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ORIGINAL RESEARCH ARTICLE

In Vitro – In Vivo Evaluation of Okra Gum: Guar Gum Compression Coated Diclofenac Sodium Tablets for Colonic Delivery: A Factorial Approach

R.M. Kotadiya[*1], V.A.Patel[2], H.V.Patel[1]

[1]*Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar-388121, Gujarat, India*
[2]*A. R. College of Pharmacy, VallabhVidyanagar-388120, Gujarat, India*

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ABSTRACT

Colon targeting would be valuable when a delay in absorption is therapeutically desirable in treatment of chronic medical condition like rheumatoid arthritis (RA). The major objective was to modulate drug release of prepared matrices to target the peak symptoms of RA in early morning. The main interest in such dosage form was to target the drug to the colon by ensuring minimal amount of drug release in the physiological environment of the upper GI tract. Preparation of compressed coated tablets was done using okra gum and guar gum followed by optimization using 3^2 full factorial designs. Tablets were evaluated for *in vitro* characterizations including detailed dissolution study, *in vivo* pharmacokinetic study and stability study. Formulation (F9) of okra gum: guar gum in 75:25 % ratios with 450mg coat weight is most likely to provide colonic delivery Diclofenac sodium. *In vivo* ingestion in rabbits showed controlled release pharmacokinetic profile of prepared formulation (F9) with C_{max} of 22.31 mcg/mL at 6h (T_{max}) compare to marketed formulation (voveran) with C_{max} of 23.64 mcg/mL at 2h (T_{max}). The insignificant changes in the physical appearance, drug content and dissolution profile of F9 formulation after storage at 40^0 C/75% RH for 3 months indicate good stability.

Kew words: Rat cecal content, in vivo study in rabbit, mathematical modeling, scanning electron microscopy, marketed formulation.

INTRODUCTION

Due to the lack of digestive enzymes and the long transit time, colon is considered as suitable site for the absorption of various drugs. Colon drug delivery system (CDDS) is useful in administering drugs that are irritant to the upper gastrointestinal tract such as non-steroidal anti-inflammatory agents, or drugs such as peptides that are degraded by gastric juice or an enzyme present in the upper gastrointestinal tract [1]. The necessity and advantage of CDDS have been well recognized and reviewed recently [2] . In view of CDDS specifically delivering drug to the colon, a lot of benefits would be acquired in terms of improving safety and reducing toxicity when treating local or systemic chronic diseases. Many approaches have been attempted for the development of colon-specific delivery systems that rely on gastro-intestinal pH, transit times, enterobacteria and luminal pressure for site-specific delivery [3]. It appears that microbially-controlled systems based on natural polysaccharides have the greatest potential for colonic delivery, particularly in terms of site-specificity and safety because of the presence of the biodegradable enzymes only in the colon [4].

Specifically CDDS would be advantageous when a delay in absorption is desirable from a therapeutic point of view, as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis [5].

Rheumatoid Arthritis (RA) is a chronic autoimmune disease of unknown etiology and a major cause of disability. It is characterized by joint synovial inflammation and progressive cartilage & bone destruction resulting in gradual immobility [6]. Diclofenac sodium, non-steroidal anti-inflammatory drugs (NSAIDs), is considered to be first line agent which generally used for the treatment of RA. Since the therapy involves frequent administration of drug, chances of patient noncompliance increase drastically. Additionally, patients suffering from rheumatoid arthritis feel

***Corresponding Author:** R.M. Kotadiya**, Email:** rajiicp@yahoo.com

more pain in the morning hours [7]. In this case taking medication at night is an obvious solution and hence drug need to be administered 4 to 6 hours before achieving their maximum benefits, as a result peak will occur at patients waking and the effect will be decline as patient start to wake up. With orally administering conventional Diclofenac sodium formulation, it is difficult to achieve the desired clinical effect, because it elicits patients' noncompliance of administration in the early morning to coordinate the rhythm of these diseases, due to rapid absorption of the conventional formulation.

It is imperative to design a drug delivery system that administered at bedtime but releasing drug during morning hours would be ideal in this case. However, in comparison to the conventional formulation, colon specific drug delivery was very effective and more convenient for administration. Thus in present investigation an orally-administered core coat tablets of Diclofenac sodium was prepared using combinations of natural polysaccharides okra gum and guar gum to ensure chronotherapeutic drug delivery in colon for the treatment of rheumatoid arthritis.

MATERIALS

Okra pods were purchased from local market, Diclofenac sodium was obtained as gratis samples from Torrent Pharma, Ahmedabad. Guar gum (GG) was obtained from Chiti chem, Vadodara. PVPK30 was purchased from HiMedia Laboratories Private Limited.

METHODOLOGY

Isolation of okra gum (OG):

Isolation of okra gum from fresh okra pods were performed as per method reported specified by[8] . A 3kg weight of fresh Okra pods, from which the stalk and apex of the pods had been removed, was weighed. The okra was sliced with a hand knife, homogenized with two times its weight of 70% (vol/vol) aqueous ethanol at room temperature. The resultant paste was then filtered to get insoluble residue. The residue was washed with double volumes of chloroform/methanol (1/1, vol/vol) with gentle stirring for 30min to remove low molecular weight (colored) compounds followed by filtration using muslin cloth to get pure residue of OG. The residue was finally washed with acetone and product was dried in an oven for 24h. Dried product (OG) was preserved for further study.

Preparation of fast disintegrating core tablets:

The fast disintegrating core tablets of Diclofenac sodium (50mg) were prepared by direct compression using variable quantity of additives to achieve disintegrating time less than 1min. Additives used were starch (disintegrant), Microcrystalline cellulose (diluent) and magnesium stearate (lubricant). Drug and additives were weighed and mixed thoroughly by passing through a mesh (100#) to ensure complete mixing. The mixture was compressed into tablet using 8mm round, flat-faced, plain punches using a 10 station tablet punching machine. The prepared core tablets were evaluated for properties like thickness, hardness, weight variation, friability, content uniformity and disintegration. Disintegration test was performed as per Indian Pharmacopoeia 2007 in pH 6.8 PBS to check the fast disintegrating property of the prepared tablets. The results are depicted in (**Table 1**).

Preparation of Compression Coated Tablets by Factorial design:

A 3^2 factorial design was employed in the present study to prepare compression coated tablets by direct compression method. In this design experimental trials were performed for all 9 possible combinations. The ratio of OG to GG (X_1) and total weight of coat (X_2) were chosen as independent variables, while amount of drug release at 5th hour (Y_5), amount of drug release at 6th hour (Y_6) were selected as dependent variables in this factorial design. The core tablets of Diclofenac sodium were compression coated using a uniformly mixed polymer blend of OG and GG in different ratios at three levels, as mentioned in the 3^2 factorial design layouts (**Table 2**). About one third of uniformly blended coating mixture were placed in 12mm die cavity, the fast disintegrating core tablets of Diclofenac sodium (8mm) was carefully positioned in the centre of the die cavity and filled with remainder of coating mixture. It was then directly compressed around the core tablets at a maximum compression force on 10 station tablet punching machine using 12mm round flat faced punches that had been surface lubricated as and when necessary with magnesium stearate and talc mixture.

A statistical model incorporating interactive and polynomial terms was used to evaluate the response (equation 1).

$$Y=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_1X_1+b_{22}X_2X_2 \text{ -------(1)}$$

Where Y is the dependent variable, b_0 is the arithmetic mean of the 9 trials, and bi is the estimated coefficient for the factor X_i. The $X_1$and X_2 are main effects, represent the average results of changing one factor at a time from its low to

high value. The interaction terms (X_1X_1) show how the response changes when two factors are simultaneously varied. The polynomial terms (X_1X_1 and X_2X_2) are included to investigate nonlinearity**.**

Evaluation of compression coated tablets (Table 3):

Hardness

Hardness of the tablets (five tablets) was determined using a hardness testing apparatus (Monseto Type) as reported in literature [9].

Friability test

The friability of the tablets (six tablets) was measured in a Roche friabilator (DBK friability test apparatus, India). Tablets of a known weight (W_0) or a sample of tablets are dedusted in a drum for a fixed time (100 revolutions) and weighed (W) again. Percentage friability was calculated from the loss in weight as given in equation as below. The weight loss should not be more than 1% w/w as reported in literature [9].

% Friability = $(W_0-W)/ W_0 \times 10$

Weight Variation Test

To study the weight variation of matrix tablets, 20 tablets of each formulation were weighed using an electronic balance and the percent variation of each tablet from the average weight of tablet was calculated.

Drug Content

Ten tablets were finely powdered and an amount equivalent to 50mg of Diclofenac sodium was accurately weighed and transferred to a 100ml volumetric flask and extracted with phosphate buffer (pH 6.8). The mixture was then filtered through a 45µ membrane filter to remove the un-dissolve particle and 1ml of the filtrate was suitably diluted and analyzed for Diclofenac sodium content at 276.0nm using double beam UV/Visible spectrophotometer.

***In Vitro* Drug Release Studies without rat cecal content:**

Drug release studies were carried out using USP Dissolution Test Apparatus (Type I, 100 rpm, 37^0C±0.5°C). The tablets were tested for drug release for 2h in 900mL, 0.1 N HCl, pH 1.2 called simulated gastric fluid (SGF) as the average gastric emptying time is about 2h. Then the dissolution medium was replaced with 900mL, Phosphate buffer saline, pH 7.4 called simulated intestinal fluid (SIF) and tested for drug release for 3h as the average small intestine transit time is about 3h. At the end of the time periods, two samples each of 1mL were taken, suitably diluted and analyzed for drug content at 276.0 nm λ_{max} using UV visible spectrophotometer.

***In Vitro* Drug Release Studies in presence of rat cecal content:**

The susceptibility of OG to the enzymatic action of colonic bacteria was assessed by reported method [10] where by drug release study was continuing in 100mL of pH 6.8 PBS containing 4% wt/vol of rat cecal contents as per the guideline of CPCSEA (Committee for the purpose of control and supervision of Experiments on animal, Ministry of Culture, Government of India) and all the study protocols were approved by the Local Institutional Animal Ethics Committee. The cecal contents were obtained from male albino rats (weighing 150–200g). Rats were sacrificed and abdomen was opened. Cecal contents was collected and immediately transferred in pH 6.8 PBS to get a final cecal dilution of 4% wt/vol called simulated colonic fluid (SCF). The drug release studies were carried out in USP dissolution rate test apparatus (Type II, 100rpm, 37°±0.5°C) with slight modification of apparatus design. A beaker (capacity 150mL) containing 100mL of dissolution medium was immersed and fixed by rubber packing in the 1000mL vessel of dissolution test apparatus. The 1000mL vessel was filled with 200mL of water to maintain 37^0C temperature of 100mL dissolution medium. The tablets were placed in the baskets and immersed in 100mL dissolution medium containing rat cecal contents. The experiment was carried out with continuous N_2 gas supply into 100mL dissolution medium to simulate anaerobic environment of the cecum. The drug release studies were carried out for 3h and 1mL samples were taken at different time intervals without a filtration and replaced with 1mL of fresh pH 6.8 PBS bubbled with N_2 gas. To the samples, 1mL of methanol was added to solubilize the released drug from tablet formulations due to break down of OG by the cecal enzymes. 1mL withdrawn sample was diluted upto 10mL with pH 6.8, phosphate buffer saline, then it was centrifuged and the supernatant was collected. The collected supernatant was filtered through a bacteria-proof filter (45µm) and the filtrate was analysed for drug content at the respective λ_{max} as procedure described in methodology section. The above study was carried out for all the formulations with rat cecal contents and also without cecal contents in pH 6.8 phosphate buffer saline called (control).

Scanning electron microscopy:

During dissolution test at pH 6.8, formulation shows controlled drug release in upper GIT with subsequent drug release in colonic fluid was taken from medium at different time intervals to check their surface integrity by Scanning Electron Microscopy. A scanning electron microscope, (Philips FE1, TGA-7DSC-PYRIS-1DTA-7, Gaseous secondary electron detector, acceleration voltage of 20 kV, chamber pressure of 0.6 mm Hg), was employed to study the morphology and topography of tablets before and after dissolution study. After predetermined time intervals tablets were removed from the dissolution apparatus and undisturbed tablets were subjected to scanning electron microscopy to study the morphological and topographical changes after dissolution in various dissolution medium. Images were taken at different time intervals viz. 2h, 5h and 6h of dissolution study.

Release Kinetics:

To study the mechanism of drug release from the prepared matrices the release data were fitted to various mathematical models like First order, zero order, Hixon crowell, Korsmeyer-peppas and matrix type using software PCP Disso V 3.0[11] (Gambhire et al., 2007). The results are depicted in (**Table 3**).

***In vivo* pharmacokinetic study:**

Promising formulation with respect to aim of study was selected for in vivo study and compared with in vivo pharmacokinetic parameters of marketed tablets (voveran) and pure drug powder using rabbit animal model. Rabbits (New Zealand, White) of either sex weighing (2.5-3.0 Kg) were used to carryout the in vivo pharmacokinetic study with prior approval from the animal ethical committee of Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar (Protocol no. IICP/PH/12-201/03). The test formulations (prepared tablet, marketed tablet and pure drug powder) were administered to rabbits with sufficient flush of water, in a group of three animals, in fasting conditions which was assisted by a local veterinary doctor. Food was withdrawn from the rabbits 12 hours before drug administration and until 12 hours post-dosing. All rabbits have free access to water throughout the study.

Blood samples (1mL) were collected from marginal ear vein before dosing (zero time) and at different time intervals after dosing, namely 1, 2, 4, 5, 6 and 8h using heparinized tubes. The collected samples were immediately centrifuged at 5000rpm for 15minutes and plasma was separated and stored at -20°C until analysis.

Analysis of plasma level of diclofenac sodium

High-performance liquid chromatography as reported by [12] was performed using mefenamic acid as the internal standard. An HPLC model L-7100 equipped with a Rheodyne injector valve with 20µL loop and L-7400 UV detector, Merck Hitachi limited, Tokyo, Japan, was used. The plasma protein was precipitated with acetonitrile. Separation was performed on a Nucleosil 100-5 (C18) column (150 × 4.6 mm). The mobile phase was acetonitrile-0.01 M potassium dihydrogen phosphate (pH 6.3) in a ratio of 35:65 (v/v), and the flow rate was 1mL/min. A wavelength of 278 nm was used to monitor the drug and the mefenamic acid. The peak area ratio served as the basis for quantification.

Stability studies:

Stabilities studies on selected formulations of Diclofenac sodium were carried out as per ICH guidelines at 40°±2°/75% RH ± 5% RH and subjected to accelerated stability studies for 6 months, as India falls under climatic Zone III. The samples were withdrawn monthly (from 0 month to 6th month) and evaluated for physical attributes of tablets such as physical appearance, percentage drug content and dissolution characteristics after each month for 6months as per the procedure described in methodology section. Results are depicted in (**Table 4**).

RESULTS AND DISCUSSION

Relatively economical reported method was selected for the isolation of OG from fresh okra pods. The product obtained was greenish black light weighted flakes of OG which were then size reduced to get a fine free flowing powder. The obtained product was suitably preserved for further study.

A direct compression method was employed to prepare Core tablets of Diclofenac sodium by using various additives as preliminary study. Based on preliminary study, core tablets of Diclofenac sodium (50mg) with MCC (50mg) and starch (50mg) showed acceptable evaluation parameters where the disintegration time was found to be less than 1min that would ensures abrupt and complete release of drug when the dosage form reaches colon (**Table 1**). Hence core tablets containing starch along with MCC were selected for further study.

Compression coated tablets were prepared by direct compression method. Preliminary trial batches containing various concentrations of okra

gum and its combination with other polymers were prepared to check their influence on in vitro characterization of compression coated tablets. It was found that as we increase the concentration of OG, the drug release for upper GIT was retarded till 9.34%. This was attributed to the increased thickness with increase OG concentration that act as resistance and thus retarded the drug release. Further batches were prepared using combinations of polymers to check the synergistic effect of various combinations. Various polymers were combining with okra gum such as HPMC, ethyl cellulose, pectin and guar gum. Prepared formulations were subjected to in vitro dissolution as per procedure mentioned in methodology section. It was found that tablets containing combinations of okra gum with HPMC and ethyl cellulose, were retarded the drug release but some of the tablets were broken down in to two halves during dissolution with subsequent complete drug release and hence they were not considered further. Combination of okra gum and pectin was found to release the drug relatively faster in upper GIT and hence not included for further study. Tablets of okra gum and guar gum combinations were found to retard the drug release in upper GIT and gave faster drug release when it reaches colon.

Thus based on this preliminary study a factorial design was employed to find out the best combination of okra gum and guar gum that minimizes the further drug release in upper GIT and upon arrival to colon that releases the drug as quickly as possible.

In the present study, the effect of percentage of OG top GG and total weight of coat on Y_5 and Y_6 were studied using 3^2 factorial design (**Table 2**). Prepared formulations were also evaluated for their in vitro characterization. The average hardness of all the compression coated tablet formulation no. F1 to F9 lies in the range of 4.23 to 5.14 kg/cm^2. The average friability of all the formulations was lies in the range of 0.45 to 0.77%. Average weight of the compression coated tablets was found to be within ± 5%. Drug content of the developed formulations was found to be in the range of 96-103%, which is within the official requirements. The data clearly indicates that the dependent variables are strongly dependent on the independent variables. Results are depicted in (**Table 3**).

The fitted equation relating the response Y_5 and Y_6 to the transformed factor are shown in equations 1, 2 and 3. The value of correlation coefficient indicates a good fit. The polynomial equation can be used to draw a conclusion after considering the magnitude of coefficient and the mathematical sign it carries (positive or negative). In order to determine the levels of factors which yield optimum dissolution responses, mathematical relationships were generated between the dependent and independent variables using the analysis tool pack (Microsoft Excel).

The equations of the responses are given below:

Release at fifth hour (Y_5)

$$Y_5 = 18.57 - 5.518X_1 - 7.7066X_2 - 2.705X_1X_1 + 0.53X_2X_2 + 1.6725X_1X_2 \text{ ----[1]}$$

R square= 0.9543

Release at sixth hour (Y_6)

$$Y_6 = 38.852 - 6.393X_1 - 9.403X_2 - 3.128X_1X_1 + 1.1366X_2X_2 + 1.34X_1X_2 \text{ ----[2]}$$

R square= 0.9628

Percentage drug released in the fifth hour (Y_5) for the 9 batches show wide variation in the response ranging from a minimum 3.5% to a maximum of 32.12%. This large difference between the minimum and maximum value indicates that the ratio of OG: GG and coating thickness of the formulation, total weight of coat, plays an important role till the formulation is in small intestine (Y_5). As, the ratio of OG: GG and coating thickness increases the drug release is also controlled at intestinal transit. The surface plot for Y_5 for all 9 formulations was shown in (**Fig 2**).

The variation in release profile of 9 batches for drug release at sixth hour (Y_6) was also found on the higher side. The response ranged from minimum of 22.31% to maximum of 55.44% drug release. When the formulation reaches the colon the coat started to dissolve and the inner core is exposed to the colonic fluid. Negative signs of X_1 and X_2 in Equation 1 and 2 indicate that the drug release in inversely proportional to ratio of OG: GG and total weight of coat. The surface plot for Y_6 for all 9 formulations was shown in (**Fig 3**).

Selection of best batches

Best batch was selected on the basis of following criteria:

1) Less than 10% release in SGF and

2) Minimum 90% release within 3h after the tablet reaches colon

Based on these criteria two batches were selected (F6 and F9). Other batches failed to prevent drug release in SGF and SIF as 12-32% drug was released at the end of 5h as shown in (**Fig 1**). In most of the batches majority of the drug release is found in the colon. Batch F6 and F9 are the only batches which showed more than 90% drug release in SCF within 3h after the tablet reaches the colon. Of these batches batch F9 can be considered as best batch since about 5.45% drug

released till 5th h and 91.22% drug released within 8hrs.

Scanning electron microscopy (SEM):

During dissolution study, formulation (F9) shows controlled drug release in upper GIT with subsequent drug release in colonic fluid was subjected to SEM study to check their surface integrity. As per SEM study an images were taken at different time intervals viz. 2h, 5h and 6h (**Fig 4**). These time intervals were selected because that reflects the change of dissolution medium namely, 2h in 0.1N HCl, pH 1.2, 5h in PBS, pH 7.4 and 6h in PBS, pH 6.8 containing rat cecal content, subsequently of dissolution study. It was found that the optimized tablet formulation (F9) was exhibited less porosity after dissolution test in 0.1N HCl, pH 1.2 for two hours. SEM image of same tablet after 5h in PBS, pH 7.4 showed increased porosity with time. This might be attributed to neutral pH of okra gum [13] . Due to its low solubility in 0.1N HCl, pH 1.2, it gave low porosity. Where as in PBS, pH 7.4 the higher porosity was attributed to its solubility at near neutral pH (pH 6.5). SEM image of same tablet after 6h in PBS containing rat cecal content, pH 6.8 showed larger pore formation after drug dissolution. The microbiota of rat cecal content might act upon the natural polysaccharides and stimulates microbial degradation that resulted in the larger pore size. The increased porosity of the surface promoted the increase of drug diffusion and the opening of the channels with abrupt release of drug. These pictures clearly show that pore formation is the most important mechanism of release.

Kinetics study:

The data obtained from in vitro dissolution studies (F9) were fitted in different models viz. zero order, first order, Higuchi (matrix) and Korsmeyer - Peppas equation, the results are shown in (**Table 4**). It was also observed that highest correlation was found for Korsmeyer - Peppas release profile ($R^2 > 0.99$). A value of diffusional exponent, n, for formulation studied here was 1.2743, indicated an anomalous behavior corresponding to swelling, diffusion and erosion mechanism. In this formulation, ratio of OG to GG (X_1) decreases the hydration of matrix and retards the release by erosion mechanism owing to its relative hydrophobic property and total weight of coat (X_2) retards the release by diffusion mechanism. Together, these polymers retard the release of drug using different mechanisms. This model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved.

***In vivo* pharmacokinetic study:**

In vivo pharmacokinetic study was performed in White New Zealand rabbits and parameters like C_{max}, maximum concentration and T_{max}, time to reach maximum concentration are the values directly obtained from the plasma concentration time curve. *In vivo* pharmacokinetic parameters for best batch (F9) were determined and compared with marketed formulation (Voveran, Novartis Pharmaceuticals Ltd, India). Plasma concentration versus time profiles for prepared and marketed formulations were shown in (**Fig 5**). Time taken to release maximum amount of drug (T_{max}) for prepared and marketed formulations were found to be 6h and 2h, respectively with peak plasma concentrations of 22.31±2.64mcg/mL and 23.64mcg/mL, respectively. The marketed tablet releases the drug conventionally where as controlled release of drug from prepared tablet in upper GIT was attributed to the protective action of OG and GG in upper GIT. As soon as the prepared tablet reaches lower GIT it might act upon by the colonic content with subsequent abrupt drug release.

A good correlation between the dissolution profiles and bioavailability was observed. In vitro-in vivo correlation was determined by plotting a graph showing the fraction of drug absorbed versus the fraction of drug released in vitro. A high value of correlation coefficient ($r^2 = 0.9394$) suggested good correlation between in vitro-in vivo data (**Fig 6**).

Stability study:

In view of the potential utility of F9 formulation for colonic release of Diclofenac sodium, stability studies were carried out by storing the formulation at 40^0 C/75% RH for 3 months to access the long term (2 years) stability. There was no change in the physical properties of F9 formulation at the end of storage period. When the dissolution study was conducted in simulated GI and colonic fluids as described above, no significant difference ($P>0.05$) was observed in the cumulative percentage of drug released from F9 stored at 40^0 C/75% RH for 3 months when compared to that released from same formulation before storage (**Table 5**). The insignificant changes in the physical appearance, drug content and dissolution profile of F9 formulation after storage at 40^0 C/75% RH for 3 months indicate that the formulation could have a minimum shelf life of 2 years.

Table 1: Formulation and evaluation of core tablets

Ingredients	
Diclofenac sodium	50mg
Starch	50mg
MCC	50mg
Magnesium stearate	5mg
Evaluation parameters	
Weight variation (mg)	155 ± 1.2
Hardness (Kg/cm^2)	2.25 ± 0.12
Friability (%)	0.45 ± 0.03
Drug content (%)	98.34 ± 1.1

Table 2: Factorial design

Code	**Coded values**	
	X1	**X2**
F1	-1	-1
F2	-1	0
F3	-1	1
F4	0	-1
F5	0	0
F6	0	1
F7	1	-1
F8	1	0
F9	1	1

Coded values	**Independent variables**	
	X_1(%)	**X_2(mg)**
-1	25:75	250
0	50:50	350
+1	75:25	450

Table 4: Release kinetics

Models	**R Square**	**K**	**Inference**
Zero order	0.9874	27.0782	Passes
First order	0.9052	-0.5514	Fails
Matrix	0.9081	40.0562	Fails
Korsmeyer-Peppas	**0.9983**	20.8684	Passes
Hixon Crowell	0.9394	-0.1398	Fails
Best fit model	**Korsmeyer-Peppas**		
Parameters			
n =	1.2743		
k =	20.8684		

Table 5: Dissolution study for stability study

Time (h)	**Cumulative percentage release (F9)**	
	Before storage	**After storage**
0	0	0
1	0	0
2	0	0
3	1.0 ± 0.03	1.2 ± 0.02
4	1.6 ± 0.03	1.8 ± 0.09
5	5.45 ± 1.2	6.14 ± 0.8
6	39.31 ± 1.1	40.31 ± 1.1
7	64.35 ± 1.2	65.33 ± 1.6
8	97.22 ± 1.3	98.14 ± 1.2

Table 3: In vitro characterization of formulations

code	**Weight variation (mg ± SD) n=10**	**Hardness (Kg/cm^2± SD) n=5**	**Friability (%)**	**Drug content (%)**	**Y_5 (%)**	**Y_6 (%)**
F1	405 ± 1.25	4.51 ± 0.08	0.45	101.2	32.12	55.44
F2	505 ± 1.25	4.62 ± 0.04	0.65	101.2	18.65	38.64
F3	605 ± 1.91	4.23 ± 0.08	0.64	99.47	14.44	34.55
F4	405 ± 1.16	5.14 ± 0.14	0.48	96.78	26.45	48.64
F5	505 ± 1.64	4.32 ± 0.14	0.57	97.97	21.44	41.55
F6	605 ± 1.82	4.64 ± 0.08	0.67	96.54	8.88	28.64
F7	405 ± 1.33	4.76 ± 0.08	0.55	102.9	16.44	37.84
F8	505 ± 1.66	5.14 ± 0.04	0.59	100.8	10.21	30.11
F9	605 ± 1.78	4.42 ± 0.08	0.77	98.94	5.45	22.31

Fig 1: Dissolution study

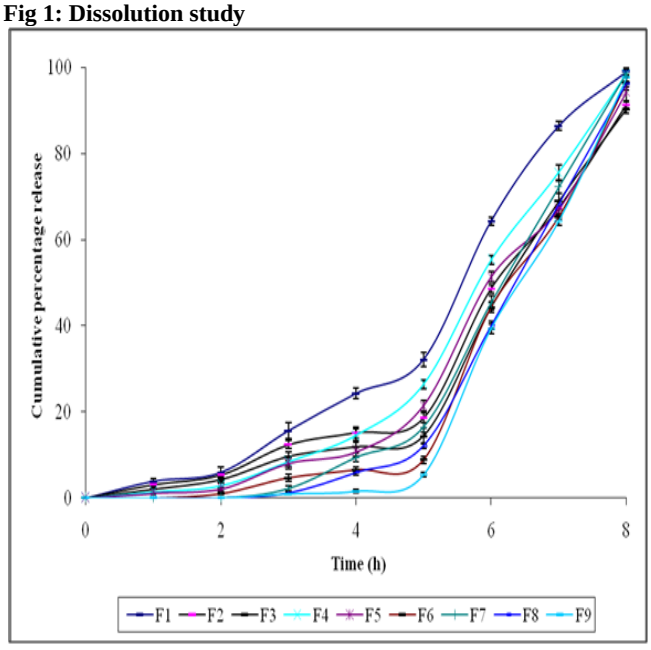


Fig 2: Surface plot for Y_5

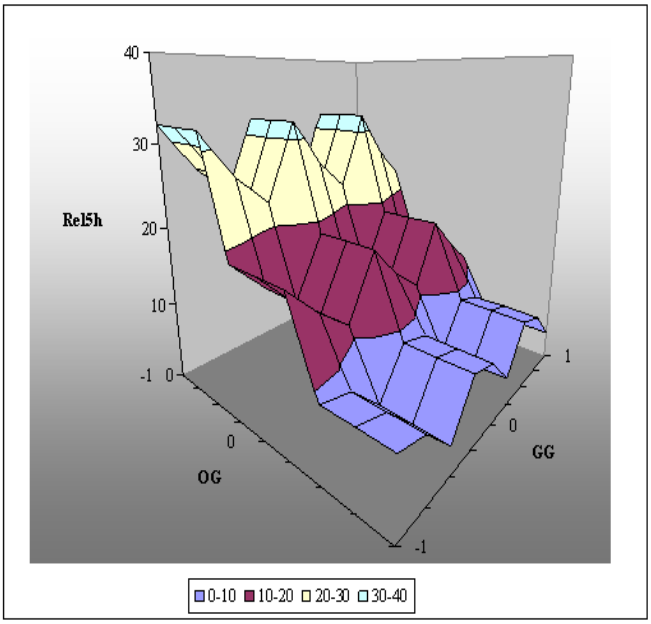


Fig 3: Surface plot for Y_6

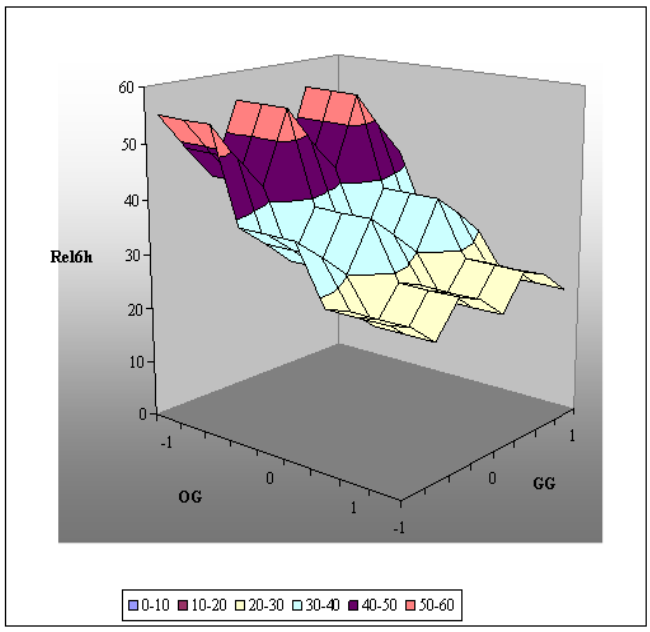


Fig 4: Scanning Electron Microscopy

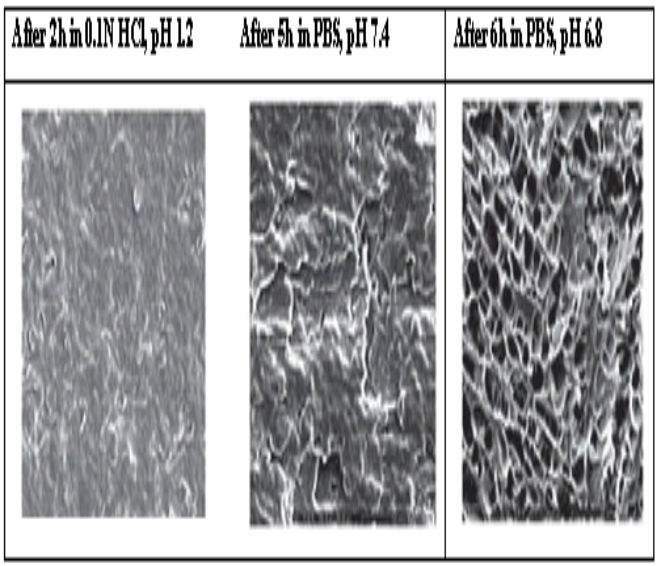


Fig 5: *In vivo* pharmacokinetic study

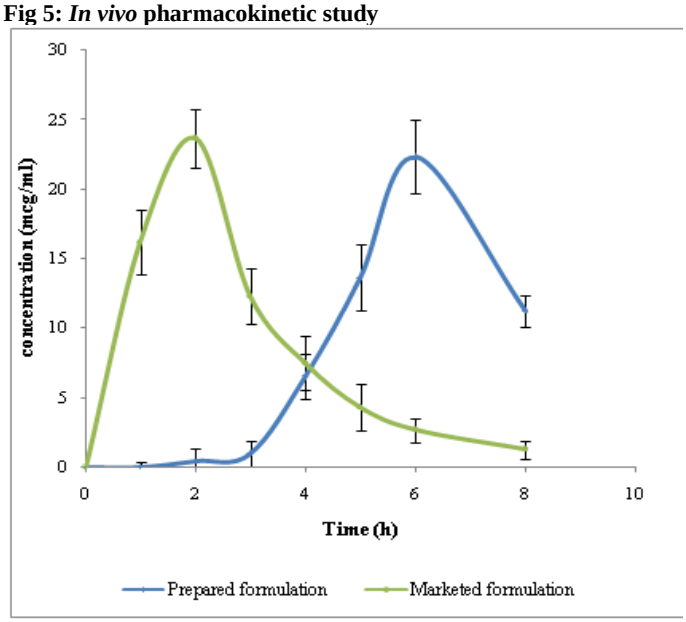
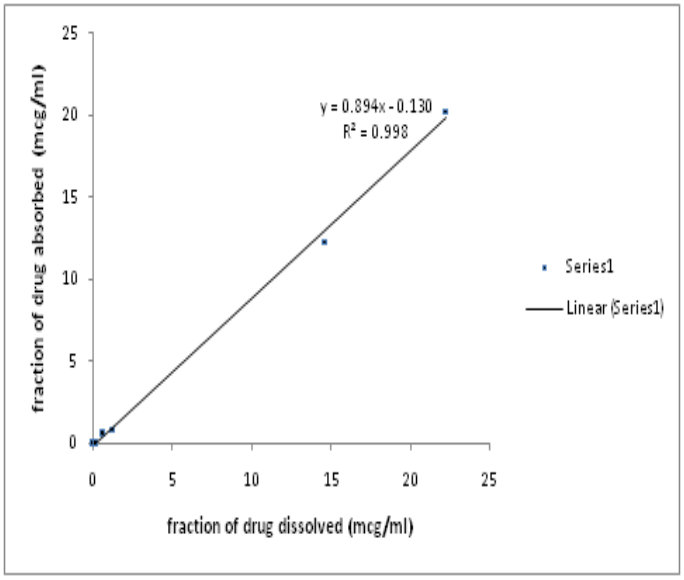


Fig 6: Fraction of drug absorbed versus fraction of drug release (F9)



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