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Formulation Optimization and Pharmacokinetic Studies of an Enteric Coated Sustained Release Mucoadhesive Tablet of Zaltoprofen

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ABSTRACT

The objective of present study was to prepare and evaluate Zaltoprofen (ZLT) enteric coated oral mucoadhesive sustained release (SR) tablet in order to improve its GI residence time and improve its bioavailability by using natural biopolymers like xanthan gum and semisynthetic polymer HPMC for its safe use in rheumatoid arthritis, osteoarthritis, ankylosing spondylitis condition. The sustained release polymers, hydroxypropyl methylcellulose (HPMC) of different viscosities and xanthan gum evaluated in different proportions as a major matrix material. Drug-polymer compatibility studies by FTIR and DSC gave confirmation about their purity and showed no interaction physically between drug and selected polymers. ZLT matrix tablets were prepared by wet granulation. The effect of polymer concentration on the drug release profile and *in-vitro* bioadhesion of the matrix tablets was studied. A 3^2 full factorial design was utilized in the optimizing the levels of HPMC and Xanthan gum. Concentration of HPMC K4M and the concentration of xanthan gum per tablet were used as the independent variables. The dependent variables were the bioadhesive strength, percent drug dissolved at 2, 6 and 10 hours. The data obtained were fit to a model and polynomial equations were generated. Response surface graph was generated based on these equations. Formulation composition with desired release characteristics and bioadhesive strength were found to be predictive using this model. The optimized factorial batch was further given the coating of Opadry® enteric (94 series) polymer in order to avoid GI disturbances. The Z-22 tablets were kept for stability study at 40°C ±2°C and 75% ± 5% RH for a period of 6 months according to ICH guidelines. The formulation was found to be stable after 6 months of study. The pharmacokinetic parameters C_{max}, T_{max}, Mean Residence Time (MRT) and Area under Curve (AUC) of developed SR tablet were found to be improved with significant difference ($p<0.05$) when compared with marketed immediate release tablets each containing 80 mg of drug. From these results, it was concluded that the enteric coated oral mucoadhesive SR tablet containing Xanthan gum and HPMC K4M can be a good alternative to immediate release single unit dosage forms to treat rheumatoid arthritis.

Keywords: Zaltoprofen, Mucoadhesion, HPMC, Xanthan gum, Bioavailability studies

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INTRODUCTION

Zaltoprofen (ZLT) is one of newly developed non-steroidal anti-inflammatory drugs, which contains phenylpropionic acid in structure[1]. It has a clear anti-inflammatory, analgesic and antipyretic effect and frequently used for rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gout. It is used in the treatment of rheumatoid arthritis and osteoarthritis as well as it has excellent effects even on post surgery and post trauma chronic inflammation[2, 3.] ZLT has short biological half-life (4.96 – 7.48 h) and blood drug level decreased in short time so that patients need frequent administration (t.i.d) [4, 5, 6]. It is newly developed NSAID which preferentially acts on cycloxygenase-2 (COX-2) at the inflammatory sites and has the minimized effects on GI tract, articular cartilage or kidney[7,1].

ZLT in doses of 80 mg three times a day has been found to be effective NSAID with very low incidence of GI adverse events in the management of acute as well as chronic pain in various arthritic and acute musculoskeletal painful conditions[8,3]. It is well absorbed throughout GI tract when administered orally. It exhibits linear pharmacokinetics and metabolism is mainly by liver. ZLT is 99.6 % bound to human plasma proteins; the free fraction in the plasma is only 0.4 % [9,10,11]. At present, most of domestic preparations of ZLT are common tablets containing 80 mg of ZLT and that need to be administered several times a day. The short biological half-life and dosing frequency (t.i.d.) of ZLT makes it an ideal candidate for sustained release tablet[12]. Hence To reduce the frequency of administration and to improve patient compliance, a once-daily sustained release formulation of ZLT is desirable [13,14,15]. Therefore it's necessary to study on ZLT sustained release mucoadhesive preparations. Based on the domestic research of pharmaceutical materials used for sustained release and controlled release muoadhesive preparations, Hydrophilic matrices were selected due to their simplicity in manufacturing processes required, stability of raw material and dosage form also the cost effectiveness and broad regulatory acceptance [16,17,18].

Hydroxy propyl methyl cellulose (HPMC), a synthetic polymer, of different viscosities mixed in certain proportion were chosen in present study as major matrix material due to its non-toxicity, capacity to accommodate high levels of drug loading, good compressibility, as well as its superior matrix building abilities [19,20,21] and also xanthan gum, a natural polymer were used alone and also in combination with HPMC. Bioadhesion is a property observed with some of these hydrogels and, therefore, they can serve the dual purpose of release control and bioadhesion. Release from a polymeric matrix is controlled primarily by the molecular weight and the solubility, whereas bioadhesion is a function of molecular weight, hydrophilicity, chain length, chain flexibility, and

functional groups [22,23]; hence, hydrogels offer a great versatility in the design of oral controlled-release dosage forms[24].The objective of present study was to prepare and evaluate ZLT enteric coated oral sustained release (SR) bioadhesive tablet, by employing factorial design, in order to improve its GI residence time and bioavailability by using natural biopolymers for its safe use in rheumatoid arthritis and osteoarthritis.

MATERIALS AND METHODS

Model drug ZLT was a gift sample from Macleods Pharmaceuticals Ltd., Mumbai, India and Nevirapine (internal standard) was gifted from Matrix lab., Sinnar, India. Xanthan gum was gifted by GlaxoSmithKline Ltd., Nashik, India. Opadry enteric white® (94 series) coating material, Hydroxypropyl methylcellulose (HPMC) E5M, K4M, HPMC K15M, and HPMC K100M (with reported nominal viscosity values of 5, 4000, 15000, and 100000 cP respectively, when present in concentration of 2% in water at 20°C) were kindly supplied by Colorcon Asia Pvt. Ltd, Goa. The marketed Zaltoprofen 80 mg tablets (Zalto® IR tablet, Batch No.KL0947) of Intas Pharmaceutical Ltd., Sikkim, India used as reference product for bioavailability studies were purchased from local market (Loni, India). All other reagents and solvents used were of analytical grade procured from Merck, Mumbai, India.

Methods

Compatibility of ZLT with different tablet excipients

Physical mixtures of ZLT with various tablets excipients namely; HPMC K4M, HPMC K15M, HPMC K100M, Xanthan gum, Opadry enteric®, Microcrystalline cellulose (Avicel PH 101), Mannitol, magnesium stearate and talc were prepared by mixing in weight ratio of 1:1. The blend was filled in a transparent glass vials and were closed by rubber closure and sealed with aluminium cap. The glass vials were stored at 40°C ± 2°C/ 75% ± 5% RH for a period of 4 weeks in a stability chamber. The blend was observed after end of 7, 14, 21 days and 30 days. The mixtures were evaluated for possible interactions via visual inspection (appearance), differential scanning calorimetry, Fourier-transform infrared spectroscopy and drug content tests.

Preparation of SR bioadhesive tablets containing ZLT

The detailed compositions of the prepared SR bioadhesive matrix tablet formulations are given in Table 1. The matrix tablets, with theoretical weight of 600 mg, containing ZLT together with other excipients were prepared by wet granulation technique. The concentration of ZLT was kept constant in all the prepared tablets (240 mg/tablet). To make powder mixtures; the drug, polymer, mannitol and avicel PH 101 were pass through sieve mesh ASTM no. 40 and thoroughly mixed for

5 min by means of a pestle in a glass mortar. The binder solution of PVP K-30 was prepared in isopropyl alcohol. The wet mass was pass trough sieve mesh ASTM no. 20. The wet granules were dried at room temperature. Thereafter, the granules were lubricated with magnesium stearate and talc for 3 min. The resultant granules of exactly 600 mg were directly compressed into tablets using a 8 station rotary tablet machine (Rotary Tabletting Machine, CMB3-16, Cadmach, Ahmedabad, India) equipped with 10 mm round, flat, and plain punches.

Table 1- Compositions of the prepared preliminary bioadhesive SR tablets containing ZLT

Sr. No.	Ingredients	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10	Z11	Z12
1	ZLT	240	240	240	240	240	240	240	240	240	240	240	240
2	Mannitol	40	40	40	40	40	40	40	40	40	40	40	40
3	Avicel PH101	167	167	167	167	197	167	137	197	167	137	107	77
4	HPMC E5 LV	120	-	-	-	-	-	-	-	-	-	-	-
5	HPMC (K4M CR)	-	120	-	-	90	120	150	-	-	-	-	-
6	HPMC (K15M)	-	-	120	-	-	-	-	-	-	-	-	-
7	HPMC (K100M)	-	-	-	120	-	-	-	-	-	-	-	-
8	Xanthan Gum	-	-	-	-	-	-	-	90	120	150	180	210
9	PVP K-30	15	15	15	15	10	10	10	15	15	15	15	15
10	Magnesium Stearate	9	9	9	9	9	9	9	9	9	9	9	9
11	Talc	9	9	9	9	9	9	9	9	9	9	9	9
12	Isopropyl alcohol	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs
	Total Weight (mg)	600	600	600	600	600	600	600	600	600	600	600	600
	Polymer conc.	20 %	20 %	20 %	20 %	15 %	20 %	25 %	15 %	20 %	25 %	30 %	35 %

Optimization of formulation using 3^2 Factorial design

After studying results from preliminary batches, 3^2 full factorial designs were prepared as shown in

Table 2 Formulation of 3^2factorial batches in weight (mg)

Sr. No.	Ingredients (mg)	Z13	Z14	Z15	Z16	Z17	Z18	Z19	Z20	Z21
1	Zaltoprofen (ZLT)	240	240	240	240	240	240	240	240	240
2	Mannitol	40	40	40	40	40	40	20	40	40
3	Microcrystalline Cellulose	47	77	107	17	47	77	7	17	47
4	HPMC (K4M CR)	90	90	90	120	120	120	150	150	150
5	Xanthan Gum	150	120	90	150	120	90	150	120	90
6	Polyvinyl pyrrilidone (K30)	15	15	15	15	15	15	15	15	15
7	Magnesium Stearate	9	9	9	9	9	9	9	9	9
8	Talc	9	9	9	9	9	9	9	9	9
9	Isopropyl alcohol	qs	qs	qs	qs	qs	qs	qs	qs	qs
	Total tablet weight (mg)	600	600	600	600	600	600	600	600	600

In this design 2 factors are evaluated, each at 3 levels, and experimental trials are performed at all 9 possible Combinations. The amounts of matrixing agent, HPMC K4M (X1), xanthan gum (X2) were selected as independent variables. The bioadhesive strength, Q2, Q6 and Q10 were selected as dependent variables (Box, 1960; William, 2000; Rekab K et al 2005). The coded and actual value of variables for each batch and the experimental design are given in Table 2. The range of a factor was chosen in order to adequately measure its effect on the response variables.

Optimization of coating process:

The optimized and finalized formulation from factorial design was subjected for enteric coating. The enteric coated polymer opadry enteric white® (94 series) was optimized for coating[25]. The composition of coating formula is as given in table 3

Table 3 Optimization of coating composition for ZLT SR tablet

Sr. No	Ingredients (mg)	ZC1	ZC2	ZC3
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 white	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

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.

Evaluation of the Designed Tablets

Physical Testing on prepared tablets

The thickness of the tablets was determined using a Vernier caliper. Five tablets from each batch were analyzed and average values were calculated. The weight variation[26] (IP 2007) 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[27] (BP 2007), 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.

Drug content estimation:

The drug content of the prepared matrix was determined in triplicate. From each batch, 20 tablets were taken, weighed, crushed and finely powdered. An accurately weighed quantity of this powder was taken and suitably dissolved under sonication in methanol and filtered through whatman filter paper no 5. The sample was analyzed after making appropriate dilutions using the UV (Shimadzu UV - 1700 UV/VISIBLE spectrophotometer with UV probe 2.10 software) and HPLC (Agilent HPLC, 1100 series, with UV detector & Chemstation Software) analytical method given in literature[28, 29].

In-vitro drug release studies

The release of ZLT from the prepared matrix tablets was performed in a USP Dissolution Tester (EDT-O8L, Electrolab Dissolution Testing Station, Mumbai, India)., Apparatus II (Rotating paddle) at a rotation of 100 rpm. The release studies were carried out in 900 mL of phosphate buffer of pH 6.8 maintained at 37 ± 0.5°C for 12 h [30].

Tablets were placed in the dissolution medium and apparatus was run. After the intervals of 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours, an aliquot (5 mL) was withdrawn and filtered through 0.45 µ millipore filter. Replacement was made each time with 5 mL of fresh dissolution medium maintained at the same temperature. The 2 mL of filtrate was diluted to 20 mL with phosphate buffer pH 6.8 and or 0.1 N HCl and the absorbance were measured at 243 nm. Drug concentrations in the samples were determined from the standard calibration curve. Cumulative percent of drug dissolved was found out at each time point.

Ex Vivo Bioadhesion Studies[31]

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.

***In-vivo* bioavailability study of ZLT 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, Letter No-PMT/PMIS/RC/2011/31). Eight healthy male volunteers in the age group of 20–35 years (56–72 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).Group A volunteers (n=4) received Zalto® immediate release tablets (Batch no. KL0947, Intas Pharmaceuticals ltd, Sikkim, dose 80 mg) whereas group B (n=4) volunteers received developed Z-22 tablet containing 80 mg of Zaltoprofen. A light breakfast was provided after overnight fasting. After 30 minutes, Sample and Zalto® IR 80 mg 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, 5, 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.

Estimation of ZLT in human serum

Analysis of ZLT in human plasma samples was done by modification of HPLC method reported by Oshima[32], Choi[33], and Manish Kumar[34] with slight modification. Standard stock solution of ZLT and Nevirapine (internal standard) were prepared by dissolving 10 mg of each drug in 100 mL of methanol in separate volumetric flasks to get concentration of 100µg/mL. The stock solution of ZLT was further diluted with methanol to get series of working standard solutions having concentration 0.05, 0.1, 0.2, 0.5, 1, 2, 3, 4, 5, 10, 15 and 20 µg/mL. 12 mL of Nevirapine stock solution was further diluted to 20 mL with methanol to get internal standard solution of concentration 60 µg/mL. 1 mL of each working standard solution of ZLT (0.01-15 µg/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 Nevirapine (60 µg/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 10 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. To 500 µL of plasma, 50 µL of Nevirapine (60 µg/mL) was added and vortexed.

The drug was extracted with 3mL of ethyl acetate followed by centrifugation at 3500 rpm/min on a centrifuge for 10 min. 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. To the residue 500 µL of mobile phase was added. After vortex, an aliquot of 20 µL was injected into HPLC system.

The Agilent Technologists HPLC system (1100 series LC) equipped with quaternary pump, degasser, auto sampler, thermostated column compartment and UV detector, a reversed phase C_{18} Chromatopak's Peerless column (250 mm × 4.6 mm x 5 µm). HPLC mobile phase was composed of 10 mM KH2PO4 (pH 3): Acetonitrile (40: 60 % v/v) at a flow rate of 1 ml/min with the detector wavelength set at 254 nm.

Pharmacokinetic data analysis

ZLT 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).

Gastrointestinal Transit (GI) Behaviour[35]

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,). The study was conducted by administering one tablet containing 240 mg of barium sulphate to a subject[35,46]. One healthy male subject (age of 28 years; weight 65 kg) participated after giving informed consent. A written consent was obtained from him. The tablet containing radio-opaque marker (barium sulphate) were prepared in a similar manner to formulation Z-22 by replacing the drug. The subject administered the tablet with 200 ml of water, after the subject had fasted overnight. Lunch was provided 5 h after administration of radio-opaque formulation. 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 of the gastric region at determined time intervals up to 6 h. All X-ray films were taken in anterior positions.

RESULTS AND DISCUSSION

Compatibility of ZLT with different tablet excipients

The Visual inspection of stored powder mixtures of ZLT with different tablets excipients did not show any change in color or appearance (e.g. discoloration, caking, liquefaction, formation of clumps). This represents a good preliminary indication of physical stability.

The compatibility of ZLT with the excipients was investigated using Differential Scanning Calorimetry (DSC-SIIO 6300, Japan) with Auto-sampler), since it is considered as a rapid method for evaluating any possible incompatibilities between drug and excipients. The DSC thermograms of pure ZLT and their 1:1 physical mixtures are shown in Figure 1. The DSC thermogram of pure ZLT exhibited a sharp endothermic peak at 140.2°C which corresponds to its melting and decomposition[1]. Regarding the DSC scans of pure excipients; the thermograms of all grades of HPMC showed a faintly endothermic effect ranging from 50–120°C that might be ascribed to their dehydration and an endothermic effect above 220°C that might be attributed to their decomposition (Figure 1). Basically, ZLT endothermic peak was evident in all the thermograms of its physical mixtures with the mentioned excipients which might indicate compatibility. However, noticeable broadening in ZLT peak intensity was observed in some thermograms. This is probably attributed to differences in geometry of the mixture samples as reported by other authors[36, 37]. In conclusion, the observed DSC results ruled out the incidence of any incompatibility between ZLT and the investigated excipients.

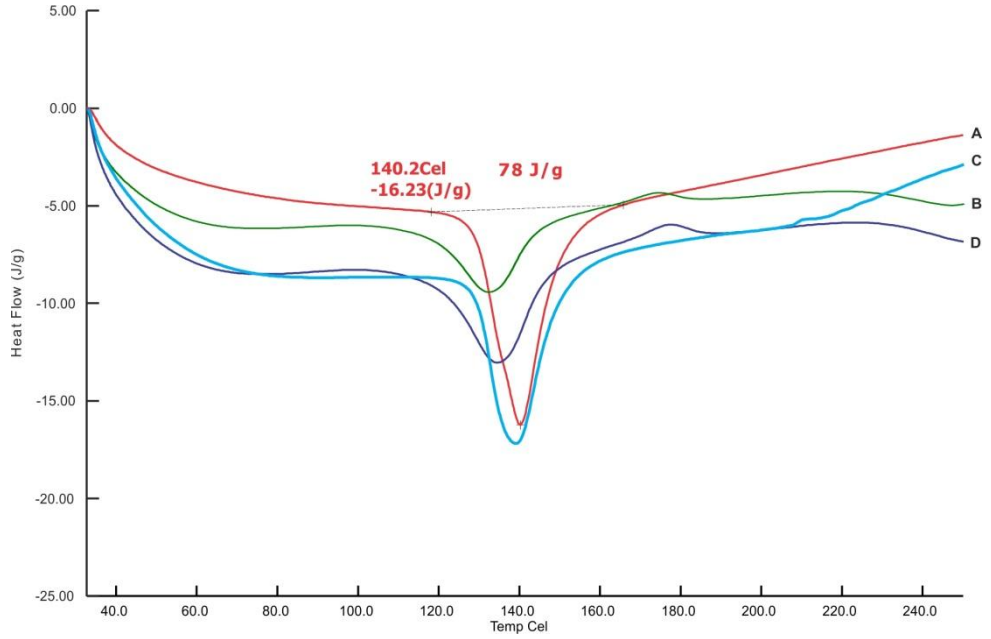

Figure 1: DSC thermograms of ZLT and its physical mixtures (1:1); A=ZLT Pure, B=ZLT+ HPMC, C=ZLT + Xanthan Gum, and D=ZLT+ Opadry® enteric.

The Fourier-Transform Infrared Spectroscopy (FTIR spectrophotometer, Alfa-T, Bruker, UK) spectrum of pure ZLT, pure excipients, and their 1:1 physical mixtures are shown in Figure 2. The FTIR spectrum of ZLT drug shows characteristic infra red absorption peak for -OH stretch (carboxylic acid) at 2977.9 cm-1 which is supported by a peak observed at 1079.9 cm-1 of –OH stretch. It also showed prominent peak at 1704.5 cm-1 due to C=O stretch of ketone and another

strong sharp peak at 1672.2 cm-1 due C=O stretch in carboxylic acid. The peaks of C=C (aromatic) stretching were observed between 1400 – -1500 cm^{-1}.

The peaks in the region 1200-1300 cm^{-1} were characteristic of C-O stretching. Peaks at 853.7 cm^{-1} suggests presents of -CH bending. It is clear evident that the FTIR spectra of the physical mixtures of ZLT with different excipients showed the presence of ZLT characteristic bands at their same positions (Figure 2). Moreover, these spectra can be simply regarded as the superposition ZLT and the investigated excipients. This could indicate the absence of chemical interaction between the drug and the excipients confirming the DSC results presented formerly.

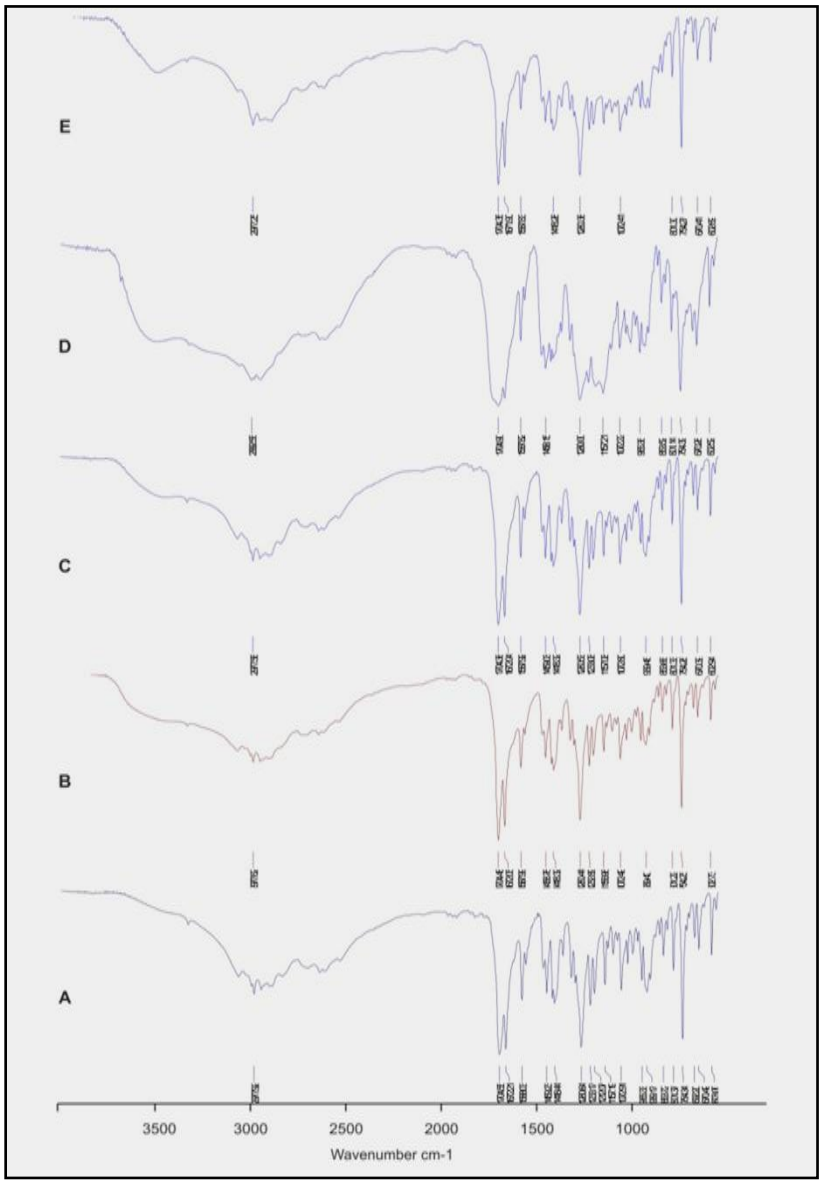

Figure 2: FTIR spectra of ZLT and its physical mixtures with different excipients; A=ZLT Pure, B=ZLT+ HPMC (1:1); C=ZLT + Xanthan Gum (1:1); D=ZLT+ Opadry enteric and E=ZLT+ Avicel PH 101 (1:1).

Physical tests for the prepared preliminary matrix tablets

The comparison of physical properties of the prepared matrix tablets are shown in Table 4. The weight of tablets formulations ranged from 598.5 – 605.2 mg. All tablets formulations prepared in this study met the pharmacopeial uniformity results were found to be good among different tablets formulations, and the percentage of drug content was more than 98.5 %. Tablets hardness is found between 5 – 8 kg/cm^2. In the present study, the percentage friability for all the formulations was below 1% indicating that the prepared tablets are of desire strength. Therefore, all the tablet formulations showed acceptable physical properties and complied with the reported pharmacopoeial specifications.

Table 4: Evaluation of preliminary bioadhesive SR tablets containing ZLT

Batch No.	Thickness (mm)	Average weight (mg)$^{\#}$	Hardness (Kg/cm^2)^t	Friability (%)	Assay-UV method (%w/w)*	Assay - HPLC method (%w/w)*
Z1	4.41	601.5	6-8	0.23	99.5	99.62±0.51
Z2	4.40	601.9	5-8	0.37	100.69	99.87±1.32
Z3	4.41	603.4	6-8	0.31	98.49	99.04±1.23
Z4	4.41	601.1	6-8	0.49	98.59	98.73±1.78
Z5	4.40	599.7	6-8	0.32	98.89	98.67±1.67
Z6	4.41	602.9	6-8	0.21	99.29	98.96±0.78
Z7	4.41	601.4	6-8	0.67	98.49	99.34±1.08
Z8	4.39	598.5	6-8	0.41	99.49	99.22±0.78
Z9	4.42	602.8	6-8	0.84	99.69	98.87±0.56
Z10	4.39	603.1	6-8	0.64	98.39	99.02±0.34
Z11	4.40	602.4	6-8	0.51	99.09	99.21±0.51
Z12	4.40	605.2	6-8	0.63	99.19	98.79±0.89

- average of 20 tablets; Weight variation test complies the IP limit; t- average of 10 tablets;

*- Average of 3 determination.

***In-vitro* Drug release of preliminary batches:**

i. Effect of Xanthan gum and HPMC Polymer Type on Drug Release

The Figure 3 shows that the percentage of drug released from the preliminary formulations of ZLT containing various grades of HPMC and Xanthan gum. It showed the drug release of Z1-Z4 formulations containing HPMC of different viscosity grade at 20 % concentration level. The Figure 3 clearly demonstrates that the release rate is greatly influenced by the formulation factors such as the type of HPMC and diluents. Slow drug release (Y_{12}=34.9 %) was observed from the Z4 tablets containing 20% w/w of HPMC K100M while relatively faster drug release (Y_4=100 %) was observed for Z1 formulations containing 20% of HPMC E5M.

In general, regardless of the polymer type, the rate and extent of drug release decreased with an increase in the viscosity of the HPMC polymer. The Z3 & Z4 formulation also showed retarding effect. When different grades of HPMC polymers are used for essentially the same purpose, such as sustained release and mucoadhesion in the current study, it is interesting to observe how the polymers compare with each other. As the HPMC level was increased, the polymer gel formed is more likely to be resistant to drug diffusion and erosion[38]. As the release rate-limiting polymer changes from a glassy state to rubbery state, a gel structure is formed around the tablet matrix, which considerably decreases the release of drug since it has to diffuse through this gel barrier into the bulk phase. The strength of gel depends on the chemical structure and molecular size of polymer[39]. The good drug release in case of formulation Z2 containing 15 % amount of HPMC K4M may be due to less tortuous diffusion path. From the results, it is clearly concluded that the drug release was dramatically retarded when the polymer level was changed from 15 % to 25 % w/w for HPMC K4M (Figure 3).

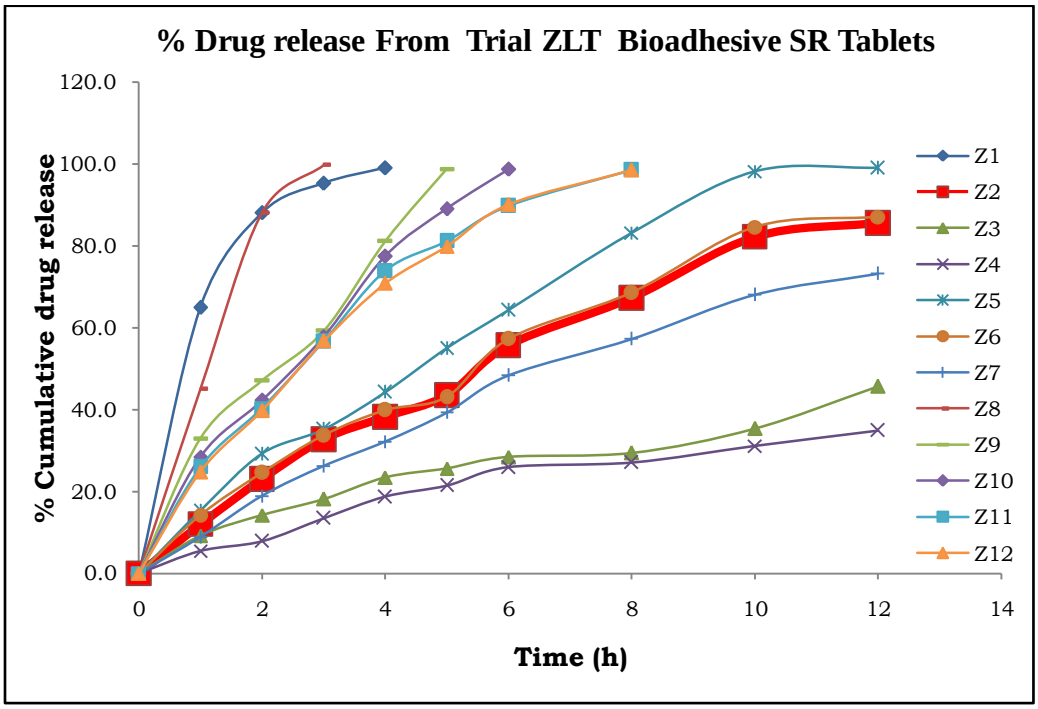

Figure 3: % Cumulative drug release (CDR) from preliminary formulations of ZLT bioadhesive SR tablets in phosphate buffer pH 6.8

The drug release profiles from the xanthan gum formulations (Figure 3), compared with those from HPMC formulations indicate a trend for rapid drug release from xanthan gum formulations. HPMC functioned as a better retardant of drug release than xanthan gum; however, the xanthan gum is a better retardant of drug release at higher concentration (>35%). Any differences in drug

release profiles between Xanthan gum and HPMC K4M tablets could also be attributable to the differences in the nature of the formation of their gel networks.

The use of two or more matrixing agents can be used to overcome the disadvantages of individual matrixing agent or to achieve desired drug release pattern[40]. HPMC forms stiff gel without quick hydration. Xanthan gum hydrates very quickly but cannot form a strong gel, causing erosion or dissolution of gel around the tablet[41]. Xanthan gum is available at lower cost and readily flowable than HPMC[42]. A logical reason to use the blend of HPMC and xanthan gum in the matrix tablet ZLT was that the drug release is superiorly explained by first order equation by both the matrixing agents, i.e., the same mechanism of drug release.

Evaluation of factorial batches:

A 3^2 full factorial design was utilized in the optimizing the levels of HPMC and Xanthan gum. Concentration of HPMC K4M and the concentration of xanthan gum per tablet were used as the independent variables. The data analysis of parameters obtained from various batches for dependent variables like bioadhesive strength (Figure 4), percent drug released at 2, 6 and 10 hours were subjected to multiple regression analysis. Positive sign of the term indicates Positive (additive) effect, while negative sign indicates negative (antagonistic) effect of the factor on the response. This design was selected as it provides sufficient degrees of freedom to resolve the main effects as well as the factor interactions. The data analysis was done by using "Stat Ease Design Expert 7.1.6 software" and fitted in equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X^2_2 + \beta_{12} X_1 X_2 \quad (1)$$

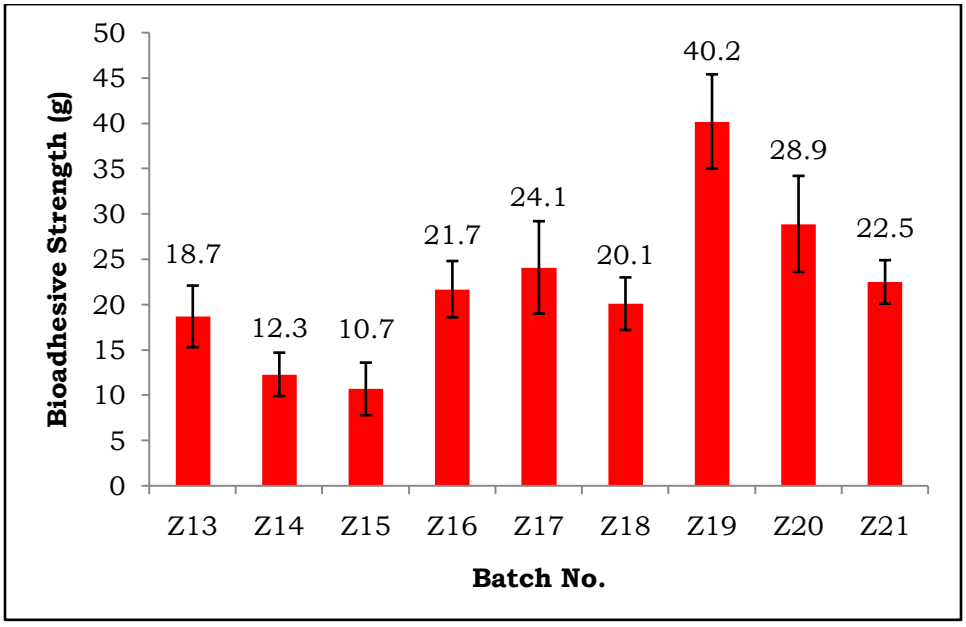

Figure 4: Bioadhesive strength (g) of factorial batches

Where, Y represents measured response; X levels of factors, and β, coefficient computed from the responses of the formulations. The high values of correlation coefficient for the dependent variables indicate a good fit. The equation may be used to obtain estimate of the response because small error of variance was noticed in the replicates. The data were also subjected to 3-D response surface methodology to study the interaction of HPMC K4M (X1) and Xanthan gum (X2) on dependent variables.

The tablets consisting of HPMC K4M(X1) and xanthan gum(X2) in different concentrations exhibited good mucoadhesive properties in the *ex-vivo* bioadhesion test observed with porcine mucosa.

$$\mathbf{Y_1 = 22.13+8.32X1+ 4.55X2 \ (r^2 = 0.8701)\ldots\ldots (1)}$$

The equation generated revealed that both, X_1 factor (HPMC K4M) and X2 factor (xanthan gum) exerted a significant influence on the mucoadhesion property. The polynomial equation clearly indicates that mucoadhesive strength was increased with increasing the amount of X1 and X2. But 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. The predictor equation generated for the geometric mucoadhesion was found to be significant with an *F*-value of 20.01 ($p < 0.05$).The influence of the main effects on the mucoadhesion of the tablet was further elucidated using the response surface plot (Figure 5A). Overall, mucoadhesion strength was found to be faster at intestinal level.

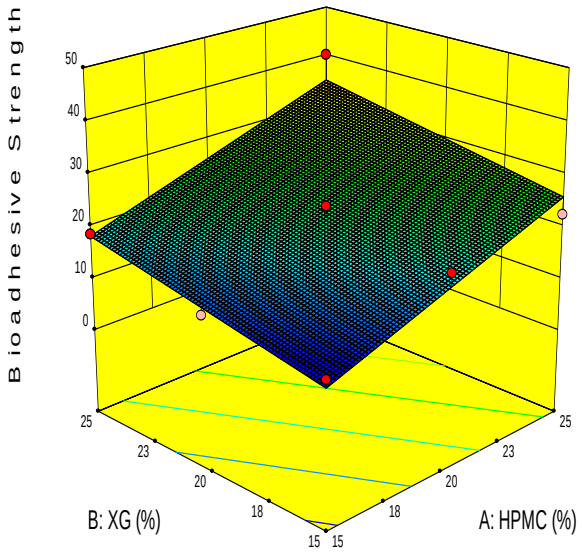

Figure 5A: Response surface graph showing effect of HPMC and xanthan gum on Bioadhesive strength

The regression equation for time Q2 (2h) taken to release of the drug in phosphate buffer pH 6.8 medium was as follows:

$Y2= 24.62-1.16\ X1-2.18\ X2\ (r^2=0.6335)$……(2)

The model term for ZLT release at the second hour was found to be significant with a probability value of 5.18 ($p < 0.05$), indicating an adequate fitting to the surface linear model. In this model, factor X_1, was found to be significant. As the ratio of polymers (X_1) increased, the amount of drug release at 1st hr found to be increased. Such a behavior of increase in the drug release at 2^{nd} hr may be attributed due to the formation of gel layer with low viscosity of polymer matrix of HPMC alone, which in turn increases the influx of water in to gel matrix leading to increased drug diffusion. Whereas in this model factor X_2 was found to be less significant, but the concentration of xanthan gum may also contribute to change in release at 2^{nd} hr. The influence of the effects on the Q2 release from the tablet was further elucidated using the response surface graph (Figure 5B).

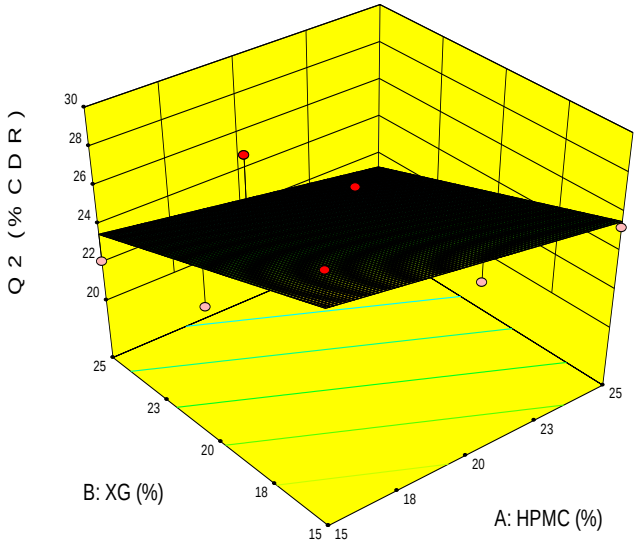

Figure 5B: Response surface graph showing effect of HPMC and xanthan gum on % drug release in Q2.

The regression equation for % drug release at 6h in phosphate buffer medium was as follows:

Y3 (Q6)= 51.76-5.45X1+0.22X2 (r2=0.6898) …….(3)

The equation generated for geometric % drug release was found to be significant with a *F*-value of 6.67 ($p < 0.05$). The model term for Q6 was found adequate fitting of the linear model. Both the factor when used in combination found to increase or decrease in % drug release at 6h.

The HPMC showed retarding effect on drug release, which might have caused slow penetration of medium in highly cross-linked matrix formation; hence slowing the drug release in buffer. Depending upon combination used for the formulation the result of multiple linear regression

analysis (linear model) reveals that, on increasing the concentration of HPMC K4M, Q6 is increased and vice versa.

The influence of main effect on the release drug after a period of 6h was further elucidated using response surface plot (Figure 5C). The figure reveals that the drug release rate was slowed after 6h.

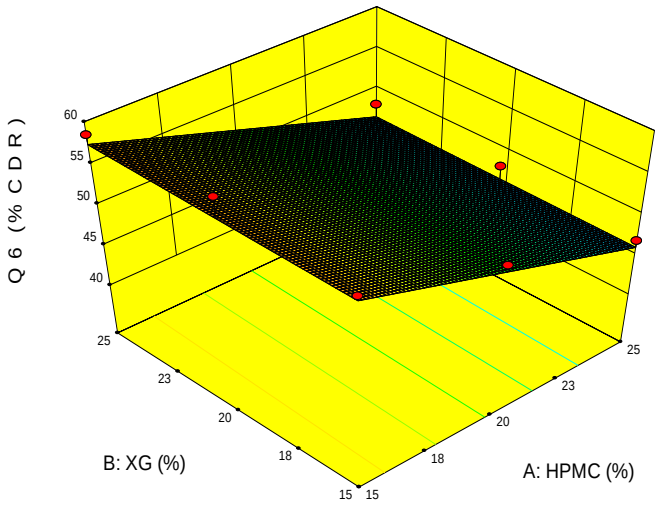

Figure 5C: Response surface graph showing effect of HPMC and xanthan gum on % drug release in Q6.in Q10.

The regression equation for % drug release at 10h in phosphate buffer medium was as follows:

Y4 (Q10) = 77.36 - 4.87X1 - 2.03X2 (r^2=0.5871).....(4)

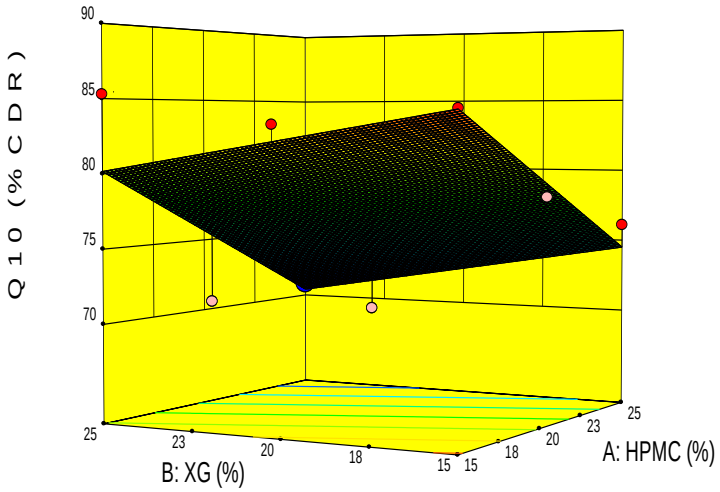

Figure 5D: Response surface graph showing effect of HPMC and xanthan gum on % drug release in Q10.

The equation generated for geometric % drug release was found to be significant with a *F*-value of 4.3 ($p < 0.05$). The model term for Q10 was found adequate fitting of the linear model. This is

further elucidated using response surface plot (Figure 5D). The HPMCK4M and xanthan gum both showed retarding effect on drug release at 10h, which might have caused slow penetration of medium in highly cross-linked matrix formation. The figure reveals that the drug release rate was slowed after 10h.

In-vitro study of Factorial batches:

The factorial design was applied to screen the optimum batch. The drug release from factorial batches is shown in figure 6. All the factorial batches evaluated for drug release up to 12 h. Formulations Z15, Z16 and Z18 shows good release profile compare to other formulations and exactly fit in the criteria for drug release. But the polynomial equations have been used to draw conclusions after considering the magnitude of coefficient and mathematical sign it carries (i.e. positive or negative). Statistical validity of the polynomials was established on the basis of ANOVA provision in the Design Expert software. Subsequently, the feasibility and grid searches were performed to locate the composition of optimum formulations. Also, the 3-D response surface graphs was generated by the Design Expert software and are shown in figure 5A, B, C & D.

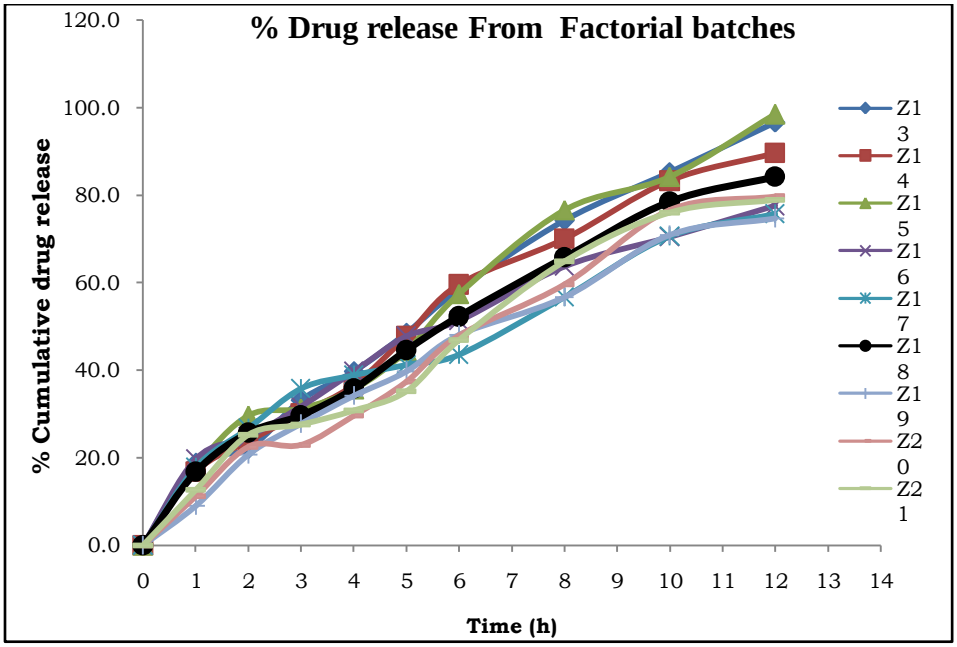

Figure 6: *In-vitro* dissolution of factorial batches of ZLT bioadhesive SR matrix tablets in phosphate buffer pH 6.8

In the present study, the drug release was analyzed by PCP Disso Version 3 software to study the kinetics of drug release mechanism (Table 5). Putting all data in different release kinetics models and comparing the coefficient of determination (r2), it was found that the release data of Z13, Z14 and Z19 obeys Hixon crowel model, where as Z15 and Z21 follows Zero order kinetics. First order

kinetics was followed by Z16 and Z18 formulation. To justify the results, power law was applied and from the diffusion coefficient value (n), it was found that all formulations follow Non-fickian diffusion transport mechanism.

Model validation:

The optimum formulation was selected based on the criteria of attaining complete and controlled drug release with highest possible bioadhesive strength (f). Upon "trading off" various response variables, the following maximizing criteria were adopted: f > 10 g; Q2 NMT 25%; Q6 > 50%; Q10 > 75%. Upon comprehensive evaluation of feasibility search and subsequently exhaustive grid searches, the formulation composition with polymer levels of 20% HPMC K4M and 17.5% Xanthan gum (Batch no. Z-22 optimized), fulfilled maximum requisites of an optimum formulation because of better regulation of release rate and higher bioadhesive strength. The formulation showed Q2 as 23.1%, Q6 as 53.7%, Q10 as 76.5% and f as 23.79 g. The said formulation, however, released the drug completely (ie, 98.7% drug in 24 hours) (Figure 7). The results showed a good relationship between the experimental and predicted values, which confirms the practicability of the model (Table 6). The release from the ZLT Optimized mucoadhesive SR matrix tablet comprising of the drug, synthetic and natural polymer, could follow three steps. First step can be the penetration of the dissolution medium in the tablet matrix (hydration). Second step could be the swelling with subsequent dissolution and/or erosion of the matrix and followed by the third step comprising of the transport of the dissolved drug, either through the hydrated matrix or from the parts of the eroded tablet, to the surrounding dissolution medium.

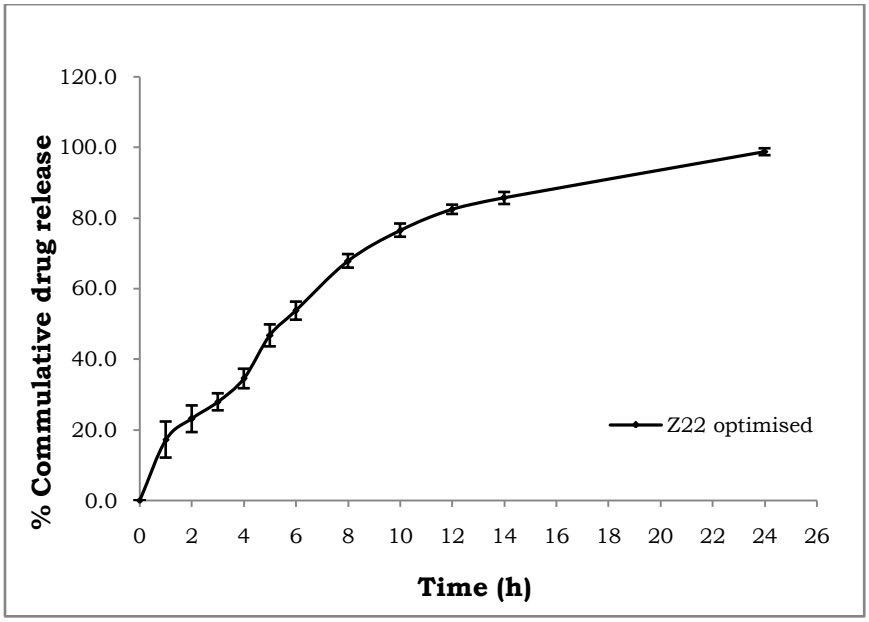

Figure 7: % Cumulative drug release from Z-22 Optimized formulation

Table 5: The correlation coefficient (r2) values for ZLT factorial formulations

Batch No.	Zero order r^2	1st order	Matrix	Hix.Crow	Peppas	n	Best Model
Z13	0.9719	0.9166	0.9700	0.9772	0.9914	0.67	Hix.Crow.
Z14	0.9746	0.9765	0.9645	0.9947	0.9914	0.72	Hix.Crow.
Z15	0.9876	0.8559	0.9453	0.9525	0.9801	0.75	Zero order
Z16	0.9194	0.9960	0.9923	0.9853	0.9952	0.57	1st order
Z17	0.9241	0.9850	0.9853	0.9796	0.9904	0.57	Matrix
Z18	0.9759	0.9822	0.9630	0.9752	0.9758	0.70	1st order
Z19	0.9773	0.9942	0.9639	0.9972	0.9954	0.85	Hix.Crow.
Z20	0.9912	0.9753	0.9389	0.9893	0.9941	0.84	Peppas
Z21	0.9873	0.9740	0.9296	0.9854	0.9807	0.82	Zero order

Table 6: Comparison of Predicted and Experimental Values for optimized formulation

Formulation	Response			
	Bioadhesive Strength	Q2 (%CDR)	Q6 (%CDR)	Q10 (%CDR)
Predicted value	19.9	23.7	52.6	78.4
Experimental value (Z-22 optimized)*	20.1	23.1	53.7	76.5
% Variation	-1.01	2.53	-2.09	2.42

* - mean ±SD (n=3).

The drug release was analyzed by PCP Disso Version 3 software to study the kinetics of drug release mechanism. Putting all data in different release kinetics models and comparing the coefficient of determination (r2), it was found that the release data of ZLT Optimized batch obeys first order release with correlation coefficient (r^2) value 0.9940. The diffusion coefficient value (n), it was found that the formulation follows non-fickian diffusion transport mechanism (n=0.70).

Enteric coating of ZLT optimized core tablets:

The selected ZLT optimized tablets were coated with enteric coating Opadry® enteric polymer (94 series). Opadry Enteric, acrylic-based coating system, is a fully-formulated, delayed release coating system for solid oral dosage forms. The 94 series of Opadry® Enteric Systems are formulated using methacrylic acid copolymer Type A (Methacrylic acid- methyl methacrylate 1:1 copolymer), which provides consistent gastro resistance and reproducible drug release. Both coating dispersions were prepared according to technical document provided by the Colorcon, Goa (Colorcon technical document, 2009)[25]. The coat was applied at a 3% weight gain. The coating process parameters were set as recommended by the manufacturer for enteric coating system.

The disintegration time of enteric coated ZLT tablets were determined according to the procedure reported in USP (USP 28, 2008). Six tablets of ZLT enteric coated tablets were 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 coating of tablets was 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 7.

From the above results, it is observed that the ZC1 formulation does not show any disintegration in 0.1N HCl medium while in phosphate buffer (pH 6.8), tablets loose coating in 5.21 min. This time is faster compare to formulation ZC2 and ZC3. The other evaluation parameters were also found within acceptable limit. Hence the final batch prepared was a combination of ZLT optimized and ZC1 (Table 8) and is designated as Z-22 batch.

Table 7: Evaluation of coated ZLT SR bioadhesive tablets

Sr.no.	Test	ZC1	ZC2	ZC3
1	Appearance	#	#	#
2	Thickness (mm)	4.61 ± 0.34	4.64 ± 0.27	4.62 ± 0.43
3	Average weight (mg) (± 5 %)	622.4	625.3	624.1
4	Disintegration test			
	1. 0.1N HCl	*	*	*
	2. Phosphate buffer (pH 6.8)	5.21	7.56	8.23
5	Assay (By HPLC)	99.57 ± 1.23	99.17 ± 0.77	98.80 ± 1.77

-White colored, circular, flat, enteric coated tablets having plain surface on both the side

* - No signs of cracking or softening on tablet surface were observed.

Table 8: Final composition of optimized formula of ZLT (Z-22)

Sr. No	Ingredients (mg)	Z-22
Core composition		
1.	Zaltoprofen	240
2.	Mannitol	40
3.	Microcrystalline Cellulose	62
4.	HPMC (K4M CR)	120
5.	Xanthan Gum	105
6.	Polyvinyl pyrrilidone (K30)	15
7.	Magnesium Stearate	9
8.	Talc	9
9.	Isopropyl alcohol	qs
	Total Weight (mg)	**600**
Coating Composition (%) (ZC2)		
10.	Opadry Enteric white (94 series)	5

11.	Red oxide of iron	0.055
12.	Lake quinoline white	0.050
13.	Yellow oxide of Iron	0.045
14.	Isopropyl Alcohol $ (80%)	qs
15.	Dichloromethane $ (20%)	qs
	Total weight (mg)	**620**

Compatibility with marketed formulations:

The FTIR spectra obtained for pure ZLT, Z-22 formulation and marketed Zalto® IR tablet are depicted in Figure 8. The characteristic peaks of ZLT are already discussed in "Compatibility of ZLT with different tablet excipients". Moreover, these spectra can be simply regarded as the superposition of pure ZLT, Zalto® and Z-22 formulation (Figure 8). The resemblance in the peaks for ZLT with the Z-22 formulation indicates compatibility with the polymers and excipients used in the formulation. There was no significant difference in the peak characteristics which indicates the stability in the formulation.

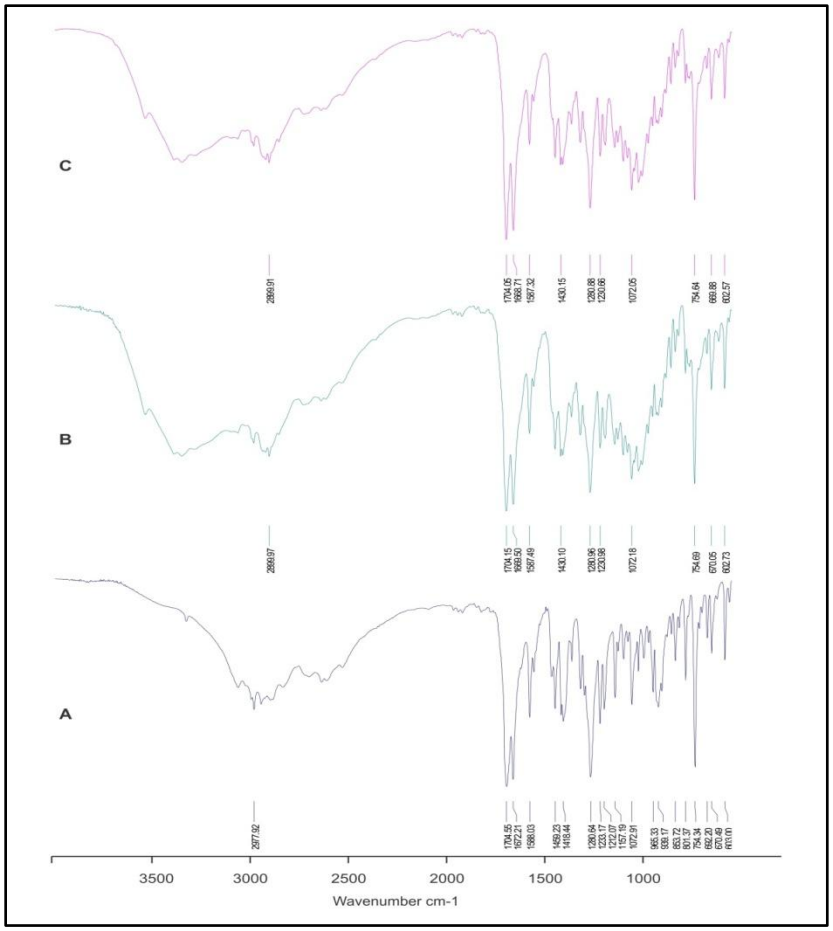

Figure 8: Overlay FTIR spectra of A= ZLT pure, B= Z-22 formulation; C=Marketed formulations (Zalto® Tablet)

In order to evaluate the physical state of a drug incorporated in the bioadhesive SR tablet, DSC analysis was performed for pure drug, Z-22 formulation and Marketed formulations (Zalto® Tablet) which are depicted in Figure 9. ZLT shows sharp melting endothermic peak at 140.2^{0}C, Z-22 formulation shows melting endotherm at 138.6ºC. However, there was a slight decrease in the melting point of drug when prepared in the form of tablet compare to pure drug. Zalto® shows melting endothermic peak at 138.4ºC, However, the intensity of the drug endothermic peak was noticeably decreased. This may be due to the great dilution of the drug caused by the presence of polymers in the formulation. A secondary endothermic peak was observed at 149.6 ºC in Z-22 and Zalto®, this could be due to excipient incorporated in formulation. The evaluation of the thermograms obtained from DSC revealed no interaction between the polymer and the drug in the Z-22 formulation.

The *in-vitro* drug release from optimized matrix tablets (Z-22) were not compared with the marketed tablets because no controlled release marketed formulations are available in Indian market.

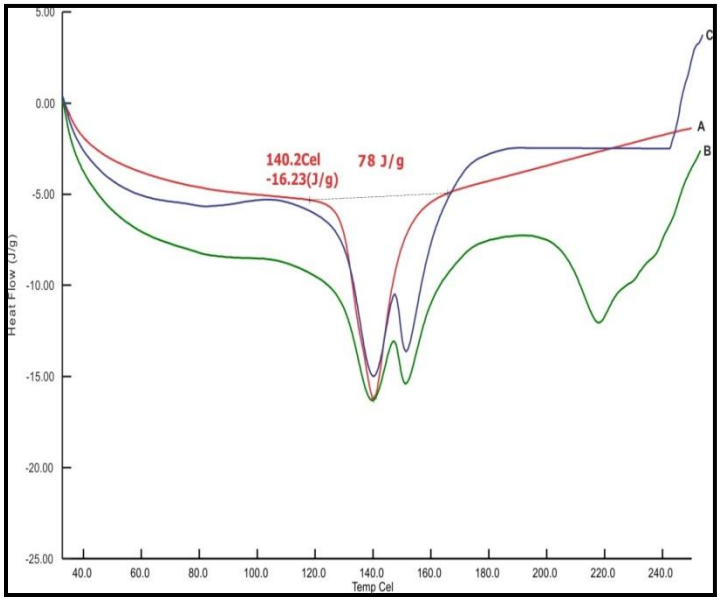

Figure 9: DSC thermograms of A= ZLT pure, B= Z-22 formulation; C=Marketed formulations (Zalto® Tablet)

Stability study for Z-22 Bioadhesive SR tablets:

A drug product may undergo changes in its physicochemical characteristics during storage and these changes can affect the bioavailability of drug from dosage forms. A unit of solid oral dosage form such as a tablet has to meet pharmacopeial specifications, such as drug content, friability, and release during its shelf life[43].

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 Z-22 was investigated as per ICH guidelines[44]. All the stored tablets didn't show any change in their colour or appearance throughout the storage period. . It is evident that the drug content of tablets belonging to formulation Z-22 remained within the acceptable limits. A slight increase in tablets friability was observed compared to the fresh ones. The release profiles of the fresh and stored tablets belonging to formulation Z-22. Evidently, a slight decrease in drug release was observed on comparing the fresh tablets to the stored tablets. However, even with this decrement, the stored tablets complied with the reported specifications of sustained-release products. This indicates that Z-22 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.

***In-vivo* bioavailability study**

The Z-22 formulation was selected for bioavailability studies due to its good bioadhesive property in both the media, while Zalto® IR 80 mg tablet was selected because no sustained release formulation of ZLT was available in Indian market. 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 ZLT and Nevirapine were 10.710 and 4.070 minutes respectively. The peak area ratios of ZLT to Nevirapine were calculated and plotted against the respective concentrations of ZLT to obtain the calibration curve. A good linear relationship was observed between the concentration of ZLT and the peak area ratio of ZLT to that of internal standard with a high correlation coefficient (r=0.997) in the range of 1-25 µg/ml. The LOD & LOQ of method was found 0.01 µg/mL and 0.05 µg/mL (RSD = 1.47%) 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 ZLT at each time point following administration of Z-22 and Zalto® are shown in Figure 10 and the pharmacokinetic parameters are listed in Table 9. The peak concentrations (C_{max}) of Zalto® tablet and Z-22 were 4.64±0.45 and 3.47±0.19 µg/mL, respectively with a significant difference of (P<0.0001), while, the time to reach peak concentration (T_{max}) was 1.56±0.32 and 5.88±1.55 hours, respectively with significant difference of (P<0.0001) with each other. The AUC_{0-24} and AUC_{total} of Zalto® and Z-22 were 22.39±3.73 and 44.23±4.91 µg.hr/mL^{-1} and 24.19±4.06 and 57.38±9.02 µg.hr/mL^{-1}, respectively with significant difference of (P<0.0001)

with each other The C_{max}, T_{max}, and AUC_{0-t} obtained with Z-22 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 one product is administered as immediate release tablet and other as bioadhesive sustained release formulation. Thus, the bioavailability was improved as compared to immediate release marketed product. This also establishes the fact that the developed Z-22 formulation was effective and better than the marketed dosage form tablet. Higher bioavailability with same dose and showing sustained effect can be considered as better qualities of the developed formulations.

Table 9: Pharmacokinetics of Zaltoprofen following oral administration of formulation Z-22 and Zalto® 80 mg tablets (n=8).

Pharmacokinetic parameters	Zalto® Mean (±SD)	Z-22 Mean (±SD)	Significant Difference (p<0.05)
C_{max} (µg/ml)	4.64 ± 0.45	3.47 ± 0.19	P<0.0001
T_{max} (h)	1.56 ± 0.32	5.88 ± 1.55	P<0.0001
AUC_{0-24} (µg.h/ml)	22.39 ± 3.73	44.23 ± 4.91	P<0.0032
AUC_{Total} (µg.h/ml)	24.19 ± 4.06	57.38 ± 9.02	P<0.0033

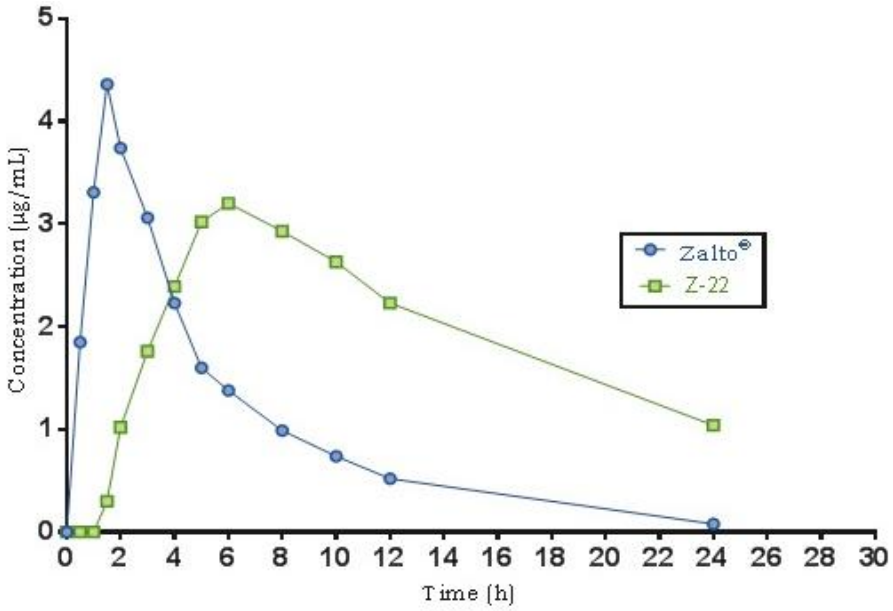

Figure 10: Mean serum levels of ZLT after oral administration of formulation Z-22 and Zalto® 80 mg tablets. Each point represents mean value ± standard deviation (n =8).

***Gastrointestinal Transit (GI) behaviour*[35]**

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[45].

Behavior of the mucoadhesion of tablet in the human intestine was observed in real time using radiographic imaging technique (Figure 11). 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. The administered formulation could not be detected after 24 h possibly due to the degradation of polymers in the colon and/or evacuation during the passage of the bowel.

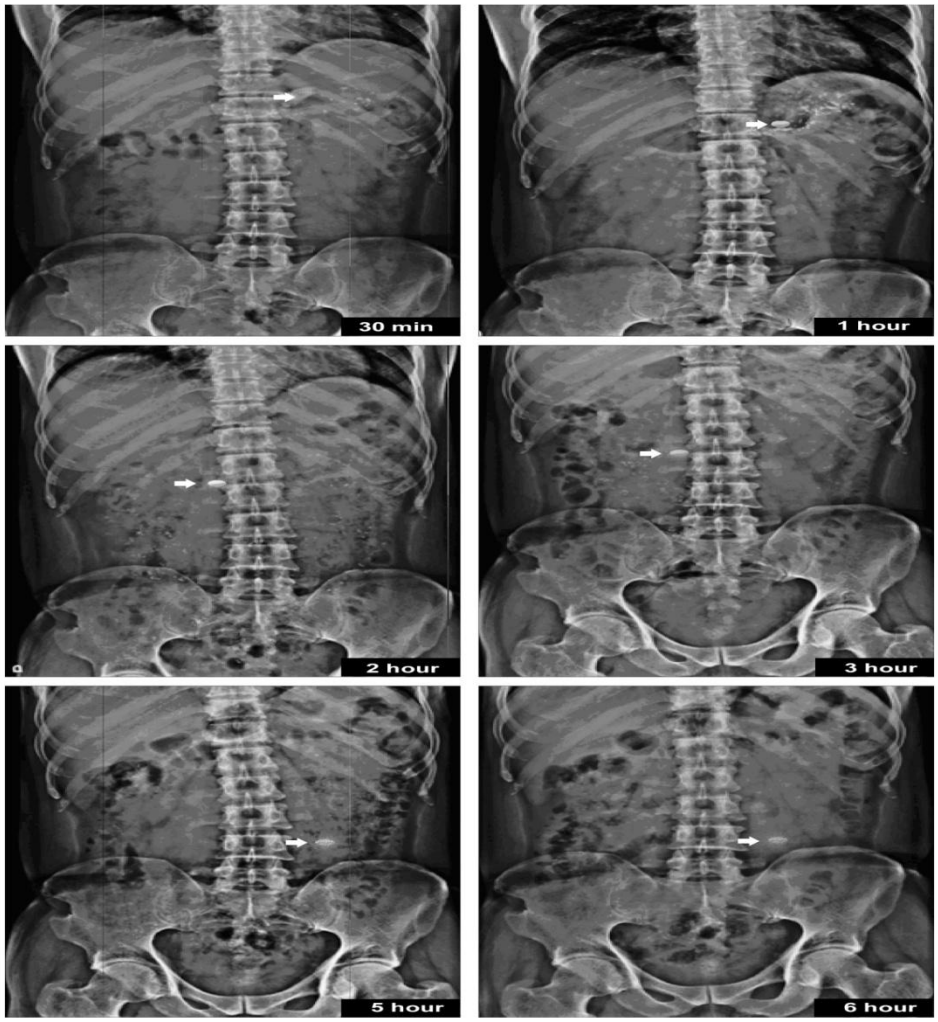

Figure 11: X-ray photographs recorded at 0.5, 1, 2, 3, 5 & 6h after oral administration of blank formulation of Z-22 in human volunteer.

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.

CONCLUSION

A ZLT bioadhesive SR tablet belonging to formulation Z-22, composed of 20% of HPMC K4M and 17.5% Xanthan gum, complies with the release specifications for SR products and also exhibited acceptable weight variation, drug content, mucoadhesive strength, friability and stability. Finally it is concluded that by adopting a systematic formulation approach, delivery of a drug from a single SR dosage form can be obtained which could improve patient compliance and give better disease management. This is further proved by *in-vivo* bioavailability study. The bioavailability of ZLT was improved compared to immediate release marketed product. The enteric coated SR bioadhesive tablet formulation Z-22 can be a useful alternative formulation in comparison with Zalto® IR tablet and it is expected to be less irritant to gastric and intestinal mucosa as xanthan gum have antiulcer and mucosal protective properties.

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