig. 4: Release profile of naproxen

RESULTS

Physico-chemical evaluation of matrix tablets

The results of the thickness, Hardness, weight variation, drug content, friability, and disintegration time of tablet shown in table 2.

Uniformity content of naproxen

ASSAY

Count and crush 20 tablets and produce powder. Shake a quantity of the powder containing 40mg of Naproxen with 70ml of methanol for 35 minutes, add sufficient methanol to produce 100ml and filter with 0.4micron. Dilute 10ml of the filtrate to 50ml with methanol and determine the absorbance at the maximum 331nm. Calculate the percentage of $C_{14}H_{14}O_3$ from the absorbance obtained by repeating the operation using a 0.01% w/v solution of naproxen in methanol and using the declared content of $C_{14}H_{14}O_3$ in naproxen (Solanki, Patel, & Suhagiya, 2011).

In vitro dissolution studies of Naproxen

Dissolution test was carried out for determine the *in vitro* test, USP apparatus type II used for determine the *in vitro* dissolution test. Phosphate buffer of ph 6.8 used in dissolution vessels, volume of vessels must be about 900ml.time for the dissolution checking was about 12 hr. USP apparatus 1 contain basket and temperature of that solution must maintain at 37±0.50^{o}C and basket revolution was set at 50 per minutes. From the vessels about 1ml solution withdraws from the centre with help of accurate pipette and check on spectrophotometer. According to delay release table of USP withdraw the sample and checked at different intervals. The absorbance of the naproxen sample was measured at λ max 331 nm using UV/visible spectrophotometer. Different concentrations were measured from standard calibrated curve of drug naproxen. Calibrated curve was measure by making the pure solution of naproxen in distilled water; cumulative % of naproxen release was measured using the equation achieved from standard curve

Swelling index of tablet

Swelling parameters are determined by percentage weight gain by the tablet. Swilling of tablet include absorption of media or liquid, which may result in increasing the size and weight of tablet. Liquid or water absorbed by the grains due to capillary action and space within the particles. After this capillary action liquid enter into the pores within the granules or hydration of molecules. When liquid enters into the molecules, swilling of the particles are going to start and due this size and weight of the tablet increase, this swilling parameters can be determined by the equation (Senapati, Srinatha, & Pandit, 2006).

Swelling index of tablet (SI) = (Wt – W0) x 100
W0
Where, Wt= Weight of tablet at time t.
W0 = Initial weight of tablet

The swelling index can be measured in 0.1M Hcl (pH 1.2) at 37^{o}C. After exact time the table was withdraw from the buffer containing beaker and eliminate the excess buffer by using filter paper, dry and weighed again up to 6 hrs.

Different kinetic or mathematical models (Dash, Murthy, Nath, & Chowdhury, 2010)

Zero order kinetics

Different types of drugs that don't disintegrate immediately and release their active materials slowly according to the specification of USP. The release can be determine by the following equation
Qt = Qo + ko t
Where, Qt= drug released amount in time't',
kt = zero order release constant
Qo = initial quantity of drug in the media,

First Order Kinetics

Gibaldi and Feldman determine the application of model 1st time for drug dissolution
In this model there are following parameters.
Log Qt = Log Qo + ktt/2.303

Table 1: different Formulation of tablet

Sr No	Ingredients	FD1	FD2	FD3	FD4	FD5	FD6
1	Naproxen granules	750	750	750	750	750	750
2	Colchicines	0.5	0.5	0.5	0.5	0.5	0.5
3	Kolidol 30	10	10	15	15	25	15
4	Mg Sterate	0.5	1.5	0.5	1.0	1.5	0.5
5	Avicel102	20	30	10	5	15	10
6	Aerosol	0.2	0.5	0.8	0.5	0.2	0.5
7	Lactose	49.2	38	53.2	58	37.8	53.5
Total weight		830	830	830	830	830	830

*All quantities are in mg.*Naproxen 500mg is equivalent to granules 750mg.

Table 2: Results of Thickness, weight variation, Hardness and Friability

Formulation	Hardness (Kg/cm^2)	Thickness (mm)	Friability (%)	Weight variation (mg)
F1	7.0	4.5	0.45	pass
F2	6.4	4.9	0.54	pass
F3	7.5	4.7	0.34	pass
F4	6.5	4.6	0.59	pass
F5	6.9	4.5	0.68	pass
F6	5.8	4.8	0.75	pass

Table 3: Drug (naproxen) content in each formulation

Formulation	Drug %
F1	94.34
F2	95.55
F3	98.48
F4	94.78
F5	99.48
F6	95.49

Table 4: Drug (colchicines) content in each formulation

Formulation	Drug %
F1	92.34
F2	93.45
F3	95.48
F4	98.78
F5	97.48
F6	95.49

Table 5: *In vitro* Release study of tablet about Naproxen

Sr No	Time (h)	F1	F2	F3	F4	F5	F6
1	1	12.5	13.5	14.5	12.4	15.3	13.4
2	2	20.5	20.4	23.4	18.4	23.4	18.9
3	3	34.5	27.4	29.4	27.4	29.2	25.6
4	4	48.4	31.3	36.4	32.4	37.1	31.3
5	5	58.4	42.4	41.4	40.4	42.4	39.5
6	6	72.9	52.4	56.5	46.4	53.4	43.6
7	7	79.8	64.2	61.4	52.4	61.2	54.5
8	8	85.4	71.4	68.6	62.3	69.4	63.7
9	9	91.3	79.4	78.3	71.3	78.2	69.0
10	10	94.3	87.4	85.4	78.3	83.4	73.7
11	11	-	91.3	94.2	82.3	89.3	81.3
12	12	-	95.5	98.4	94.4	99.4	83.5
13	13						88.9
14	14						95.4

Table 6: Accelerated Stability studies of formulation F4 (Naproxen).

Time (hr)	Cumulative % drug release	
	Initial	3 months
1	18.3	16.3
2	27.4	23.4
3	29.2	28.9
4	37.1	35.1
5	42.4	41.4
6	53.4	50.4
7	61.2	58.9
8	69.4	62.4
9	78.2	77.2
10	83.4	80.4
11	89.3	87.3
12	99.4	91.4

Table 7: Accelerated Stability studies of formulation F4 (colchicines)

Time (hr)	Cumulative % drug release	
	Initial	3months
1	14.3	16.3
2	23.4	26.4
3	28.2	29.9
4	37.1	37.1
5	42.0	42.4
6	53.4	55.4
7	61.2	61.9
8	67.4	69.4
9	78.2	77.2
10	84.4	86.4
11	89.3	91.3
12	99.4	98.4
Hardness	6.81	6.83
Friability	0.85	0.84
Drug contents	Pass	Pass

Table 8: Kinetics of Drug Release Study of F 4

Formulation	Zero order (R_2)	First order (R_2)	Higuchi's P lot (R 2)	Hixson Crowell (R 2)	Korsmeyer-peppas model		
F4	0.891	0.939	0.989	0.977	(R 2)	(n)	Order release
					0.407	0.860	Non - Fickian

Table 9: Swelling Index of Tablets of Batch F1 toF6

Formulation	Time (hr)					
	1	2	3	4	5	6
F1	54.5	67.3	75.4	93.4	104	111
F2	34.4	54.4	71.4	87.5	98.5	103
F3	44.5	65.4	77.5	92.4	101.3	112
F4	35.4	55.2	68.5	79.1	93.2	98.3
F5	35.4	65.3	78.4	89.3	102.3	114
F6	51.3	63.3	73.2	82.1	93.3	101

Where, Qo = primary amount of drug in media,
kt = first order release constant
Qt = quantity of drug released in time't',

All types of drugs that can soluble in water, and release the drug in that way the proportional all the quantity of drug remain in its internal core.

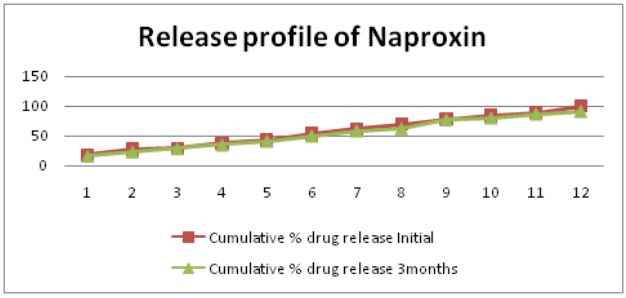

Fig. 5: Cumulative drug release initial and after 3 months

Higuchi model

This model contain several theoretical concept of drug release, Higuchi produce this model for the study of release rate of drugs that are contain in matrix system.
Following equation expressed this model
ft = kH t1/2
Where, kH = Higuchi diffusion constant,
ft = fraction of drug soluble in time 't'.
Higuchi mentioned that Fick's law based on drug release as a diffusion process and square root time dependent. This relation describes the drug dissolution from different types of modified release drugs.

Korsmeyer-Peppas model

Korsmeyer describe a simple, semi-empirical-geometrical model, which relate the drug release to the elapsed time (t);ft = atn
Where, a = constant incorporating structural and geometric characteristics of the drug dosage form,
ft = Mt/M∞ = fraction of drug release.
n = release exponent

Comparison of dissolution profiles

We found the dissolution profile and release profile of the F4 and F5 very suitable and according to time line. The dissolution profile of F5 is between 0 to 90,the dissolution profile for both formulation were as similar to reference drug of sustain release.

Stability studies

The stability study of the formulation also important and is need for producing the effective formulation. We carry accelerated stability studies for our formulation. We found F4 formulation best in this time frame of stability. We checked its safety and efficiency during accelerated stability study. this satiability study was for 3months at 40c and 75% RH. (Nazzal & Khan, 2006).

CONCLUSION

From the above formulation study we concluded that formulation of combinational drug of naproxen and colchicines are well optimized formulation and can be use for the gout treatment, all the trial batches give good result for this combinational drug .now a days the combinational therapy is very successful and have good synergistic effect and this is easy to take only one time for the patient. in this research we estimate the in-vitro release profile for two different drug with different release profile one was sustain release and other was conventional release. There was no incompatibility for both drugs during release and action in this study we found that F4 have beat result and good release profile.

DISCUSSION

In the present study, the proposed tablets were confirmed to be a successful tool for providing the fast-release of colchicine and the desired sustained-release of naproxen sodium for prolonged period of time up to 12□h. These tablets were composed of sustained-release granules and prepared using of Methocel K4 and HPMC K15.These tablets showed acceptable physical properties and elicited

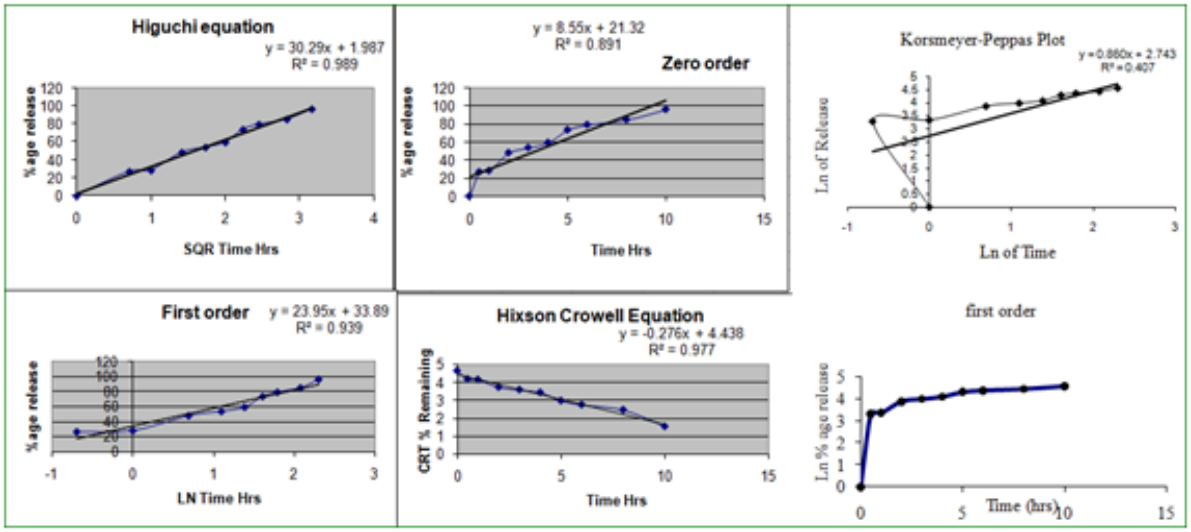

Fig. 6: Kinetic release of naproxen F4

the required *in-vitro* release pattern that coincides with the purpose set for this study. This method is easy and cost effective. Characterization of pallets and final tablets were performed. Final tablets were evaluated for all tests like appearance, friability, dissolution, hardness, assay, weight variation and in-vitro release study performed. The results obtained were satisfactory and complies with USP specification. Formulation containing combination of Methocel K4M and HPMC K15 showed good sustain release profile for 12 hours. In this research we estimate the in-vitro release profile for two different drugs with different release profile one was sustain release and other was conventional release. There was no incompatibility for both drugs during release and action in this study we found that F4 have beat result and good release profile.

Fig. 4 represents the release profiles of naproxen sodium, from the prepared tablets. The tablets showed fast-release of more than 60% of colchicine in 0.1M HCl during 2h of the release study. This was attributed to the prompt disintegration of the fast-release. It is evident that the sustained-release layer of bilayer tablets showed the sustained release of naproxen sodium. The drug release profile parameters for sustained-release products were calculated as per USP specification: after 1h, 18-20% of the naproxen sodium is released; after 6h, 43-72% of naproxen sodium is released; and finally, after 12h, remaining drug is released. For assessment and comparison to these release specifications, the percent of naproxen sodium released from the prepared tablets after 1, 6 and 12h was extracted directly from the release data and was graphically presented in fig. 4. It is evident that the sustained-release granules containing 220mg of Methocel K4 and 4.5g of HPMC K15.As can be seen, these tablets also illustrated a fast-release of colchicine more than 60% during the first 2h of the release study, so they are expected to overcome the disadvantages associated with the delayed dissolution of colchicine in acidic conditions.

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