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Research Article

Development of Enteric Coated Bioadhesive Matrix Tablet of Lornoxicam: *in Vitro* – *in Vivo* Evaluation in Healthy Human Volunteers

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Abstract

The objective of present study was to prepare and evaluate sustained release bioadhesive tablet of LXM is prepared by direct compression technique using various semi synthetic (HPMC different grades) and natural polymer (Xanthan gum) in order to improve its GI residence time and improve its bioavailability for its safe use in different arthritic conditions. Various formulations were developed by using release rate controlling and gel forming polymers like HPMC (K4M), HPMC (K15M) and xanthan gum by direct compression method. The prepared tablets were subjected to physicochemical and *in-vitro* drug release study. The physicochemical properties of tablets were found within the limits. The effect of polymer concentration on the release profile and *in-vitro* bioadhesion of the matrix tablets was studied. The tablet containing HPMC K4M (20%) and MCC along with talc and magnesium stearate (2 % w/w, each) was considered as an optimum formulation (L-7). The most important side effects of LXM include peptic ulceration, nausea, etc in stomach hence it was decided to develop an enteric coated LXM tablet formulation to minimize the gastric intolerance caused by LXM on long term use and to improve patient compliance by utilizing Opadry® enteric (94 series) polymer. The optimized formulation L-12 was subjected to physicochemical, *in-vitro* drug release and the dissolution profile was compared with the marketed product Flexilor® SR tablets. The L-12 formulation exhibited almost similar drug release profile in different dissolution media as that of marketed tablet Flexilor SR® Tablet. Formulation L-12 was found stable at accelerated conditions 40± 5^{0}C/75 % RH for a period of 6 months. The pharmacokinetic parameters C_{max}, T_{max}, MRT and AUC of developed SR tablet were found to be improved with significant difference ($p<0.05$) when compared with product Flexilor® SR tablets. The gastro-intestinal transit behavior of L-12 in the human was observed in real time using radiographic imaging technique (X-ray), which demonstrated that tablets passed from stomach without adhering to intestine. The tablets were detected up to 6 h in human body.

1. INTRODUCTION

Lornoxicam (LXM, 6-chloro-4-hydroxy- 2-methyl-N-2-pyridinyl-2H-thieno-[2,3-e]-1,2-thiazine-3- carboxamide 1,1-dioxide) is a novel non-steroidal anti-inflammatory drug (NSAID) in the enolic acid class of compound with analgesic, anti-inflammatory and antipyretic properties.[1,2] LXM, which is commercially available as an 8 mg tablet, is used to treat inflammatory diseases of the joints, osteoarthritis, pain after surgery, and sciatica[2]. It works by blocking the action of cyclooxygenase, an enzyme involved in the production of chemicals, including some prostaglandins, in the body. All NSAIDs reduce inflammation caused by the body's own immune system and are effective pain killers[3].

Non-steroidal anti-inflammatory drugs (NSAIDs) are considered to be the first-line drugs in the symptomatic treatment of rheumatoid arthritis, osteoarthritis and Ankylosing spondylitis. LXM is one of the emerging NSAID molecules for arthritis treatment. The successful treatment of arthritis depends on the maintenance of effective drug concentration level in the body for which a constant and uniform supply of drug is desired. Sustained release dosage forms deliver the drug at a slow release rate over an extended period of time and achieve this objective. The short biological half-life (about 3-4 h) and dosing frequency is TID hence LXM becomes an ideal candidate for sustained release.[2] To reduce the frequency of administration and to improve patient compliance, a once-daily sustained release formulation of LXM is desirable. [4,5,6] Sustained release bioadhesive dosage forms with prolonged residence times in the GI tract are highly desirable for drugs with narrow absorption windows.[7] For sustained release systems, the oral route of drug administration has, by far, received the most attention as it is natural, uncomplicated, convenient and safer route.[8,9] Matrix tablets composed of drug and release retarding material (e.g. polymer) offer the simplest approach in designing a sustained release system. Matrix tablets are prepared by either wet granulation or direct compression method. Currently available sustained matrix tablets are generally prepared by wet granulation method. The tablets prepared in the present study by direct compression method. The method has advantages over the tablets prepared by wet granulation in terms of time saving and resources utilization, thus making it possible to formulate tablets at a lower cost.[10] Because of their flexibility, hydrophilic polymer matrix systems are widely used in oral controlled drug delivery.[11] Among the hydrophilic polymers, HPMC is frequently used because of its non-toxic nature, easy compression, swelling properties and accommodation to high levels of drug loading.[12] Additionally HPMC is a pH independent material and hence drug release from HPMC matrix formulations is generally independent of processing variables.[13] There sustained release coupling with bioadhesion characteristics to dosage form and developing bioadhesive tablets systems have advantages such as efficient absorption, enhanced bioavailability of drugs, a much more intimate contact of drug with the mucus layer, and specific targeting of drugs to the absorption site. [14, 15]

The objective of present study was to prepare and evaluate enteric coated sustained release bioadhesive tablet of LXM is prepared by direct compression technique using various semi synthetic (HPMC different grades) and natural polymer (Xanthan gum) in order to improve its GI residence time and improve its bioavailability for its safe use in different arthritic conditions.

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2. MATERIALS

The authentic samples of LXM and Thiocolchicoside (internal standard) were obtained from Glenmark Pharmaceutical Ltd, Sinner, India and Matrix lab, Sinner, India respectively. Opadry enteric white® (94 series) coating material, Hydroxypropyl methylcellulose (HPMC) K4M and K15M (with reported nominal viscosity values of 5, 4000 and 15000 cP respectively, when present in concentration of 2% in water at 20°C) were kindly supplied by Colorcon Asia Pvt. Ltd, Goa. Xanthan gum (1200 cP) was gifted by GlaxoSmithKline Ltd., Nashik, India. The marketed Lornoxicam 16 mg tablets (Flexilor® SR tablet, Batch No. 05901272) of Glenmark Pharmaceutical Ltd., Baddi, India used as reference product for bioavailability studies were purchased from local market (Loni, India). Acetonitrile (HPLC grade), Potassium dihydrogen phosphate (KH_2PO_4) (AR grade) and whatman no 5 filter paper were purchased from Modern science Pvt Ltd. (Nashik, India). The 0.45 µm pump Nylon filter was obtained from Advanced Micro Devices (Ambala Cantt, India). Milli-Q water was used throughout the work. Other chemicals used were analytical or HPLC-grade and glasswares used were Class A grade.

3. METHODS

3.1 Preparation of Preliminary LXM tablets

Matrix tablets of LXM were formulated using direct compression technique. The different proportion of HPMC K4M, HPMC K15M and Xanthan gum polymers were used to prepare LXM matrix tablet. The compositions of the tablet formulations are given in 1. Core tablets containing 16 mg LXM were prepared by direct compression method. All ingredients are initially sifted through seive ASTM no. 40 sieve. The magnesium stearate and talc were sifted through sieve no 60. Weighed amounts of LXM, HPMC, Xanthan gum and diluents (DCP & MCC) were again sieved through Seive ASTM no. 40 sieve and mixed in stainless steel mortar to get a uniform mixture. The mixture was then blended in laboratory blender with magnesium stearate and talc (2 % w/w each) for 2 min. The mixture was compressed into tablets employing direct compression method (8 Station tablet compression machine, Cadmach, Ahmedabad, India) using 8 mm concave punches.

Table 1: Composition of preliminary SR tablet of LXM

Sr. No	Ingredients (mg)	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
1	LXM	16	16	16	16	16	16	16	16	16	16
2	DCP	50	50	50	50	50	50	50	50	50	50
3	MCC	149	136.5	124	111.5	149	136.5	124	111.5	149	136.5
4	Xanthan Gum	25	37.5	50	62.5	-	-	-	-	-	-
5	HPMC K4M	-	-	-	-	25	37.5	50	62.5	-	-
6	HPMC K15M	-	-	-	-	-	-	-	-	25	37.5
7	Magnesium Stearate	5	5	5	5	5	5	5	5	5	5
8	Talc	5	5	5	5	5	5	5	5	5	5
	Total Weight (mg)	**250**	**250**	**250**	**250**	**250**	**250**	**250**	**250**	**250**	**250**
	Polymer Conc.	**10%**	**15%**	**20%**	**25%**	**10%**	**15%**	**20%**	**25%**	**10%**	**15%**

3.2 Optimization of coating process

The optimized and finalized formulation was subjected for enteric coating. The enteric coated polymer opadry enteric white® (94 series) was optimised for coating.[16] The composition of coating formula is as given in table 2.

The coating suspension was prepared by Disperse opadry® enteric white in isopropyl alcohol in a vessel to form a vortex without drawing air into the liquid, soak it and then pass through colloidal mill. Dispersed Lake quinoline white, yellow oxide of iron in dichloromethane. Then passed this mixture through colloidal mill. The above soaked solution is added to dichlomethane mixture and stirred well for 30 min. Weight gain during the tablet coating was considered as 4 % of initial tablet weight. Visual inspection was performed on tablet in order to minimize the defects. The coated tablets were collected in a cleaned double polybag and evaluated for appearance, uniformity of weight, average weight, assay and *in-vitro* dissolution test.

Table 2: Optimization of coating composition for LXM SR tablet

Sr. No	Ingredients (mg)	L11-C1	L11-C2	L11-C3
1	Opadry® Enteric white (94 series)	5.0%	7.5%	10%
2	Red oxide of iron	0.055	0.055	0.055
3	Lake quinoline yellow	0.050	0.050	0.050
4	Yellow oxide of Iron	0.045	0.045	0.045
5	Isopropyl Alcohol $ (80%)	qs	qs	qs
6	Dichloromethane $ (20%)	qs	qs	qs

$- Evaporated

3.3 Physicochemical characterization of tablets

The thickness and diameter of the tablets (n=6) were determined using digital vernier calipers. Five tablets from each batch were analyzed and average values were calculated. The weight variation[17] was determined by taking weight of 20 tablets using an electronic balance (Mettler Toledo, XP 105,). Tablet hardness was determined for 10 tablets using a Monsanto tablet hardness tester. Friability was determined According to the BP specifications[18]; a sample of 20 tablets was placed in the drum of a tablet friability test apparatus (EF2, Electrolab, Mumbai). The drum was adjusted to rotate 100 times in 4 min then the tablets were removed from the drum, dedusted and accurately weighed. This process was repeated for all tablets formulations and the percentage weight loss was calculated.

3.4 Ex Vivo Bioadhesion Studies[19]

Bioadhesion studies were conducted, using a modification of the assembly described by Singh et al., 2002 with porcine gastric mucosa as the model membrane. The mucosal membrane was excised by removing the underlying connective and adipose tissue, and equilibrated at 37°C ± 1°C for 30 minutes in isotonic PBS before the bioadhesion evaluation study. The tablet was lowered onto the mucosa under a constant weight of 5 g for a total contact period of 1 minute. Bioadhesive strength was assessed in terms of the weight in grams required to detach the tablet from the membrane.

3.5 In-vitro dissolution studies

The study was carried out using dissolution apparatus USP Type-I (basket) The *in-vitro* dissolution studies were performed up to 12 hours and more using dissolution apparatus (Electrolab, EDT-O8L, Mumbai, India). The 900 mL of dissolution medium was added to each of six flasks and equilibrated it at 37 ^{0}C ± 0.5 ^{0}C. Weighed six individual tablets. Then transfer one tablet to each of six vessels. The apparatus was started. After each time interval, 10 mL sample was withdrawn from a zone midway between the surfaces of the dissolution medium. (Replaced 10 mL with fresh dissolution medium) and then filter through Millipore membrane filter paper only. Discarded first 2-3 mL solution and take the reading on UV at 378 nm using dissolution medium as a blank. Drug concentrations in the samples were determined from the standard calibration curve. Cumulative percent of drug dissolved was found out at each time point.

3.6 Drug content of LXM [20]

The drug content of the prepared matrix tablet was determined in triplicate using reported method in literature[20]. From each batch, 20 tablets were taken, weighed, crushed and finely powdered. A powder equivalent to 10 mg of LXM of weighed accurately and transfered to 100 mL volumetric flask. Add about 70 mL diluents (mobile phase: acetonitrile 1:1) and sonicate for 30 minutes with intermittent shaking, cool to room temperature. Make up the volume with diluent and mix. Filter it trough whatman no. 5 filter paper. (Conc. 100 µg/ml). Pipette out 5 mL above solution & dilute to 50 mL with diluent and mix well (Conc. 10 µg/ml). The final solution was injected in HPLC, chromatogram was recorded at 291nm and area was measured.

3.7 Stability Studies[21]

The stability of all the formulations was carried out at different temperature sas per ICH guidelines. The selected formulations were wrapped in aluminum foil and placed in stability chamber. A stability study for the present work was carried out 40 ± 2°C/ 75 ± 5 % RH for period of 6 months and evaluated for their physical characteristics, *in-vitro* drug release and drug content.

3.8 In-vivo bioavailability study of LXM Formulations in Healthy Human Volunteers

The bioavailability protocol was approved by the Institutional Ethical Committee (Pravara Institute of Medical Sciences, Rural Medical College, Loni, Maharashtra, India). Eight healthy male volunteers in the age group of 21–35 years (61–73 kg) participated in the study. A crossover single dose study was followed. The volunteers were divided into two equal groups (group A and group B) (Table 9.2). Group A volunteers (n=4) received Flexilor® sustained release tablets (Batch no. 05901272, Glenmark Pharmaceutical Ltd, Baddi (dose 16 mg) whereas group B (n=4) volunteers received developed L-12 tablet containing 16 mg of LXM. A light breakfast was provided after overnight fasting. After 30 minutes, Sample and Flexilor® 16 mg SR tablets were administered to each subject with 200 mL of water. Lunch was provided 4 hours after drug administration. Blood samples of 3 mL were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h. The samples were allowed to clot and centrifuged at 3500 rpm for 15 minutes. Serum was separated and stored at -20^{0}C until analysis.

3.9 Estimation of LXM in human serum

Analysis of LXM in human plasma samples was done by modification of HPLC method reported by Susanna Radhofer-Welte *et al.,* 2009 and Pankaj Kumar *et al.,* 2012. [3,22] The standard stock solution of LXM and Thiocolchicoside (internal standard) were prepared by dissolving 10 mg of each drug in 100 mL of acetonitrile in separate volumetric flasks to get concentration of 100 µg/mL. The stock solution of LXM was further diluted with acetonitrile to get series of working standard solutions having concentration 20, 50, 100, 200, 500, 750 and 1000 ng/mL. 0.5 mL of Thiocolchicoside stock solution was further diluted to 250 mL with acetonitrile to get internal standard solution of concentration 200 ng/mL.

1 mL of each working standard solution of LXM (20-1000 ng/mL) was transferred in a series of eppendorf tubes (Eppendorf-Netheler-Hinz, Hamburg, Germany) containing 1 mL of human plasma, separately. In each flask, 0.5 mL stock solution of Thiocolchicoside (200 ng/mL) was added and 3.5 mL of ethyl acetate was added for complete precipitation of proteins. The tubes were vortex-mixed for 1 min, and then centrifuged for 15 min at 3500 rpm. The supernatant layers were filtered through a Millipore 0.45 µm filter into 10 mL tubes and evaporated while immersed in a 40^{0}C water bath. Each sample was reconstituted with 500 µL of mobile phase and vortexed for 30s. 20 µL sample was injected into the HPLC system. The Agilent Technologists HPLC system (1100 series LC) equipped with quaternary pump, degasser, autosampler, thermostatted column compartment and UV detector, a reversed phase C_{18} Hypersil BDS column, (250 mm × 4.6 mm x 5 µm). HPLC mobile phase was composed of 10 mM KH_2PO_4 (pH 6): Acetonitrile (70: 30 %v/v) at a flow rate of 1 ml/min with the detector wavelength set at 290 nm.

3.10 Pharmacokinetic data analysis

LXM plasma concentration-time data were analyzed for each subject using non-compartmental methods. Basic pharmacokinetic parameters required for the comparison of bioavailability, such as peak serum concentration (C_{max}), time to reach the peak serum concentration (T_{max}), and area under serum concentration time curve (AUC) for the drug under observation were obtained in each subject from plasma concentration versus time profile using KINETICA 5.0 software (Inna Phase Corp., 2000).

3.11 Gastrointestinal Transit (GI) Behaviour [23]

The GI transit behaviour of the formulation was visualized using fluoroscopy (low energy, Konica Minolta, Siemens, Germany) under the supervision of a radiologist. The study was approved by the institutional Ethical Committee (Pravara Institute of Medical Sciences, Rural Medical College, loni, India). Three healthy male subjects (age of 20 - 30 years; mean weight 65 - 72 kg) participated after giving informed consent. The study was conducted by administering one tablet containing 16 mg of barium sulphate to a subject[23]. The subjects swallowed the tablet with 200 mL of water after overnight fasting. The subjects received standard breakfast after 3h. During the experiments the subjects remained in a sitting or upright posture. In each subject the position of the tablet was monitored by X-ray photographs (Konica Minolta, Siemens, Germany) of the gastric region at determined time intervals up to 6 h.

4. RESULTS AND DISCUSSION

The results of physicochemical evaluation of tablets are given in Table 3. The tablets of different batches were found uniform with respect to thickness (4.04-4.24 mm), diameter (8 mm) and hardness (6.0 to 7.5 kg/cm^2). The friability (%) and weight variation of different batches of tablets were found within the prescribed limits (friability: 0.20 to 0.60%; deviation of weight variation test: 2.12 to 4.21%). A good and uniform drug content (>98%) was observed within the batches of different tablet formulations. The tablets consisting of HPMC K4M, K15M and xanthan gum in different concentrations exhibited good mucoadhesive properties in the *ex-vivo* bioadhesion test observed with porcine mucosa. The increase in HPMC concentration causes strong bioadhesion, while decrease in amount reduces the bioadhesive property. This indicates that HPMC possesses a large number of carboxyl of hydroxyl groups that are responsible for adhesion and thus results in increase in mucoadhesive properties of tablets. Hence, the tablets containing drug, HPMC, XG, DCP/MCC, talc and magnesium stearate could be prepared satisfactorily by direct compression method.

Table 3: Evaluation of preliminary SR tablet of LXM

Batch No.	Thickness (mm)	Diameter (mm)	Average weight (mg)	Hardness Kg/cm2	Friability (%)	Assay (n=3) (by HPLC) (%w/w)	Bioadhesive Strength (g)
L1	4.01±0.02	8	251.3	6-8	0.41	99.85±0.55	08
L2	4.02±0.04	8	253.2	5-8	0.59	99.77±0.32	11
L3	4.01±0.04	8	250.8	5.5-8	0.35	99.95±0.53	13
L4	4.01±0.04	8	252.3	6-8	0.20	99.15±0.87	14
L5	4.00±0.06	8	254.1	6-8	0.44	99.27±1.12	19
L6	4.01±0.07	8	250.1	6-8	0.60	99.25±0.23	21
L7	4.00±0.03	8	250.7	6-8	0.33	100.24±0.43	23
L8	4.01±0.05	8	252.4	6-8	0.37	101.25±0.51	24
L9	4.01±0.10	8	253.3	5.5-8	0.46	99.22±0.13	22
L10	4.01±0.08	8	254.7	6-8	0.52	100.5±0.92	26

± *Standard deviation*

4.1 In-vitro dissolution testing of preliminary batches

Dissolution testing is an *in-vitro* method of assessing the rate and extent of drug release for all batches of a development, new or commercially available products. The tests should be sensitive enough to demonstrate any small variable in manufacturing of a product as well as the type and level of excipients used. Therefore, it is possible that an over-discriminatory test, although in-vivo irrelevance might be suitable for these purposes.[24] Dissolution testing is a valuable tool when developing a SR drug product as dissolution profiles of test formulations can be compared to those of the reference product

In order for a LXM to exert an appropriate pharmacological effect *in-vivo*, it must initially be released from a dosage form and the dissolve, to be made available for absorption.

The release profiles of preliminary formulations (L1 to L10) are given in figure 1. The rate of drug release was found to be inversely related to proportion of the polymer present in the matrix structure, i.e. the drug release increased with lower viscosity grade and polymer proportion in the matrix tablet. The L1-L4 formulation contains XG as a polymer and is employed in a concentration of 10 - 25 %w/w (L1 - L4). The LXM showed rapid release from the formulation. The complete drug release occurs within 8 hours. The L5 – L8 formulation contains HPMC K4M as a polymer and is employed in a concentration of 10 - 25 %w/w. The L-5 showed 100 % drug release in 6 hours while L-6 showed 100 % drug release in 10 h. The formulation L-7 showed good release pattern and the release is as per targeted SR tablet.

The L-9 and L-10 formulation contains HPMC K15M in a concentration of 10 % and 15 %; the time required for initial release of drug is low. This could be due to more time required for wetting the tablet. As a result, more time was required for the formation of diffusion layer leading to higher percentage of drug release initially. Afterwards, drug has shown retardant release due to viscous nature of HPMC (figure 1).

In-vitro dissolution studies showed that the drug release from xanthan gum matrix was fast. Even though increasing concentration of xanthan gum from 10-25% (L-1 to L-4) decreases the drug release. The formulation L-4 containing 25% of xanthan gum showed 100% drug release in 8 h. Xanthan gum viscosity is relatively independent of pH. Tablets containing xanthan gum underwent greater surface erosion at early times in simulated gastric fluid. Once the matrix hydrates and swells the rate of erosion slows down. The net effect of initial rapid erosion, diffusion and swelling of the matrix occurs with xanthan gum. Hence xanthan gum was excluded from further study.

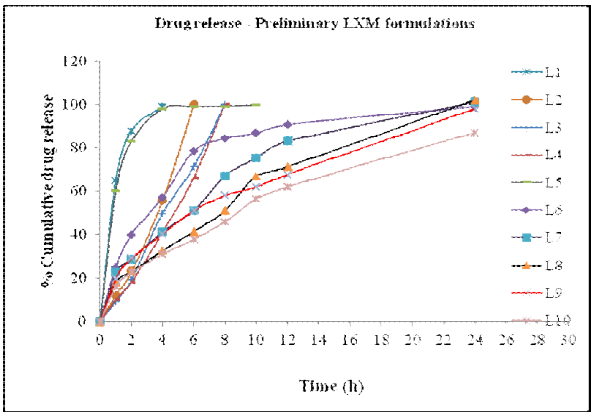

Figure 1: % Cumulative drug release profile of preliminary LXM formulation in phosphate buffer pH 6.8

But it was clearly observed that as the HPMC K4M proportion increases in the formulation the release rate decreased (L-5 to L-8). The formulation containing 20 % of HPMC K4M releases around 83.23 % of drug in 12 hours. The polymer proportion of 25 % of HPMC K4M releases around 72 % in 12 hours. The HPMC K15M, proportion of 10 % (L-9) releases only 67.78 % of drug in 12 hours and subsequently L-10, containing 15% HPMC K15M showed 62.12% drug release in 12h. The lowest drug release rate was obtained with L-9 and L-10 in phosphate buffer media. The HPMC K15M builds up an excessively viscous gel around the tablet. This is more resistant to water penetration and erosion.

4.2 Drug release kinetics

In order to study the exact mechanism of drug release from tablet, drug release data was analyzed according to Zero Order[25], First Order[26], Korsmeyer-Peppas[27], Hixson-Crowell and Matrix model.[28]

Table 4: *In-vitro* drug release kinetic studies of LXM formulations

Batch No.	Zero order	1st order	Matrix	Hix.Crow	Peppas		Drug transport mechanism
	r^2					n	
L-6	0.3480	**0.9941**	0.9396	0.9905	0.9655	0.46	Non-Fickian Transport
L-7	0.7681	0.9707	0.9900	0.9763	**0.9907**	0.51	Non-Fickian Transport
L-8	0.8998	0.9158	0.9872	0.9410	**0.9902**	0.57	Non-Fickian Transport
L-9	0.8005	0.9276	0.9993	0.9858	**0.9990**	0.50	Fickian diffusion
L-10	0.8706	0.9954	0.9944	0.9854	**0.9957**	0.54	Non-Fickian Transport

The model with the higher correlation coefficient was considered to be the best model.[29] Putting all data (Table 4) in different release kinetics models and comparing the coefficient of determination (r^2), it was found that the release data of L-6 obeys First order kinetics, where as L-7 to L-10 follows Peppas kinetics. To justify the results, power law was applied and from the diffusion coefficient value (n), it was found that L-6, L-7, L-8 and L-10 formulations follow Non-fickian diffusion transport mechanism, while L-9 follows Fickian diffusion.

The results obtained from the evaluation of tablet characteristics were utilized in the selection of optimized formulation. Hence formulation containing 20% of HPMC K4M (L-7) were selected for further development process because higher than 20% HPMC K4M showed a very less release and lesser than 20% HPMC was expected to give immediately rapid drug release. Only L-7 formulation was following decided pattern. The directly compressible diluents like DCP and MCC were used in different concentrations in order to reduce the rigidity of swollen matrix. Additionally it also helped to increase the flow ability of LXM. The usage of SDL as diluent was ruled out considering its incompatibility associated with LXM.

4.3 Enteric coating of LXM optimized core tablets

The selected L-7 tablets were coated with enteric coating Opadry® enteric polymer. Both coating dispersions were prepared according to technical document provided by the Colorcon, Goa (Colorcon technical document, 2009).[16] The coat was applied at a 4% weight gain. The coating process parameters were set as recommended by the manufacturer for enteric coating system.

The disintegration time of enteric coated LXM tablets were determined according to the procedure reported in USP.[30] Six tablets of LXM enteric coated tablets were weighed individually and placed in acid medium (0.1 N HCl) for 2 h in a USP basket rack assembly (Electrolab, 2DT) after which they were removed and inspected for cracking or disintegration. The same tablets were then placed in phosphate buffer, pH 6.8 and observed for disintegration.

Results showed that there were no signs of cracking, peeling or disintegration in 0.1 N HCl (pH 1.2) however the coatings of coated tablets were completely removed in 5–8 min in phosphate buffer (pH 6.8). The coated tablets were also evaluated other physical parameters like appearance, average weight, disintegration time, assay and content of isopropyl alcohol. The results are given in Table 5.

From the above results, it is observed formulation L11-C2 and L11-C3. The other evaluation parameters were also found within acceptable limit (Table 7:25). Hence the final batch prepared was a combination of L-7 and L11-C1. The final optimized batch was prepared and labeled as L-12 which contains 20% HPMC K4M polymer in core and 5% opadry® enteric material (94 series) in a coat.

Table 5: Evaluation of coated LXM tablets

Sr. No.	Test	L11-C1	L11-C2	L11-C3
1	Appearance	#	#	#
2	Thickness (mm)	4.43 ± 0.21	4.54 ± 0.30	4.48 ± 0.31
3	Average weight (mg) (± 5 %)	261.1	263.3	262.5
4	Disintegration test			
	1. 0.1N HCL	*	*	*
	2. Phosphate buffer (pH 6.8)	5.41	7.23	7.54
5	Assay (By HPLC)	99.57 ± 1.23	99.17 ± 0.77	98.80 ± 1.77

 # -Yellow colored, circular, concave, enteric coated tablets having plain surface on both the side
** - No signs of cracking or softening on tablet surface were observed.*

4.4 Comparison of *In-vitro* Release Profile of Optimized LXM matrix Formulation (L-12) with Marketed Formulation

Dissolution testing was carried out on USP dissolution apparatus I (basket) for L-12 matrix tablets (optimized formulation) with marketed tablets (Flexilor®), which was showed Figure 2.
The drug release of Flexilor® (Film coated tablet) was compared with L-12 formulation (Figure 2). The release of the drug from marketed preparation started instantaneously and more than 20% of drug was released within 120 minutes in 0.1N HCl and followed Peppas model. The release pattern of L-12 was slow to that of marketed preparation. Drug release was 0 % in 0.1N HCl after 2 h. This is due to enteric coating of L-12 formulation. But in phosphate buffer pH 6.8, the release was quick initially and showed sustain release afterwards. The drug release from L-12 and marketed Flexilor® tablet followed Matrix model (Table 6). The swelling behavior of L-12 formulation at different time interval is shown in figure 3.

Table 6: Comparison of *In-vitro* drug release kinetic studies of L-12 and marketed Flexilor® SR Tablet formulations

Batch No.	Zero order	1st order	Matrix	Peppas		Drug transport mechanism
	r^2				n	
L-12	0.8310	0.9172	0.9229	0.8618	1.8	Super case II transport
Flexilor	0.7611	0.9649	0.9748	0.9809	0.71	Non-Fickian Transport

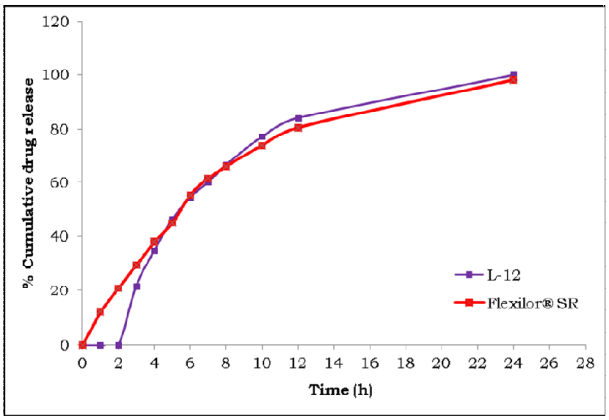

Figure 2: % cumulative drug release profile of L-12 formulation and Flexilor® in 0.1N HCl and Phosphate Buffer pH 6.8

The dissolution profile comparison may be carried out using model independent or model dependent method. In this study, the dissolution profiles of L-12 and Flexilor® were subjected for model independent methods proposed by Moore.[31] In order to consider the similar dissolution profile, the f1 values should be close to 0 (0-15) and values f2 should be close to 100 (50 -100). The difference factor (f1) and similarity factor (f2) were calculated.[32]

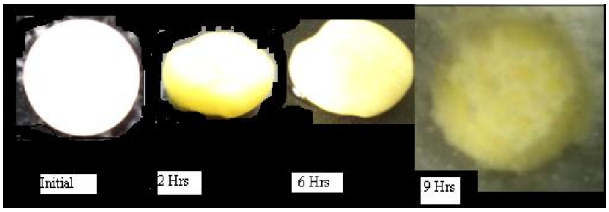

Figure 3: Swelling of LXM tablets (L-12) during dissolution (pH 6.8) at different time intervals

Flexilor® SR tablets and optimized LXM formulation (L-12) exhibited pH dependent drug release based upon LXM solubility in the dissolution media. When compared with marketed tablet Flexilor® SR tablet, f1 and f2 values were found to be 13.2 and 56.3 respectively, indicating a slightly equivalence between these two formulations.

4.5 Stability studies

Accordingly, the effect of storage at 40°C/75% RH for 6 months on the physical properties and *in-vitro* release of tablets belonging to formulation L-12 was investigated as per ICH guidelines.[21] All the stored tablets didn't show any change in their colour or appearance throughout the storage period. The friability and hardness was found within specified limit during the study period. The drug content (n=10; Mean ± SD) was found above 99.0 % at the end of 6 months (initial: 100.02±0.45%; 1 month: 99.86 ±0.30%; 2 month: 99.67±0.63%; 3 month: 99.91±0.43%; 6 month: 99.21±0.61%). This indicates that L-12 tablet is fairly stable at accelerated storage condition. However real time stability studies for a period of 2 years are required to establish the stability of developed product.

4.6 In-vivo bioavailability study

Formulation (L-12) was selected as a suitable formulation for the bioavailability study in human volunteers, because of its slow drug release, ability to swell in pH 6.8 and bioadhesive ability as showed in *in-vitro* testing. The values of pharmacokinetic parameters were tested for equality of variance; on acceptance of the hypothesis, paired *t*-test was used to test the significance of the observed difference in pharmacokinetic parameters; else, the t-test with unequal variance was used to test the significance.
The retention times of LXM and Thiocolchicoside were 14.52 and 5.86 minutes respectively. The peak area ratios of LXM to Thiocolchicoside were calculated and plotted against the respective concentrations of LXM to obtain the calibration curve. The calibration was carried out with prepared serum samples (ranging from 75-1000 ng/mL). Linear least square regression line of the constructed standard curve was computed and the correlation coefficient was found to be 0.9997. The LOD and LOQ of LXM were found to be 48 ng/mL & 62 ng/mL respectively. The method was found to be precise (intra- and inter-day variation was found to be less than 2%) and accurate (mean recovery 99.8%).
The mean plasma concentrations of LXM at each time point following administration of L-12 and Flexilor® are shown in Figure 4 and the pharmacokinetic parameters are listed in Table 7. The peak concentrations (C_{max}) of Flexilor® SR tablet and L-12 were 856.5 ±54.51 and 842.8 ±72.44 ng/mL, respectively with a significant difference of ($P<0.0001$), while, the time to reach peak concentration (T_{max}) was 2.38 ± 0.52 and 4.38 ± 0.52 hours, respectively with significant difference of ($P<0.0001$) with each other.
The AUC_{0-24} and AUC_{total} of Flexilor® and LXM 12 were 5845 ± 685.66 and 7497.48 ± 833.52 ng.hr/mL^{-1} and 6075.15 ± 731.58 and 7936.35 ± 940.87 ng.hr/mL^{-1}, respectively with significant difference of ($P<0.0001$) with each other. The T_{max}, AUC_{0-t} and AUC_{Total} obtained with L-12 and reference marketed product when studied with paired *t*- test showed significant difference ($P<0.05$) between the two formulations. This difference may be due the reason that formulated product is administered as delayed release bioadhesive sustained release formulation (L-12) and other as a sustained release tablet.

Table 7: Pharmacokinetics of Lornoxicam following oral administration of formulation Flexilor® 16 mg and L-12 tablets (n=8).

Pharmacokinetic parameters	Flexilor® Mean (±SD)	L-12 Mean (±SD)	Significant Difference (p<0.05)
Cmax (ng/mL)	856.5 ± 54.51	842.8 ± 72.44	P<0.0001
AUC $_{0-24}$ (ng h/mL)	5845.94 ± 685.66	7497.48 ± 833.57	P<0.0001
AUC $_{Total}$ (ng h/mL)	6075.15 ± 731.58	7936.35 ± 940.87	P<0.0033
T $_{max}$ (h)	2.38 ± 0.52	4.38 ± 0.52	P<0.0033

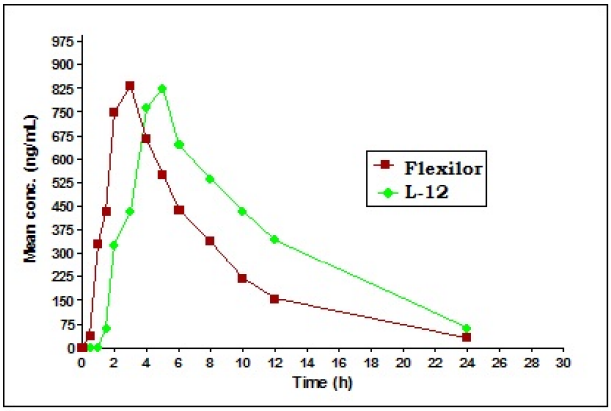

Figure 4: Mean serum levels of Lornoxicam after oral administration of formulation L-12 and Flexilor® 16 mg tablets. Each point represents mean value ± standard deviation (n =8).

Based on the statistical analysis of LXM on 8 subjects, the study results clearly demonstrate that the C_{max} of Test product is nearly bioequivalent and AUC_{0-24} and AUC_{Total} values shows that bioavailability was improved compared to the reference product Flexilor® SR tablet manufactured by Glenmark pharmaceutical ltd, mumbai under fasting conditions

4.7 Gastrointestinal Transit (GI) behaviour

As Gamma scintigraphy is a technique whereby the transit of a dosage form through its intended site of delivery can be non-invasively imaged *in-vivo* via the judicious introduction of an appropriate short lived gamma emitting radioisotope. The observed transit of the dosage form can then be correlated with the rate and extent of drug absorption. As this technique was not available hence radiographic imaging technique was used.[33]

Behavior of the mucoadhesion of tablet in the human intestine was observed in real time using radiographic imaging technique (Figure 5). In radiographic images made at 30 minutes after the administration of tablets. The drug was not liberated from tablet and does not observe in the human stomach. In the next picture taken at 1 h, no significant changes were detected. After 2 h, the tablet had seen in duodenum part of small intestine and it does not altered its position and remain adhered in next pictures. This provided evidence that the tablet adhere to the intestinal mucosa. Additionally the tablet was visualized in the subsequent X-ray films very well.

Thus, from the radiographic images taken up to 6 h, it can be concluded that the tablet passed the stomach after 2 h and adhere to intestine region. However the *in-vitro* results showed good adhesion in intestine, and *In-vivo* studies showed 2h gastric retention thus indicating that the developed tablet showed bioadhesive characteristics.

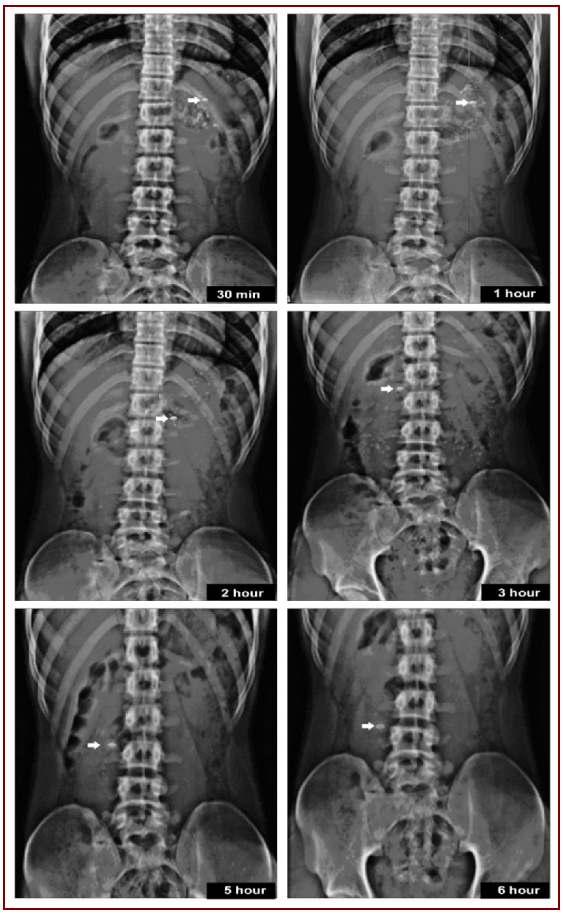

Figure 5: X-ray photographs recorded at 0.5, 1, 2, 3, 5 & 6h after oral administration of blank formulation of L-12 in human volunteer.

5. CONCLUSION

On the basis of present study it was observed that enteric coated sustained release bioadhesive tablets of LXM prolong the time for absorption, bioavailability and thus better patient compliance with minimal side effects can be achieved. The sustained release formulations of LXM developed in this investigation was found to be better when compare with marketed formulation (Flexilor® SR Tablet), based on *in-vitro* release studies. Hence the L-12 formulation was subjected for *in-vivo* study and detail investigation showed that L-12 can be a useful alternative formulation in comparison with Flexilor® SR tablet. The prepared formulation is expected to be less irritant to gastric and intestinal mucosa as it bypass the stomach region and the polymer, xanthan gum have natural mucosal protective properties.

6. ACKNOWLEDGMENT

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7. CONFLICT OF INTEREST

The authors report no conflicts of interest.

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